An Anatomical Study Of
The Development Of Cortical Visual Pathways
In The Wallaby (Macropus eugenii)

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Declaration

All experiments and analysis in this thesis are my own work, except for a set of slides from which analysis of the interhemispheric connections at postnatal days 122 was made. These were lent by Dr. L. R. Marotte. This work is supported by an Australian National University PhD Scholarship. Experiments were approved by the Australian National University Committee on Animal Experimentation, under Proposals R. BB. 28. 84, R. BB. 31. 85 and R. BB. 33. 85.

[Signature]
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Abstract

The present experiments were designed to investigate in the tammar wallaby (Macropus eugenii):

(1). The afferent and efferent connections to and from the visual cortex in the adult.

(2). The time course of the formation of these visual cortical connections during normal development. and,

(3). The distribution of thalamocortical axons and corticothalamic cells in the visual cortex during the ontogeny of the cortical lamination.

In either the adult and the pouch young, wheat germ agglutinin conjugated horseradish peroxidase was injected in the visual cortex, the dorsal lateral geniculate nucleus, the lateral posterior nucleus, and the superior colliculus, followed by histochemical processing to demonstrate the anterogradely labelled terminals and retrogradely labelled cells.

In the adult, the $\alpha$ segment of the lateral geniculate nucleus projects mainly to layers IV and VI of area 17 of visual cortex. The $\beta$ segment projects to area 18 and the ventral temporal cortex as well. In area 17 it projects principally to layers I, IIIb and upper V. In area 18 and ventral temporal cortex it projects moderately to layer IV. Cortical cells in layer VI project to both segments of the geniculate. The lateral posterior nucleus projects to all three areas, most densely to layers I and IV of area 18. Cells projecting back to the lateral posterior nucleus are mainly in layer V as are those projecting to the superior colliculus. Intracortical connections are found in all layers except I and II. The interhemispheric connections are restricted to the 17/18 border in layers III and V. This organization is similar to that in placental mammals.
During development thalamic projections reach the occipital cortex by 15 days after birth when both diencephalon and telencephalon are extremely immature. Descending cortical projections to the thalamus are formed from 48 days, those to the superior colliculus from 71 days while intra- and interhemispheric connections are last, beginning at 99 days.

The future visual cortex begins as a cortical plate which comprises a thin superficial cell compact zone and a lower, less packed zone of increasing depth. From 22 to 65 days, axons from the geniculate and lateral posterior are evenly and densely distributed in the less densely packed zone. By 82d, with the formation of the layers IV, V and VI, the thalamic axons become more concentrated into bands corresponding to the cytoarchitectonically identifiable cortical layers. By 99d, the geniculocortical axons become defined principally in layers IV and VI, their major destinations in the visual cortex in the adult. This laminar distribution of thalamocortical projections becomes adult-like by 118 days when the mature pattern of cortical cellular lamination is also recognizable. Cortical cells projecting to the thalamus are first seen at 45d in the cell compact zone. At subsequent times, these cells are disposed in two bands in the deeper part of the cortical plate, eventually becoming recognizable as layers V and VI.

These sequences resemble those described during development of the cortex in placental mammals with two main differences. The first afferent connections to the cortex form relatively earlier than in placentals. The cortical plate, except for a thin superficial cell compact zone, is evenly and densely innervated from the beginning, well prior to lamination of the cortex, unlike placentals where afferents do not invade the cortical plate until relatively late in development. The observed differences may be due to the technical advantages of the use of more sensitive, direct rather than transneuronal labelling of thalamocortical connections, greatly facilitated by pouch access to the marsupial young, or they may indicate that the marsupial uses a slightly different developmental timetable and plan to achieve the same end.
## List of Abbreviations

<table>
<thead>
<tr>
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<th>Definition</th>
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<tr>
<td>CP</td>
<td>cortical plate</td>
</tr>
<tr>
<td>d</td>
<td>days postnatal</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IMZ</td>
<td>intermediate zone</td>
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<tr>
<td>LGNd</td>
<td>dorsal lateral geniculate nucleus</td>
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<td>LGNv</td>
<td>ventral lateral geniculate nucleus</td>
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<tr>
<td>LP</td>
<td>lateral posterior nucleus</td>
</tr>
<tr>
<td>LPI</td>
<td>lateral division of lateral posterior nucleus</td>
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<tr>
<td>LPm</td>
<td>medial division of lateral posterior nucleus</td>
</tr>
<tr>
<td>MG</td>
<td>medial geniculate nucleus</td>
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<tr>
<td>MZ</td>
<td>marginal zone</td>
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<tr>
<td>OR</td>
<td>optic radiation</td>
</tr>
<tr>
<td>PY</td>
<td>pouch young animal</td>
</tr>
<tr>
<td>SC</td>
<td>superior colliculus</td>
</tr>
<tr>
<td>SGS</td>
<td>stratum griseum superficiale</td>
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<tr>
<td>SO</td>
<td>stratum opticum</td>
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<tr>
<td>SP</td>
<td>subplate</td>
</tr>
<tr>
<td>SVZ</td>
<td>subventricular zone</td>
</tr>
<tr>
<td>SZ</td>
<td>stratum zonale</td>
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<tr>
<td>TMB</td>
<td>tetramethylbenzidine</td>
</tr>
<tr>
<td>VC</td>
<td>visual cortex</td>
</tr>
<tr>
<td>V-TP</td>
<td>area ventral temporal</td>
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<tr>
<td>VZ</td>
<td>ventricular zone</td>
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<td>WGA-HRP</td>
<td>wheat germ agglutinin conjugated horseradish peroxidase</td>
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Chapter 1. Introduction

About Marsupials

Before entering the subject of this thesis, it is justifiable to have a brief description on marsupials and their unique mode of reproduction, from which the experimental advantages for developmental studies are utilized in the present study.

Emergence of marsupials.

It is believed that marsupials, metatherian mammals, originated as an early offshoot from the main mammalian line in the cretaceous period about 135 to 64 million years ago. They may have the same ancestor, the pantotherian mammals, with the placentals, eutherian mammals. It is still unclear at present why only marsupials existed in the continent of Australia before the first human inhabitants came about 40,000 years ago. There are currently two theories for this evolutionary miracle. The first speculates that the marsupials originated in North and South America. From there they moved to Antarctica and Australia, which were unseparated. Then came two continental drifts that caused the separation of South America from Africa and of Antarctica-Australia from Africa and, finally, of Australia from Antarctica (Hoffstetter, from Marshall, '80); The second hypothesis argues that the origin of the marsupials occurred in Australia, followed by the dispersion of marsupials to North and South America and other continents (Kirsch, '77). In either case, marsupials survived only in Australia and, less successfully, in America. The assumption that both Australian and American marsupials share a common origin is supported by several lines of evidence (see Mayner, '85 for a summary of this).

Marsupials are divided into two radiations, the polyprotodont and diprotodont. While in America only polyprotodonts exist, both are present in Australia.

The wallaby is a diprotodont species. As kangaroos, they belong to the subfamily Macropodidae included in the family Macropodoidea and are grazing animals. The tammar wallaby, Macropus eugenii, lives in abundance in Kangaroo
Island off the coast of South Australia. They are also distributed in smaller numbers in mainland South Australia and West Australia. They can be easily raised semidomestically under humane conditions.

**Reproduction in the tammar wallaby.**

The tammar wallaby is a seasonal breeding animal. The annual breeding begins during summer solstice (December 21-22) in Australia, probably triggered by the longest exposure to the daylight of the year (Tyndale-Biscoe, '73). The first birth normally occurs in late January to early February in the breeding colony in Canberra. After birth, the young grasps its way on the fur of the mother by well developed foreclaws to the pouch. In a matter of minutes it completes the journey and becomes attached with one of four teats. The young remains in the pouch until September.

About 8-16 hours after the production of a young, the female enters oestrus (McConnell, '84). If it is fertilized, the conception will result in the development of the embryo into a unilaminar blastocyst. However, the removal of the developing young from the pouch, before June, can reactivate the blastocyst and result in the birth of another young in 26-28 days (Renfree and Tyndale-Biscoe, '78), followed by another post-partum oestrus. Between June and August, the reactivation of the blastocyst can no longer be triggered by the removal of the pouch young, but the production of the new pouch young is facilitated pharmacologically by a single injection of bromocriptine (Sandoz, Australia) into the female at 5mg/kg body weight. After August, the blastocyst may also be reactivated by the alteration of the light/dark cycle, but with less reliability (Tyndale-Biscoe, personal communication).

There are two ways to estimate the age of the pouch young of male and female tammar wallaby. The first one is simply counting from the assumed date of birth, 26-28 days after the removal of the previous pouch young; The other is measuring the head length of the pouch young for the comparison with a table of measurements of the head length of animals of known age and sex (W. E. Poole, CSIRO).
Use of marsupials in developmental studies.

Two main experimental advantages for developmental studies on the visual pathways are emerging from the unique mode of the reproduction in tammar wallaby, and are further enhanced by the possibilities of manipulating the time of birth and determining the age of the pouch young. First, in tammar, unlike the most widely used laboratory animals such as monkey, cat and rat, the young is born at an extremely immature stage in that the retinal axons have not grown into the primary visual centres in the thalamus and midbrain at birth (Wye-Dvorak, '84). The pouch young are able to tolerate an artificial pouch environment, with the supply of the water, for a reasonably long period of time sufficient for most anatomical and physiological experiments. Great accessibility is thus offered by this model to carry out certain kinds of experimental operations concerning events occurring in early development, which may be not so feasible to do in the uterus of the placental animals. Second, the time span from the beginning of the gestation to the eye opening is very prolonged in the tammar more so than in most of the placental mammals. With an average of 162 days, this time span in tammar is nearly 40 days longer than that of macaque monkey, 100 days longer than that of cat, and 130 days longer than that of rat. This unique feature provides a better resolution in time to the continuous series of developmental changes in this period.

In the rest of this chapter, I intend to review what I consider to be so far the essential features of the anatomical description of the mature pattern of the main visual structures and pathways at different levels, from the retina to the striate cortex in the brain and their ontogeny during normal development. Unfortunately, although conceptually inseparable, a large body of the knowledge about the electrophysiological properties of the mammalian visual system in either mature or immature animals is not included within the scope of the following review, it being outside the nature of the present study.
Primary Visual Pathways in Mammals

The Retina

The Retinal Structure.

The basic plan of the construction of the retina is similar in all mammalian species, virtually in all vertebrates (Ramon y Cajal, '11). Thus the description of the retinal structure can be generalized. Transversely, the retina has three nuclear (cellular) layers, interspersed with two synaptic (plexiform) layers. Rays of light pass through the optics of the eye, which includes the cornea, aqueous humor, pupil, lens and vitreous body, and most of the retinal layers, focusing on photoreceptors, the outer most elements at the very back of the eye. Cones are mainly distributed in the center of the retina, whereas rods dominate the periphery. Rods and cones have complementary properties. Rods are highly sensitive to light with low intensity but insensitive to colour; cones handle sharp vision and colour vision in good lights, and can be only stimulated by light with relatively high intensity. Next is the inner nuclear layer containing three major classes of neuronal bodies: the horizontal, bipolar, and amacrine cells. Bipolar cells receive inputs from rods and cones by means of synaptic connections in the outer plexiform layer, then pass on to the ganglion cells by synaptic relationship with dendrites of ganglion cells in inner plexiform layer. The horizontal cell bodies are mainly distributed in the junction of outer plexiform layer and inner nuclear layer. The amacrine cells are found between inner nuclear layer and inner plexiform layer, but also with a distribution in the third nuclear layer, the ganglion cell layer, in which ganglion cells process the visual information primarily then pass it on into the brain via their axons traversing through optic disk, optic nerve and optic tract.

Ganglion Cells.

In Golgi stained preparations, ganglion cells can be subdivided on the basis of morphological features. In cat, at least three groups of ganglion cells with distinct morphological differences are described: In the α group, cells are characterized by
large somata (about 30µm in diameter) and large dendritic trees which increase in diameter from center (200µm) to periphery (1,000µm); In the \( \beta \) group, cells have medium-sized somata (20µm) and a small but bushy dendritic tree that is about 25µm across in the center and about 300µm in the periphery; In the gamma group, cells are smallest in somatic size (16µm) but with large, sparsely branched dendritic trees (300-800µm from centre to periphery). A subgroup, called delta cells, is further divided from gamma group by their more prolifically branched dendritic trees (Boycott and Wassle, '74).

Histologically it is also evident that the ganglion cells have an uneven distribution across the retina in that the density of ganglion cells is much higher in the central retina than in the peripheral retina, with the peak density found in area centralis (Hughes, '75a, '81; Stone, '65, '78). The distribution of each particular class of ganglion cells is approximately similar in most parts of the retina and is consistent with the distribution pattern of the entire ganglion cell population across the retina. There is some correlation between morphological classification and functional classification. For example, \( \alpha \) cells have been identified as the morphological correlates of the physiological Y cells (nonlinear, brisk-transient) (Cleland et al., '75; Peichl and Wassle, '81), \( \beta \) cells comprise the physiological X cells and gamma the W cells (Fukuda and Stone, '75).

In the tammar, ganglion cells with various somatic sizes are recognized, though not classified into specific group types due to the lack of information of their dendritic patterns and the lack of evidence of the existence of clearcut somatic size groups as seen in the cat (Wong et al., '86). In other marsupial species, various degrees of the central-to-peripheral gradient of ganglion cell density similar to that reported in placental mammals is also observed (Hughes, '75b; Freeman and Tancred, '78; Beazley and Dunlop, '83). It is argued that the habitat requirements may to a certain degree determine or regulate the ganglion cell isodensity contour map such that in mammals adaptational to an extended terrain in animal's visual habitat, the ganglion cells tend to accumulate with great density in a horizontal zone passing the area
crossed and uncrossed pathway.

In mammals, the optic nerves exchange a portion of their axons in the optic chiasm before continuing into the brain as the optic tract. This enables each hemisphere to survey the opposite side of the visual field and integrate visual information received by both eyes.

The degree of cross-over of the retinal axons is largely related to the laterofrontal positions of the eyes, and related to the determination of the extent of the visual field (VF) presented to opposite brain. In more laterally eyed animals such as some rodents, more than 95% of the retinal axons cross over in the chiasm and project into the contralateral brain; only a small proportion of axons originating from ganglion cells situated at the far end of the temporal retina project ipsilaterally, forming a small binocular representation in the visual structures in the brain (Takahashi et al., '77; Hsiao, '84). On the other hand, in animals with more frontally placed eyes such as cat and monkey, great numbers of the retinal axons are uncrossed, forming a large binocular segment in the visual brain structures.

The ganglion cells giving rise to the crossed and uncrossed axons originate from particular loci in the retina. It is shown in both cat (Stone, '66) and monkey (Stone et al., '73; Bunt et al., '75) that uncrossed axons are come from ganglion cells in the temporal half of the retina, where the VF of the other side is perceived, and that the crossed axons are originated from ganglion cells of the nasal half of the retina mainly receiving stimuli in the ipsilateral VF. However, the sharpness of the separation of the crossed and uncrossed cells in the ganglion cell layer is different in cat and monkey. In cat, there are bands along the vertical middle line of retina, in which the crossed and uncrossed cells intermingle, whereas in monkey such bands are very much reduced in width. As a result, there are still 25% of the cells in temporal retina remain crossed in cat, while in monkey all ganglion cells in temporal retina project ipsilaterally (Stone et al., '73; Bunt et al., '75). In rat, the uncrossed cells are distributed in a intermixed
fashion with crossed cells in approximately one quarter of the inferior temporal retina (Cowey and Frnzini, '79; Cowey and Perry, '79; Lund et al., '79).

Retinal Projections to the Dorsal Lateral Geniculate Nucleus

Morphology of the LGNd.

The most noticeable morphological feature of the mammalian LGNd is, perhaps, the laminar organization in which cells of particular cytological characteristics are organized into cytoarchitecturally distinct layers. However, the visibility of such laminar organization in Nissl-stained preparation varies from species to species. In rodents, the LGNd is composed of a fairly homogenous cell population and lamination is hardly recognizable (Jones, '85), while in carnivores and high primates, the highly laminated LGNd can be clearly appreciated. In cat, three overt laminae separated by interlaminar fibre plexuses are recognized in parallel to the optic tract. The innermost two are designated as laminae A and A1 which occupy roughly two thirds of the nucleus. The third lamina consists of sublaminae C, C1, C2, and C3 (Guillery, '66).

In monkey, the LGNd is more overtly laminated and was first described by Clark ('32) as consisting of 6 cellular layers separated by cell sparse interlaminar zones. He numbered these layers from the superficial. Layers 1 and 2 are composed of large cells and are called magnocellular layers, while layers 3 to 6 are composed of small cells and referred to as the parvocellular layers. This scheme was later adopted by Kaas et al. ('78) not only on the basis of the position of the layers and retinal inputs, but also on the basis of homologies of the cells described in a number of other primate species. According to this system, the basic pattern of primate LGNd can be described as containing two parvocellular layers (PE and PI: external and internal), two magnocellular layers ME and MI), and two poorly developed superficial layers (SM and SI) which were not considered in Clark’s nomenclature system ('32). In rhesus monkey, the two parvocellular layers are further divided into four leaflets, corresponding to Clark’s layers 3, 4, 5, and 6.
In marsupials, various cellular arrangements in the LGNd have been reported. While in some species the laminar organization is poorly distinguished (Royce et al., '76; Sanderson., '79), other species have a high degree of laminated cellular organization visible in Nissl-stained preparations (Goldby, '41; Hayhow, '67; Pearson et al., '76; Royce et al., '76; Sanderson and Pearson, '77, '81; Haight and Nelson, '78). The basic plan for the construction of the laminated LGNd in various marsupial species seems common in that the LGNd can be divided into two segments on the basis of cytoarchitectonics: the lateral $\alpha$ segment with closely packed and deeply stained cells organized in distinct cellular layers, and the medial $\beta$ segment which contains scattered cells in an unlaminated fashion with poor staining quality. However, the complexity of such lamination varies a great deal from species to species according to the further subdivision of the $\alpha$ segment. In the tammar, up to six cellular laminae are identified in the LGNd with the $\alpha$ segment further divided into five sublaminae (Sanderson et al., '84).

**Retinal Input to the LGNd.**

The LGNd is the major recipient of the retinal input in the thalamus. The retinal input in the LGNd is characterized by the high degree of the laminar specificity according to the eye of origin and the high degree of topographic specificity in a given lamina.

In laminar specificity, the retinal axons from different eyes are exclusively or largely separated by terminating into different laminae of the LGNd. In monkey, it has been shown that each of the geniculate layers receives retinal input from one eye only: layers 1, 4, and 6 (ME, PE) receive retinal axons from the contralateral eye, and layers 2, 3, and 5 (MI, PI) from the ipsilateral eye. Of the two superficial layers, the SI receives retinal inputs from the contralateral eye and the SE from the ipsilateral eye (Kaas et al., '72, '78).

In cat, Hickey and Guillery ('74) showed that layers A, C, and C2 receive exclusively the retinal input from the contralateral eye, whereas layers A1 and C1 from the ipsilateral eye.
In rat, the cytoarchitectonically unidentified laminar arrangement is revealed by the selective terminal label of retinal axons. It is shown that there are basically three layers of retinal terminations in the rodent LGNd, an outer and an inner layer containing terminals of axons from the contralateral eye, and a much shorter central layer containing axons from the ipsilateral eye, forming a small binocular segment in the dorsal part of the nucleus (Lund et al., '74; Wise and Lund, '76). A certain degree of intermixture of axons from both eyes in the LGNd is noticed in rat (Hickey and Spear, '76), and some other rodents (Takahashi et al., '77; Collewijn and Holstege, '84).

In marsupials, the complexity of the pattern of retinal input distribution in the LGNd varies greatly among different species. For example, in polyprotodont marsupials, the number of eye specific layers of retinal input is relatively less and the considerable amount of overlap of ipsilateral and contralateral retinal input is reported (Lent et al., '76; Royce et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79). In diprotodont marsupials, on the other hand, more eye-specific terminal bands are found and the retinal inputs from different eyes are more sharply segregated in the LGNd (Hayhow, '67; Pearson et al., '76; Sanderson et al., '78; Sanderson and Pearson, '81; Sanderson et al., '84). As many as ten separate layers of retinal input, the most ever reported in mammals, is observed in gray kangaroo (Sanderson et al., '84). In tammar, nine bands of eye specific layers of retinal termination are described. Of these, four are from the contralateral eye, and five from the ipsilateral eye. The interleaving ipsilateral and contralateral terminal bands are in the dorsal and caudal part of the LGNd, forming the binocular segment of the nucleus (Sanderson et al., '84).
Retinal Projections to the Lateral Posterior(LP)-pulvinar Complex

Morphology of the LP-pulvinar Complex.

The dorsal part of the medial-caudal thalamus is generally referred as the lateral posterior in rodents and marsupials, the LP-pulvinar complex in carnivores and primates.

In monkey, the LP-pulvinar complex consists of a single lateral posterior nucleus and at least four pulvinar nuclei (Jones, '85). The LP is relatively small and is constituted by a homogeneous population of moderately packed, palely stained, and medium-sized cells, whereas the pulvinar is composed of four distinct nuclei called medial, lateral, inferior, and anterior pulvinar nucleus (Plm, Pll, Pli, and Pla). In the lower primates, the pulvinar is fairly homogeneous and the further division of the nucleus solely on the basis of cytoarchitectonics is not possible. Thus, connectivity is used as the major category to subdivide this nucleus into a dorsal or superior pulvinar nucleus which connects with the superior colliculus, and a ventral or inferior pulvinar nucleus which connects with the cerebral cortex (Glendenning et al., '75; Symonds and Kaas, '78; Carey et al., '79; Raczkowski and Diamond, '81).

In contrast to the situation in monkey, the LP-pulvinar complex is dominated by the lateral posterior nucleus in cat. Three constituent nuclei are recognized in the LP cytoarchitectonically: the lateral LP (LPl), the medial LP (LPm) and the intermediate LP (LPi). The pulvinar nucleus, on the other hand, is very much reduced to a single homogeneous structure (Jones, '85).

In most rodents, the lateral posterior nucleus is virtually a single homogeneously celled structure. The pulvinar nucleus can not be recognized on the basis of cytoarchitectonics (Jones, '85).

In marsupials, according to the histological description, the lateral posterior nucleus consists of two subdivisions: the medial and lateral divisions (Goldby, '41; Haight and Neylon, '78). In tammar, Mayner ('85) recently argued that the pulvinar
exists in this species and is situated medially to the lateral posterior, and in some other marsupials was treated as the medial division of the LP.

**Retinal Input to the LP-pulvinar Complex**

The retinal projection only terminates in the contralateral LP-pulvinar complex and its distribution in the nucleus is rather restricted. The information about the topographical order of the retinal axons in the structure is limited.

In monkey, the direct retinopulvinar projection is only evident in the most lateral part of the inferior pulvinar nucleus (Nakagawa and Tanaka, '84).

In cat, the direct retinal input to the LP-pulvinar complex is similarly restricted to the most lateral part of the pulvinar nucleus (Berson and Graybiel, '78; Graybiel and Berson, '80).

In rodents, retinal axons from the contralateral eye are also limited to the lateral most part of the LP (Montero and Guillery, '68; Hickey and Spear, '76).

In marsupials, the retinal projection in the contralateral LP is not significant in amount and is largely limited to the most dorso-lateral edge of the nucleus, although a restricted but concentrated retinal input is observed at the medial margin of the nucleus (Pearson et al., '76; Royce et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79, '84). In the tammar, retinal projection is also confined to the dorsolateral margin of the nucleus (Wye-Dvorak, '84).

**Retinal Projections to the Superior Colliculus**

**Morphology of the SC.**

The mammalian superior colliculus (SC) lies on the dorsal surface of the midbrain. The different mammalian species share a similar fundamental scheme for the anatomical organization of the SC (Stein, '81). In Nissl-staining preparation, the whole thickness of the SC can be divided into seven distinct laminae with an alternation between cell-rich and fiber-rich. From the topmost the lamina I is fiber-rich and is called stratum zonale (SZ); lamina II is called stratum griseum superficiale
(SGS); and Lamina III, stratum opticum (SO); These three superficial laminae form the upper division of the SC, which is directly involved in the visual function.

**Retinal Input to the SC.**

The organization of the retinal input in the SC is characterized by its laminar distribution and its eye-specific segregation. In monkey, the retinal axons to both contra and ipsilateral SC terminate most heavily in the upper margin of the stratum griseum superficiale (SGS), and substantially, but less pronouncedly in the stratum opticum (SO). In the stratum zonale (SZ) only scattered retinal terminals are found. In SGS, the retinal termination tends to segregate into clumps in an eye-specific fashion (Hubel et al., '75). In cat, the situation regarding the laminar distribution of the retinal axons and the pattern of their segregation into the eye-specific patches is similar to that found in monkey. Besides, it is observed that, while the projection from the contralateral retina is distributed in the entire extent of the SC, the projection from the ipsilateral retina is excluded from the rostral and caudal poles of the SC (Graybiel, '75; Harting and Guillery, '76).

In rat, the whole extent of the contralateral SC receives intensive retinal input, which terminates heavily in the SGS and SO, and to a much less degree in the SZ. The retinal inputs to the ipsilateral SC seems only to be present in the rostral pole of the SC and, in laminar distribution, is faintly concentrated in the SGS and SO, but largely absent from the SZ (Wise and Lund, '76; Land and Lund, '79).

In marsupials, the retinal projection terminates intensively and extensively in the SGS and SO in the contralateral SC, with the highest aggregation found in the SGS. In ipsilateral SC, the retinal projection is largely reduced in intensity and is only present in rostral parts of the SC, in the form of series of patches located in lower part of the SGS (Pearson et al., '76; Royce et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79; Wye-Dvorak, '84). However, there is also faint termination of retinal input in the SZ of ipsilateral SC in American opossum (Royce et al., '76).
Retinal Projections to Other Structures

Apart from the above subcortical structures, the retinal projection also terminates in the suprachiasmatic nucleus (see Moore, '82 for review), the ventral lateral geniculate nucleus (LGNv) (Collewijn and Holstege, '84), the pretectal complex (Royce et al., '76; Berman, '77; Benevento and Standage, '83; Collewijn and Holstege, '84), the nucleus of the optic tract (Royce et al., '76; Berman, '77; Collewijn and Holstege, '84), and the accessory optic system (Erickson and Cotter, '83; Collewijn and Holstege, '84; Simpson, '84). These minor projections although functionally important are not considered further.

Cortical Visual Pathways in Mammals

Cortical Visual Areas

On the basis of cytoarchitectonics, anatomical connectivity, and the retinotopic representation, the mammalian visual cortex is described as consisting of multiple areas in which the visual field (VF) is separately represented, either completely, or partly.

In primates, as many as 20 cortical areas out of a total of 36 are reported as to be the candidates for being exclusively or largely visual in function; 10 of them are electrophysiologically demonstrable as such (Van Essen, '85). The most prominent one is the highly developed striate cortex, called V1 retinotopically and area 17 cytoarchitecturally. The adjective striate refers to the striae of Gennari, a band of fibres overlying the granule cell zone of layer IV composed of thalamocortical axons. These are visible to the naked eye in slices of unfixed primate brain. Striate cortex is commonly used synonymously with area 17 and V1 in other mammals. The striate cortex occupies about 15-17% of the total neocortical surface, covering virtually the
whole occipital lobe and extending rostrally to the lunate sulcus and laterally beyond the calcarine sulcus. There is an orderly and complete representation of the contralateral visual hemifield with the more central parts of the visual field (VF) being represented on the smooth outer surface (operculum) (Daniel and Whitteridge, '61). Bordering this and extending rostrally on the cortex are some extrastriate areas.

Cytoarchitectonic area 18 contains parts of at least 5 topographically organized areas and is mainly occupied by V2 (Van Essen, '85). It has a long common border with V1 and a visual representation in mirror image to that in V1 (Zeki, '69; Gattass et al., '81).

Further rostrally there are many visual areas containing visual representations of all or part of the visual fields formed by neurones with various receptive field properties (Van Essen, '85).

In cat, the visual cortex is reported to contain more than a dozen of separate visual areas (Rosenquist, '85). Among these, the most prominent, area 17, lies on the crown of the lateral posterolateral gyri and along the medial bank of the lateral gyrus down to the superior bank of the splenial sulcus. It extends posteriorly the tentorial surface of the postlateral gyrus down to the splenial sulcus. The entire contralateral visual hemifield is represented in area 17 such that the upper VF is represented in the caudal part of area 17, the lower VF is represented in the rostral part of area 17, the contralateral temporal VF is represented in the ventral bank of the cingulate gyrus and the contralateral nasal VF represented in the dorsal bank of the cingulate gyrus (Tusa et al., '78).

Both electrophysiological and cytoarchitectonic studies identified two regions (areas 18 and 19) with roughly mirror symmetrical representations of a proportion of the VF. Area 18 lies lateral to area 17 on the crown and medial bank of the lateral sulcus and on the posterior bank of the postlateral sulcus; area 19 lies lateral to area 18 on the medial bank of the lateral sulcus and on the anterior bank of the postlateral sulcus (Tusa et al., '79). More visual areas are described in postlateral sulcus,
posterior suprasylvian gyrus, and lateral suprasylvian region (Rosequist, '85). They are not considered separately here.

Rat visual cortex contains 7 areas revealed by microelectrode mapping technique (Espinoza and Thomas, '83). The main representation (V1) is located in cytoarchitectural area 17 with upper visual field (VF) projecting to caudal aspect and lower VF projecting to rostral aspect of area 17. Medial to this in area 18 there are two additional visual areas and laterally in area 18a there are 4 more.

In marsupials, the lack of a retinotopic mapping study makes the discovery of cortical areas incomplete. Nevertheless, the knowledge of cytoarchitecture and connections of visual cortex with other visual structures has been used to distinguish three different areas of visual cortex in the possum: the striate cortex the peristriate band and the posterior parietal area (Benevento and Ebner, '77a; Heath and Jones, '71; Haight et al., '80). In wallaby, Mayner ('85) identified in the occipital cortex the cortical areas 17 and 18 on the basis of cytoarchitecture and argued the similarities of the visual cortical organization in tammar to that of grey squirrel (Kaas et al., '72b).

Cellular Organization of the Visual Cortex

There is a common basic plan for the anatomical organization of the primary visual cortex of all mammalian species in which cortical cells are organized into a six-layered laminar structure according to cytoarchitecture and cytology.

In monkey, according to the lamination scheme of Lund ('73), layer I of the visual cortex is relatively narrow, consisting mainly of fibre terminals and terminal branches with few scattered stellate cells; layer II and III merge gradually into one another, containing both pyramidal and spined stellate cells; layer IV consists of 4 sublaminae: IVa, IVb, IVc,α and IVc,β. Both layer IVa and IVb contains stellate cells, as well as pyramidal cells. Being the most prominent cell condensation in area 17, layer IVc contains stellate cells only and can be further divided into sublaminae IVc,α and IVc,β on the basis of the different axonal distribution: the axons from Y-
type cells in LGNd terminate in sublayer IVc,α while those from X-type cells in LGNd terminate in sublayer IVc,β. There are two sublayers in layer V: Va and Vb, both containing pyramidal neurons only, but those in sublayer Vb are relatively larger and more sparsely located; layer VI contains both stellate and pyramidal cells (Lund et al., '75, '79).

In cat visual cortex, immediately below the pia lies layer I, a zone almost free of cell bodies. Layer II and III, both composing mainly of small pyramidal cells, are commonly considered together because of the difficulty in separating them. Layer IV has two sublayers: IVa and IVb, with IVb having smaller, most closely packed neurons within visual cortex. Layer V is also further divided two sublayers: Va and Vb, the cell bodies of the largest pyramidal neurons in the cat visual cortex lie at the boundary of sublayer Va and Vb. Layer VI contains small and medium pyramidal cells. (Lund et al., '79).

In Nissl-stained sections, the six layered organization of area 17 of rat cortex also can be visualized. Layer I is thin and mainly contains fibres, except a sparse distribution of small non-pyramidal cells. It is believed that layer I receives a direct input from the LGNd. Layer II and III can not be delimited exactly in either Golgi or Nissl preparations. Both small pyramidal and non-pyramidal cells are present in either layers, while the intermediate and moderately large pyramidal cells are found in layer III only. Layer IV contains both pyramidal and non-pyramidal cells. The abundance of the cell bodies of the small pyramidal and non-pyramidal neurons make this layer granular in appearance. Layer V is characterized by the large pyramidal cells which usually occur in the lower half of the layer. Layer VI is predominated by small and medium sized pyramidal cells, as well as pyramidal-like cells (Ribak and Peters, '75; Feldman and Peters, '78).

In marsupials, the anatomy of the primary visual cortex is similarly characterized by a six layered laminar organization (Heath and Jones, '71; Haight et al., '80; Mayner, '85). In both possum and wallaby, the layer II is particularly
prominent as a rather narrow but closely-packed and deeply-stained collection of medium-sized pyramidal or round cells (Haight et al., '80; Mayner, '89a).

Cortical Connections With The LGNd

Recent horseradish peroxidase studies have revealed that in mammals, the visual cortex has connections with more brain structures than previously thought (Tigges et al., '82, '83; Tigges and Tigges, '85). However, only those connections with the LGNd, the pulvinar-LP complex, the SC, and the opposite visual cortex, will be reviewed in following sections, since they are generally thought to be the major sites in visual processing and are relevant to the topic of this thesis.

In mammals, the pathway through the LGNd is the major system transmitting visual information from the retinal to the neocortex. The massive reciprocal connections between the LGNd and the visual cortex, in particular the specificities of such connections at regional, laminar, and cellular levels, have been studied intensively in the past few years.

Areal And Laminar Distribution Of The Geniculocortical Axons In The Visual Cortex.

In primates, it is generally agreed that in both Old World and New World primates the LGNd projects exclusively to the striate cortex (Wiesel et al., '74; Kaas et al., '76; Hubel et al., '77; Henderickson et al., '78). The projections from S lamina of the geniculate also terminate in the striate cortex (Wong-Riley, '74; Ogren and Hendrickson, '76). However, there are conflicting reports claiming the existence of a geniculate projection to the extrastriate cortex, although the amount of this projection is insignificant (Wong-Riley, '76; Benevento and Yoshida, '81; Yukie and Iwai, '81). In laminar distribution, Hubel and Wiesel ('72) showed that axons from the parvocellular layers of the LGNd terminated in a thick band in sublayer IVc,β, and in layer I and sublayer IVa as well, while those from the magnocellular layers terminated in sublayer IVc,α. These results were confirmed by later studies. In addition, a small
band of termination probably arising from both magno- and parvocellular layers of the geniculate was found at the junction of layers IV and V of the cortex (Wiesel et al., '74; Glendenning et al., '76; Kaas et al., '76; Hubel et al., '77; Hendrickson et al., '78). The axons arising from S laminae are found terminating in layer III or more superficial layers (Fitzpatrick et al., '83; Weber et al., '83).

In cat, it is now clear that the LGNd projects to all of areas 17, 18 and 19, with axons from laminae A and A1 terminating in areas 17 and 18, and axons from lamina C (including C1, C2) terminating in areas 17, 18 and 19. Those from MIN terminate in area 18 only (Gilbert and Kelly, '75; Maciewicz, '75; LeVay and Gilbert, '76; Hollander and Vanegas, '77; Updyke, '77). It is also established that axons from the A laminae terminate in the low part of the layer III, layer IVa and IVb and layer VI, whereas those from lamina C terminate in layer I, and two bands above and below the layer IV granule cell zone including the lower part of layer III and the upper part of the layer V. (LeVay and Gilbert, '76; Raczkowski and Rosenquist, '80; Conley, '88).

In rat, the geniculocortical projection is distributed heavily in area 17, and less substantially in areas 18 and 18a, with the principle projections terminating in layers IV and the deeper parts of layer III, and less significantly in layer I and VI. (Ribak and Peters, '75; Hughes, '77; Coleman and Clerici, '80; Perry, '80).

In marsupials, the LGNd projects to all three areas cytoarchitectonically identified as striate, peristriate and posterior parietal areas. (Benevento and Ebner, '71b; Haight et al., '80; Coleman and Clerici, '81). In possum, after cortical injection of horseradish peroxidase, Haight et al. ('80) demonstrated that the α segment has concentrated projections to striate and peristriate cortex, while β segment has a less pronounced projections to striate, peristriate and posterior parietal areas. In opossum, an LGNd lesion resulted in terminal degeneration in layer IV, with some expansion into adjacent portions of layer III and layer VI. A few degenerated fibres are also present in layer I. However in the opossum there seems no LGNd projection in
extrastriate cortex (Benevento and Ebner, '71b), as further confirmed by later study using cortical HRP injections in both striate or extrastriate cortex (Coleman et al., '77).

**Areal And Laminar Distribution Of The Corticogeniculate Projection.**

Fibres from the visual cortex project to the LGNd to make the geniculocortical connection reciprocal.

In monkey, area 17 is the major source of the corticogeniculate projection, which terminates in all layers of the entire LGNd (Lund et al., '75; Ogren and Hendrickson, '76; Lin and Kaas, '77; Graham, '82). Axons to the magnocellular layers originate from the deepest part of layer VI, while those to the parvocellular layers originate from more superficial parts of layer VI (Lund et al., '75). Sparse projections from area 18 to the magnocellular and S layers were reported, which also originate from layer VI (Wong-Riley, '76; Lin and Kaas, '77).

In cat, Updyke ('75, '77, '83) established that area 17 projects to all laminae of the LGNd and MIN intensively. Area 18 projects to all laminae as well, but more substantially in C laminae than in A laminae; area 19 mainly projects to laminae C1-C3. The corticofugal fibres to the LGNd originate from pyramidal cells in layer VI (Gilbert and Kelly, '75; Updyke, '75).

In rats, cortical projections to the LGNd arise from large pyramidal cells in layer VI of areas 17, 18 and 18a (Sefton et al, '81).

In possum, injections of tritiated amino acid in each of three cortical areas produced label in the LGNd (Haught et al., '80). It is, however, not established what cortical cells belonging to which layer of which area give rise to the corticogeniculate projection.

**Cortical Connections with LP-pulvinar Complex**

As the major thalamic structure in the extrageniculate pathway, the LP-pulvinar complex has extensive afferent and efferent connections, including reciprocal connections with the visual cortex.
Areal and Laminar Specificity of the LP-pulvinar Projections to the Visual Cortex

In monkey, numerous studies have shown that the lateral and inferior pulvinar nuclei project to the striate cortex and most of the visuotopically mapped extrastriate cortical areas (Benevento and Rezak, '75, '76; Ogren and Hendrickson, '76, '77; Trojanowski and Jacobson, '76, '77; Wong-Riley, '77; Curcio and Harting, '78; Rezak and Benevento, '79; Benevento and Standage, '83). In striate cortex, the axons from PLl and PLi terminate principally in the cortical layer I, and less significantly in layer II and III. In extrastriate cortex, they terminate mainly in layer IV and adjacent part of layer III (Benevento and Rezak, '75; Ogren and Hendrickson, '76, '77). In lower primates, both ventral and dorsal division of the pulvinar project to cortical layer I of the striate cortex and the layer IV of several extrastriate cortical areas (Glendenning et al., '75; Carey et al., '79).

In cat, the much less prominent pulvinar only projects to area 19, and six other extrastriate visual areas, plus a nonvisual area in the anterior part of the cingulate gyrus (Marcotte and Updyke, '81; Symonds et al., '81; Raczkowski and Rosenquist, '83; Updyke, '83). In LP, each division projects to a number of cortical visual areas, but only the lateral division of the LP (LPl) projects to areas 17 and 18, with its termination predominantly in the layer I, and in the lower part of layer III and upper part of layer V as well. LPl also projects to area 19 and several other extrastriate areas and terminates heavily in the cortical layer I, except in area 19 where the heaviest termination of the LPl axons is found in the layer IV (see Rosenquist, '85 for review). The interior division of the LP (LPi) projects to ALLS, PLLS, DLS, PS, 20b, 21b, and medial division (PLm) projects to EVA, area 7 and 5b.

Cytoarchitectonically, the LP in the rat is a homogeneous entity. However, on the basis of anatomical connections, it can be divided into a caudal tectorecipient region and a rostral corticorecipient region (Perry, '80; Mason and Groos, '81). The rostral LP projects to both striate (area 17) and surrounding extrastriate cortex (areas 18 and 18a). The heaviest LP axons termination is in the cortical layer IV of area 18a.
In area 17 LP axons terminate in layer I and in area 18 in layer IV (Hughes, ’77; Herkenham, ’80; Mason and Groos, ’81).

In marsupials, Haight et al. (’80) showed that in possum, both lateral and medial divisions of the LP project to each of the striate, peristriate and posterior parietal areas. However, the projection from LPl is more pronounced in the striate cortex, while those from LPm mainly terminate in the peristriate cortex. Data concerning the laminar distribution of the LP axons in the cortical visual areas are currently not established. In opossum, study by cortical HRP injection shows that the LP projects to both striate and peristriate areas, with a much heavier termination in the peristriate area (Coleman et al., ’77).

**Cortical Projections to the LP-pulvinar Complex**

In monkey, both PLi and PLl receive projections from the striate area and a few extrastriate areas which receive pulvino-cortical projections originating from PLi and PLl (Benevento and Davis, ’77; Graham et al., ’79; Lin and Kaas, ’79; Graham, ’82). The cortical projections to the LP originate from layer V in the striate cortex and from layer VI in the extrastriate cortex (Lund et al., ’75; Ogren and Hendrickson, ’77; Trojanowski and Jacobson, ’77).

In cat, virtually all of the cortical areas receiving projections from LP project back to the LP. Cortical projection to LPl is from cortical layer V in areas 17 and 18, and in layer VI in area 19 (Kawamura et al., ’74; Updyke, ’81).

In rat, by retrograde labelling with fluorescent dyes and HRP, Mason and Groos (’81) demonstrated that it is the layer V pyramidal cells in areas 17 and 18a that project to the rostral part of the LP.

In possum, cortical axons coming to the LPm are from the peristriate cortex, while those to the LPl are from the rest of the visual cortex (Haight et al., ’80).
Cortical Connections with the SC

The superficial layers of the SC receive substantial afferent input from the visual cortex, in topographic alignment with the retinal inputs in the SC.

In rhesus monkey, the corticocollicular projection arises from areas 17, 18, and 19, plus some extrastriate areas. In area 17, the corticocollicular cells are situated in layers V and VI, while in areas 18 and 19 and other extrastriate areas, they are only found in layer V. In SC the corticocollicular axons mainly terminate in the superficial layers including SZ, SGS, and less substantially in SO (Graham, '82; Fries and Distel, '83; Fries, '84).

In cat, the corticocollicular axons originate from the layer V cells of areas 17, 18 and 19, plus other visual areas (Hollander, '74; Updyke, '77; Segal et al., '83; Segal and Beckstead, '84). Their terminals are principally distributed in SZ and SGS of the SC (Kunzie et al., '76; Kunzie and Akert, '77; Segal and Beckstead, '84).

In rat, it has been demonstrated by collicular injection of HRP that all cortical areas 17, 18 and 18a project to the SC (Sefton et al., '81). A combined study of collicular HRP injection with cortical tritiated proline injection suggests that each of 7 physiologically identified cortical visual areas project separately to the SC (Olavarria and Van Sluyters, '82). The corticocollicular fibres arise from pyramidal cells of the cortical layer V and terminate substantially in SZ, SGS, and faintly in SO.

By cortical tritiated proline and leucine injection, it is shown in possum that both striate and peristriate areas send axons to SZ, SGS and, with smaller concentration, SO of the SC. By collicular HRP injection, it has been shown that the cells in layer V are the origin of the corticocollicular projection (Haight et al., '80).
Intrahemispheric Connections

The intrahemispheric connections between area 17 and other cortical areas have been the subjects in a number of studies in both placental and marsupial mammals.

In monkey, area 17 has reciprocal connections with at least four associated areas including the V2, V3, MT, and V4 (see summarized version by Van Essen, '85). While projections from area 17 mainly terminate in IV and lower III, cells projecting to area 17 are localized in layers II/III and V/VI (Rockland and Pandya, '79; Tigges et al., '81; Lin et al., '82).

In tree shrew, it was shown by a WGA-HRP study that area 17 is reciprocally connected with area 18, the temporal dorsal (TD) and the temporal posterior (TP) areas, and the posterior limbic cortex areas. Projections from area 17 terminate in layers II-V in area 18, with the heaviest in layer IV, and in layers II-IV in TD. Cortical cells projecting to area 17 are mostly situated in layer III in all cortical areas, but are also seen in layer V and, to a lesser extent, in layer VI (Sesma et al., '84).

In cat, area 17 has extensive connections with other visual areas including areas 18, 19, 20a, 21a, 21b, and the posterior lateral lateral syrapsylvian (PLLS), the posterior medial lateral syrapsylvian (PMLS), and the anterior medial lateral syrapsylvian (AMLS) (Symonds and Rosenquist, '84a,b). In areas 18, 19, 21b and PLLS, cells projecting to area 17 are situated in either layers II/III and, in less numbers, in V/VI, except in area 19, a small proportion of intrahemispheric cells projecting to area 17 are from layer IV. In areas 21a and PMLS, cells projecting to area 17 are mostly from layers V and VI, with some also from layers II and III; In areas 20a and AMLS, the cells projecting to area 17 are situated in layers V and VI only (Symonds and Rosenquist, '84a,b).

In rat, area 17 is reciprocally connected with the areas 18 and 18a (Montero et al., '73; Olavarria and Montero, '81), and certain areas in parietal and temporal cortex as well (Miller and Vogt, '84). It is known that axons from area 17 largely terminate
in layers II and III in areas 18 and 18a, and cells in the same layers of areas 18 and 18a project to area 17 (Olavarria and Montero, '81).

In opossum, it was reported that the striate cortex (area 17) sends projections to layers I-IV of some extrastriate visual areas in temporal cortex (Benevento and Ebner, '71a).

In possum, the striate cortex was found reciprocally connected with some cortical areas in the posterior parietal, the medial temporal, and the lateral temporal cortex (Crewther et al., '84). In addition, there was a suggestion that the striate and peristriate cortex were also reciprocally connected although this was obscured by the spread of label at the injection site (Crewther et al., '84). It appears that the axons from the striate cortex largely terminate in layers III and IV in extrastriate areas, and that the cells projecting back to the striate cortex are situated mostly in layers II and III, but also in layers V and VI (Crewther et al., '84).

Interhemispheric Connections

The major interhemispheric connections in placental mammals occur via the corpus callosum, an extensive and massive bridge of fibres between the two cortices. Through the corpus callosum, axons transfer information between two cortical hemispheres and, based upon such transformation of sensory information, conduct the processes of learning from one cortex to the other (Gazzaniga, '67; Sperry, '70).

In visual processing, it is known from the pioneering work of Myers and Sperry ('53) that the corpus callosum is capable of conducting interocular transfer, through which the visually uninformed hemisphere can acquire the information obtained previously by the other hemisphere.

The general rules in the organization of the interhemispheric connections seem to be that, first, the distribution of the callosal cells and axons in main visual cortical areas such as areas 17 and 18 is largely limited to a restricted region (Benevento and Ebner, '71a; Heath and Jones, '71; Wong-Riley, '74; Innocenti, '80; Cusick and Lund,
and second, the intercortical connections are always organized in a point-to-point topographic order such that a defined cortical locus is precisely connected reciprocally with the homotopic locus in the contralateral cortical hemisphere.

In monkey, the interhemispheric connection via the corpus callosum is largely concentrated into a restricted region across the areas 17/18 border (Wong-Riley, '74; Kaas and Lin, '77; Weller and Kaas, '81), which retinotopically represents the vertical meridian of the VF (Van Essen and Zeki, '78). Callosal axons originate from the large pyramidal cells in the deeper part of layer III, and terminate mainly in layers II and III (Wong-Riley, '74; Winfield et al., '75; Kaas and Lin, '77; Weller and Kaas, '81).

In cat, each of twelve visual cortical areas are involved in the callosal connections (Segraves and Rosenquist, '82a,b). In area 17, 18 and 19, the callosal neurons are located along the areas 17/18 border and the lateral border of area 19. Retinotopically, these two bands of callosally connected regions represent the vertical meridian (Hubel and Wiesel, '65). The callosal cells in the 17/18 border region are mainly within the deeper part of the layer III, and less significantly in upper layer VI. In other visual cortical areas, the distribution of the callosal cells is more pronounced in layer V and VI. The majority of the callosal axons are concentrated in layers II, III, and V (Shatz, '77; Innocenti, '80; Segraves and Rosenquist, '82a).

In rat, the interhemispheric connections are confined to a circumferential band around the edges of area 17, and in the conjunction with the neighbouring areas 18 and 18a. The callosal axons originate from layers II, III, and V, and terminate in the conjunction of layers II and III, and of layers V and VI. In the border region between areas 17 and 18a, where the heaviest concentration of the interhemispheric connection is found, the terminals of callosal axons aggregate densely across all laminae (Cipolloni and Peters, '79; Cusick and Lund, '81; Miller and Vogt, '84).

In marsupials, the corpus callosum is absent (Owen, '1837). Its function of interocular transfer is replaced by the fasciculus aberrans and the anterior commissure (Nelson and Lende, '65; Putnam et al., '68; Heath and Jones, '71; Robinson, '82).
Interhemispheric connections are again found restricted to area 17/18 border region in opossum (Benevento and Ebner, '71a) and possum (Heath and Jones, '71; Crewther et al., '84).

Development of Primary Visual Pathways

Morphogenesis of the Retina

Embryogenesis of the Retinal Ganglion Cells

The formation of the eyes starts from the outpushing of the lateral wall of the anterior part of the cephalic neural tube, called the optic vesicles. At early stages of development, the optic vesicles keep protruding outwards, becoming eventually connected with the brain only by a retaining optic stalk, which later becomes the optic nerve. Later the optic vesicles invaginate and are transformed into a double-layered cup: an outer layer gives rise to the pigment epithelium, and an inner layer becomes the neural retina. The germinal cells in retinal epithelia divide at the basement of the inner epithelium layer, then migrate inwards either becoming daughter cells and assuming their appropriate locations within the differentiating retinal layers, or remaining as germinal cells and moving back to the basement of the epithelium for another phase of division. There is an inner-to-outer gradient for cell generation and differentiation such that the glial cells enveloping the ganglion cell layer in the innermost part of the retina generate and differentiate first, followed by ganglion cells, which form the innermost ganglion cell layer of the retina. The next neural layer that differentiates is the inner nuclear layer containing amacrine, bipolar and horizontal cells.

After Sidman's pioneering ('61) study using tritiated thymidine autoradiography to label permanently the neurons undergoing mitotic division, a good deal of information regarding the timing of ganglion cell generation during development is available in a number of species. In monkey, the ganglion cells generation occurs
between embryonic day 36 and 62 (E36-62) (Rakic, '77c); in cat between E20-35 (Polley et al., '81; Kliot and Shatz, '82; Walsh et al., '83); in mice between E11-18 (Sidman, '61). In quokka, the generation of the retinal ganglion cells starts before birth and lasts until postnatal day 25 (Beazley et al., '88).

In all species studied, generation of ganglion cells seems to follow a central retina-first, peripheral retina-last order.

The mature pattern of the central-to-peripheral gradient of ganglion cell density is not present early during development. Instead, the ganglion cell density is uniform across the central and peripheral retina at E34. The distribution of ganglion cell density across the retina does not resemble the adult pattern until E57 (Stone et al., '82). Similar progress is also observed in the quokka during the ontogeny of the retina (Dunlop and Beazley, '85).

It appears that the cell death in the retinal ganglion cell layer is the immediate cause for the onset of the central-to-peripheral gradient of ganglion cell density from the fact that the cell death occurs concurrently with the formation of the central-to-peripheral gradient of ganglion cell density in the retina and the fact that cell death happens more frequently in peripheral than in central retina (Sengelaub and Finlay, '82; Stone et al., '82; Beazley and Dunlop, '83)

One of the explanations for the cell death in the retina and axonal loss in the optic nerves during normal development is the competition between axons of afferents for available synaptic sites which once occupied allow for the retrograde transport of a survival factor from the postsynaptic structures (Cowan, '73; Jacobson, '78; Hamburger and Oppenheim, '82; Bennett, '83; Oppenheim, '85). The competition could occur between axons from different eyes for available territory in the target structures (e.g. LGNd and SC) or among cells with different topographic specificity.

In the early development of the retina of the tammar ganglion cells from the retinal periphery have a smaller region of central projection than those from the central retina. This inequality is present as soon as the axons of ganglion cells can be detected in the brain and before the unequal density of the area centralis and visual streak is
apparent in the ganglion cell layer of the eye. Trophic support for peripheral ganglion cells may be less than that available for more centrally lying cells leading to more death in the periphery (Marotte 1989).

Disproportionate growth of retina in size may also contribute to the onset of the central-to-peripheral gradient in ganglion cell density. In cat, the peripheral retinal region grows more than central retina (Mastronarde et al., '84), and the large increase of retinal size occurs well after the final genesis of cells destined for the ganglion cell layer (Rapaport and Stone, '83; Mastronarde et al., '84).

Development of the retinogeniculate projection

Embryogenesis of Diencephalon

Soon after the closure of the anterior region of the neural tube, a number of constrictions appear along its wall and form a series of swellings or neuromeres upon which the prosencephalon (forebrain), the mesencephalon (midbrain), and the rhombencephalon develop. The prosencephalon, becomes divided into two parts, the anterior telencephalon and the more posterior diencephalon, soon after the optic vesicles form on its lateral walls. The dorsal wall of diencephalon becomes thalamus by generation, migration, aggregation and differentiation of neuroblast cells generated in the ventricular zone.

The description of a fundamental plan of diencephalic histogenesis came from Herrick's ('18) morphological studies in which the diencephalon was described as consisting of a roof plate, a floor plate and a pair of lateral plates. Lateral plates pushed out and, as a result, became grooved on their ventricular surfaces by dorsal, middle, and ventral diencephalic sulci which outline four regions of cellular proliferation called epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus. There are two phases of thalamic growth: an early phase of cellular proliferation during which the epithalamus, dorsal thalamus, and central thalamus differentiate from
each other, followed by a later phase during which the individual nuclei differentiate within these large subdivisions. (Bergquist and Kallen, '54; Coggeshall, '64).

**Generation of LGNd**

This has been extensively studied since the introduction of tritiated thymidine autoradiography by Sauer('59), Sidman and Miale ('59), in rat (Lund and Mustari '77; Bruckner, '76), cat (Hickey and Hitchcock, '84; Hitchcock et al., '84; Shatz, '81), monkey (Rakic, '77b), and quokka (Harman and Beazly, '88). Two principles arise from these studies. First, all LGNd cells are generated at an early stage of development within a relatively short period. Second, there is a gradient of neurogenesis in the LGNd, which seems different in direction from species to species.

In monkey, Rakic ('77b) found that virtually all geniculate neurons are born between embryonic day 36 (E36) and E43 from a particular portion of the ventricular wall, which also gives rise to other nuclei adjacent to the LGNd. It seems that the generation of the LGNd follows a "pushing-out" pattern in such that the neurons in the outermost part of the LGNd, e.g. those forming future magnocellular layers are generated first, and the neurons in the innermost layer are born later. The monkey LGNd shifts its position from the dorso-lateral aspect to the ventrolateral aspect of the thalamus during development between E48-97 as the result of the tremendous expansion of the dorso-medial part of the thalamus, as pulvinar, in this species during this period (Rakic, '77b). Thus the initially lateral-to-medial axis of the nucleus becomes a ventral-to-dorsal one. The differentiation of the initially homegenous neuronal population into the laminated cellular layers takes place between E90-130 with the outermost magnocellular layers differentiating first.

In cat, the generation of LGNd neurons takes a relatively longer period between E22-32. Unlike in monkey and rat, a spatiotemporal gradient of cell birth for cat’s LGNd has not been reported. It seems that the size of cells, rather than their final position in the LGNd, determines the sequence of cell birth. For example, early born cells have either large or small soma sizes, while later born cells are only of small sizes (Hickey and Hitchcock, '84). As the large cells are scattered among small cells in the
innermost A lamina of the nucleus, this may be some indication of an inside-out pattern of neurogenesis during the histogenesis of cat’s LGNd (Shatz, '81).

In rat, the neurons in LGNd are born over a 2-day span between E12-14. Like monkey, the generation of cells also follows a lateral-to-medial gradient such that the neurons situated outermost in the nucleus, adjacent to optic tract, are born first, while those in the medial part of the nucleus are born later (Lund and Mustari, '77; McAllister and Das, '77).

In quokka (Harman and Beazley, '88), the generation of geniculate neurons starts before P2, the earliest time tritiated thymidine was injected, and ends by P10. There are lateral to medial, ventral to dorsal, and caudal to rostral progressions in neurogenesis.

Emergence of eye-specific layers of retinal input in the LGNd

The adult pattern of segregation of retinal inputs into eye-specific layers or regions is not present in the LGNd early during development. Instead, the retinal inputs from different eyes are more extensive than at maturity and overlap with each other to form an intermixed distribution pattern (monkey: Rakic, '77; cat: 'Shatz, '83; rat: Bunt et al., '83; hamster: So et al., '78, '84; opposum: Calvacante and Rocha-Miranda, '78; quokka: Harman and Beazly, '86; tammar: Wye-Dvorak, '84). A most detailed description of this transition of distribution pattern of retinal inputs in LGNd from an intermingled to a segregated state comes from studies on prenatal and neonatal cats (Shatz, '83; Sretavan and Shatz, '84, '86).

After intraocular injections of tritiated amino acids into one eye and the HRP into the other in embryonic animals of various ages, a gradual segregation of retinal inputs of different eyes from an initially intermixed pattern to an eye-specific one is observed between E32-57. This process is similarly reported in monkey (Rakic, '77c), rat (Bunt et al., '83) marsupials (Calvacante and Rocha-Miranda, '78; Wye-Dvorak, '84; Harman and Beazley, '86) and other species (So et al., '78, '84; Frost et al., '79; Card-Linden et al., '81; Godement et al., '84). By filling single axons with HRP, the development of individual retinal ganglion cell axons during the process of
segregation of retinal inputs into eye-specific layers in the LGNd was described (Sretavan and Shatz, '84). It was found that, corresponding to the progress of the segregation of the retinal inputs from different eyes into eye-specific layers, the morphological features of ganglion cell axons in the LGNd undergo a remarkable change between E32-57. During the period when retinal inputs from both eyes are intermixed in LGNd, the individual axons which traverse across the nucleus have many side branches with equal density in all layers. At subsequent stages, the side branches in appropriate layers to the eye of axonal origin are allowed further prolongation, while those in inappropriate layers are largely reduced such that they virtually disappear (Sretavan and Shatz, '84).

From the ultrastructural study, Campbell et al., ('84) have shown in hamster that the ganglion cell axons are able to produce transient synaptic connections in inappropriate layers, which is also found in electrophysiological experiments on tissue slices of the LGNd of embryonic cats (Shatz and Kirkwood, '84).

In determining the mechanisms underlying the processes of segregation of retinal inputs into the eye-specific layers in LGNd, several hypotheses are formulated. The most popular one seems to be the hypothesis of binocular competition, in which it is assumed that ganglion cell axons from different eyes have competitive interaction for postsynaptic territory within the LGNd. This hypothesis is supported by a number of studies of monocular enucleation. After one eye is removed, the projections from the remaining eye extend their distribution well into the layers designated for retinal inputs from the enucleated eye and persist with this widespread distribution into maturation (Godement et al., '80; Rakic, '81; So et al., '84; Sretavan and Shatz, '84, '86; Shatz and Sretavan, '86; Williams et al., '86).

However, the weight of binocular competition as a factor in the formation of eye-specific distribution of the retinal input in LGNd layers is questioned by developmental studies of the morphological features of the individual retinal axons during the segregation of ganglion cell axons into appropriate laminae (Sretavan and Shatz, '86). It is observed that, coincident with the segregation of retinal inputs into
their own target LGNd laminae, the morphology of individual axons traversing across the LGNd undergoes a dramatic change such that in inappropriate layers the side branches of the retinal axons are largely retracted or eliminated, whereas in appropriate layer the axons develop an elaboration of terminal arborization. It is therefore argued that the retraction of sidebranches, rather than the elimination of axons themselves, may be the main cause contributing to the formation of eye-specific termination of the ganglion cell axons during development (Shatz and Sretavan, '86; Sretavan and Shatz, '86).

In monocular enucleation, some of the retinal axons of the remaining eye terminate and arborize in laminae designated for the removed eye. The extension of arborization of retinal axons in territories either for the remaining eye or for the removed eye is greatly increased (Sretavan and Shatz, '86). This also suggests a kind of competition between neighbouring ganglion cells from the same eye for space in the target structures. Previously, it has been shown that removal of one eye could increase the size of terminal arborization of particular classes of cells (Sur, '82).

The results of recent experiments in the tammar on the effects of very early monocular enucleation play down the importance of binocular competition. Terminal laminae are seen in adult animals from which one eye had been removed before ganglion cell axons had arrived in the LGNd. Lamination is normal in the contralateral monocular segment. The whole geniculate is shrunken particularly the monocular segment ipsilateral to the remaining eye. In the binocular segment contralateral to the remaining eye lamination is still seen although the layers are wider, suggesting that there may have been a loss of cells normally receiving ipsilateral input and/or a limited expansion along the lines of visual projection. Retinotopy of the remaining projection is normal (Marotte et al., '89).

Whatever forces control the segregation of retinal inputs into eye-specific layers in the LGNd, it seems that the spontaneous activity of ganglion cells is a crucial factor mediating interactions between their axons. It is recently reported that these activities exist in developing mammalian retinas at early developmental stages (Galli and
Maffei, '88) and application of tetrodotoxin (TTX), to block spontaneous activity, can prevent the segregation of the retinal inputs into eye-specific layers (Shatz and Stryker, '88). At an individual axonal level, this produced abnormal widespread terminal arborizations extending into layers designed for another eye (Sretavan et al., '88).

Emergence of topographic specificity of the retinogeniculate projection

In adult cat, there is only a very coarse topographic order present in the optic nerve (Horton et al., '79; Guillery, '82). After passing the chiasm and entering the optic tract, ganglion cell axons are topographically more orderly organized (Torrealba et al., '81, '82). Thus, on arrival in the LGNd, the ganglion cell axons have already achieved some degree of topographic specificity. This is experimentally proved in a recent study suggesting that retinal axons achieve a certain degree of retinotopic order before they segregate into appropriate laminae (Jeffery, '85). In the tammar, this is more directly examined by a recent study in the marsupial wallaby (Marotte, '89) in which a defined region of the retina was ablated by laser beam and the deficit in labelling was examined in the geniculate and the superior colliculus (see next section) after an intra-ocular injection of HRP. Topography can be demonstrated very early in development but undergoes refinement with time as evidenced by the sharpness of the border of the label deficit. This suggests that, early during development, there is probably an overlap of retinal inputs from individual ganglion cells of the same eye within one specific lamina, in tangential plane, in addition to the overlap of the retinal inputs from different eyes across the laminae in the LGNd. The continuous refinement of the ganglion cell axonal terminals may be the result of the retraction of side branches of terminal axons away from the topographically inappropriate loci, as in the situation of segregation of ganglion cell axons into eye-specific layers in the LGNd (Sretavan and Shatz, '84,'86).

The development of the retinal projection to the LP-pulvinar complex has received little attention so far and will not be reviewed here.
Development of Retinocollicular Projection

Embryogenesis of Mecencephalon

The mesencephalon (midbrain) becomes distinct soon after a number of constrictions appear along the wall of the neural tube to form a series of swellings (or neuromeres). During early embryonic life, the mesencephalon remains undivided and simple relative to other brain regions. At a later stage, constrictions appear on the dorsal aspect of the midbrain, thus forming two pairs of swellings. The rostral pair is called the tectum in lower vertebrates and the superior colliculus in mammals.

Generation Of The Superior Colliculus

In mammals, the neurons designated for future superficial tectal layers SZ, SGS and SO are generated from the wall of the ventricle.

In monkey (Rakic, '77c; Cooper and Rakic, '81), the generation of cells destined for the future SC occurs from E30 to E56, with peak proliferation from E38 to E43. Only a weak ventrodorsal gradient of neurogenesis is detectable. There seems to be virtually no mediolateral or rostrocaudal gradients.

In rat (Brukner et al., '76; Mustari et al., '79), the generation of neurons destined for the SC occurs during the embryonic period between E12 to E17. The cells in rostrolateral colliculus are born earlier than those in caudomedial parts. Furthermore, dorsoventrally, there seems to be an inside-out gradient of neurogenesis in that cells of deeper laminae are born earlier than cells of superficial laminae.

In quokka (Harman and Beazley, '88), collicular cells of the superficial layers are generated during a period starting before P2 and ending by P20, the peak of the collicular neurogenesis occurring at P5. It appears that the neurogenesis in the superior colliculus follows the lateral to medial, ventral to dorsal, and rostral to caudal gradients.

Segregation Of The Retinal Input Into The Eye-Specific Patches

Like the case of the LGNd, the adult pattern of retinal input distribution into the eye-specific patches is not present early during development. Axons from both retinae
tend to innervate the entire SC (Cavalcante and Rocha-Miranda, '78; Frost et al., '79; Land and Lund, '79; Williams and Chalupa, '82; Shatz, '83). The establishment of the mature pattern involves a process of growth and retraction similar in all species.

In monkey (Rakic, '76, 77c), at the earliest time tested at E78, the retinal axons are concentrated uniformly over the superficial layers of both contralateral and ipsilateral SC, indicating the overlap of fibres from the two eyes, as in the LGN, at this stage. At E124, the distribution of retinal fibres in the SC appears in a pattern of alternating dense and light concentration, corresponding closely in number and pattern to the ocular dominance patches observed in adult, suggesting the emergence of the complementary segregation of projections from the ipsilateral and contralateral eyes, which is present by E144 in a pattern similar to that in mature monkey.

In cat (Williams and Chalupa, '82), the retinocollicular projections in prenatal animals was studied by ocular injection of HRP and/or tritiated leucine. At the earliest time (E38) tested, both contralateral and ipsilateral SC have intensive retinal axonal termination distributed uniformly across almost the entire SC. As development proceeds, clear gaps of label in SGS of contralateral SC are evident, which, as revealed by double labelling, quite closely match with patches of label on the ipsilateral side. The segregation of the retinal axons from different eyes starts to resemble the mature pattern several days before birth. The ipsilateral terminal field becomes excluded from the rostral and caudal tectal poles shortly before birth.

In rat, (Land and Lund, '79; Bunt et al., '83), optic fibres are first seen on the tectal plate of the contralateral side at E16.5 and of the ipsilateral side by E17. Contralaterally, the retinal input covers the whole extent of the SC at E17 and later, reaches the adult-like thickness by birth. Ipsilaterally, the retinal input is much less concentrated, but also distributed across the whole SC. The ipsilateral projection only becomes restricted into more rostral and medial SC by P7.

In marsupials, it has been shown in a number of species (opposum: Cavalcante and Rocha-Miranda, '78; tammar; Wye-Dvorak, '84; quokka; Harman and Beazley, '86) that, as in placental mammals, there is an early phase of more extensive and
overlapping projections from both eyes followed by progressive segregation of retinal inputs of different eye origin into the eye-specific patches and retraction of the ipsilateral projection into the rostral part of the SC.

What determines and regulates the segregation of retinal inputs into eye specific patches is currently not known. It is postulated that, as in the retino-geniculate system, binocular competition plays an important role during development. It has already been shown in a number of studies that removal of one eye during pre- or postnatal life alters the projection from the intact eye to the SC such that the ipsilateral projection retains its initial wide termination into adulthood (Lund and Lund, '71; Frost and Schneider, '76; Land et al., '76; Sanderson et al., '78; Finlay et al., '79; Land and Lund, '79; Godement et al., '80; Lent and Mendez-Otero, '80; Rhoades and Chalupa, '80).

The ganglion cell death may also underly the retraction of the ipsilateral projection (Frost et al., '79; Cunningham et al., '81; Jeffery and Perry, '82; Insausti et al., '84; O'Leary et al., '86).

Another factor that may be at play in the retraction of the ipsilateral retinocollicular projection is the elimination of axonal collaterals terminating in inappropriate regions (Land and Lund, '79; Ivy and Killackey, '81). This is also reported in a number of studies concerning the development of callosal connections (Innocenti, '81; Innocenti and Clarke, '84; Lund et al., '84; Dehay et al, '88).

After arrival in appropriate parts in the SC, further fine-tuning in the disposition of ganglion cell axons may be required for the achievement of the precisely aligned retinotopical maps. In both LGNd and SC, the progressive refinement of the retinal axons from a diffused distribution in tangential plane of the target structures to a defined point-to-point relationship is described in tammar by combining the laser ablation in a highly restricted retinal region and ocular HRP injection (Marotte, '89). It is yet to be determined what the role of factors such as death of ganglion cells, elimination of retinal axons or collaterals, and retraction of side branches of individual ganglion cell axons take in this process.
Development of Visual Cortical Pathways

Morphogenesis Of The Telencephalon

Embryogenesis

The telencephalon is the derivative of the anterior part of the prosencephalon. It is further divided into two parts, anterior olfactory lobe which in lower vertebrates becomes the dominant part of the telecephalon, and the posterior cerebral hemispheres derived from a pair of bulbous projections, which in higher vertebrates become the dominant part of the telencephalon.

Generation Of The Cortical Neurons

This has been studied in a number of representative mammals by exposing the young of various ages to tritiated thymidine and examining later in the adult the distribution of radioactive cells in the visual cortex with respect to particular timing of application of tritiated thymidine. The cortical cell generation starts at almost the same stage as the geniculate cell generation in a given species, but ends at a relatively later stage in monkey and cat (Rakic, '74, '76; Luskin and Shatz, '85b).

The generation, migration and differentiation of the cortical cells in the developing cortex in many species follows an inside-out pattern such that the cells destined for deeper laminae are generated and complete their migration to the appropriate destination earlier than those destined for more superficial layers.

The cortical cells are generated in the ependymal layer of the embryonic cerebral ventricle and then migrate radially to accumulate in the cortical plate by passing through the subventricular zone, the intermediate zone and the subplate zone. They stop on the surface of the cortical plate and then are displaced inwards as later-born cells arrive. The cortical structure thus increases its thickness by continuous addition of the later-born cells on top of the CP.

By a combined Golgi electromicroscopic and tritiated thymidine autoradiographic analysis, the migrating postmitotic neurons are found following the
shafts of radial glial cells stretching across the fetal cerebral wall (Rakic, '74). The attachment between migrating cells and glial cells may involve certain cell-cell recognition and adhesion (Rakic, '81).

Rakic recently ('88) postulated that the ventricular zone may consist of proliferative units containing columns of precursor or stem cells defined by glia septa from neighbouring proliferative units. These proliferative units provide a proto-map of prospective cytoarchitectonic areas and the number of these units determine the size of the cytoarchitectonic areas. The proliferative units then become the polyclones for further production of cohorts of postmitotic cells migrating along the common radial glial guides, forming the ontogenetic columns giving rise to the functional unit in the cortex. There is evidence that cortical afferent inputs can regulate the number of ontogenetic columns, thus regulating the size of specialized areas (Rakic, '88). This postulation was challenged by a recent study in rat’s visual cortex using an in vivo retroviral vector carrying the Escherchia coli B-galactosidase gene, which can be copied by daughter cells faithfully and can be easily visualized after processing. It was shown that, while clonally related neurons do tend to remain in radial alignment in the intermediate zone, they often scatter into different cortical radii in the cortical plate. Thus clonally related neurons do not necessarily follow a single radial glial fibre or, as referred to by Rakic, the ontogenetic columns (Walsh and Cepko, '88).

Morphogenesis Of The Visual Cortex

The fundamental plan for the morphogenesis of the visual cortex is the same in all mammals. Five stages in the development of telencephalon can be observed on the basis of cytoarchitectonics. At stage 1, the telencephalon is single-layered with the ventricular zone containing precursor and stem cells. At stage 2, the marginal zone is forming, overlying the ventricular zone, and mainly contains the outmost cytoplasmic parts of the ventricular cells. At stage 3, some postmitotic cells start to migrate into the low part of the marginal zone, forming the intermediate zone. At stage 4, some postmitotic cells have reached more superficial parts of the marginal zone, starting to form the cortical plate. In the meantime, the subventricular zone is forming at the
junction of the ventricular zone and intermediate zone (The Boulder Committee, '70). Stage 5 is a prolonged period during which the cortical plate increases in thickness greatly, as the result of continuous addition of new-born cortical cells onto the dorsal surface of the CP, and undergoes a phase of cellular differentiation, starting upwards from the deepest part of the cortical plate. The cortical afferent and efferent connections, plus the intrinsic connections, are also forming and progressively acquiring the mature pattern of their areal and laminar distribution during this period, contributing to the eventual establishment of the adult pattern of the laminar organization of the visual cortex.

Whilst a large proportion of cells generated in the ventricular zone become the future cortical neurons in different laminae subject to the timing of generation, a small proportion of earliest born cells are only transiently present in the telencephalon, forming a cellular zone called the subplate, at the junction of cortical plate and intermediate zone (Raedler and Raedler, '78; Kostovic and Rakic, '80; Luskin and Shatz, '85a). In adulthood, the subplate zone and its occupants, the earliest-generated cells, virtually disappear (Luskin and Shatz, '85b).

The fact that the arriving afferent axons accumulate in the subplate zone for a long time while the cortical plate is undergoing a phase of differentiation, and the fact that this zone only transiently exists during development suggests that the subplate might provide a transient target structure to accommodate temporarily the geniculocortical axons and thus resolve the mismatch in timing between the innervation of geniculocortical axons and the formation of the cortical lamina IV, the principal destination of the geniculocortical axons (Shatz et al., '88). The evidence that cells in the subplate contain substantial amounts of synapses during the waiting period of geniculate axons in the region (Kostovic and Rakic, '80; Chun et al., '87) seems in favor of this postulate, although no direct evidence, for example, identified geniculate axon terminals synapsing on the subplate cells is available in this regard.

The cytoarchitectonic features of the telencephalon during the morphogenesis of the cortex in the marsupials seems different from the placentals in that the cell
compact zone, a parcellation of very densely packed pre- and postmigratory cells just beneath the marginal zone, is much thinner in marsupials than in placentals. For example, the cell compact zone in tammar wallaby is about 4-6 cells in thickness (Reynolds et al., '85), whereas in monkey, cat and rat (Rakic, '74; Lund and Mustari, '77; Luskin and Shatz, '85a,b) it is about 20-30 cells in thickness. This forms part of the experimental work of this thesis and will be further discussed in chapter 4.

Development Of Cortical Connections With The LGNd

Formation Of The Geniculocortical projection

The geniculocortical axons start to enter the telencephalon at a very early stage when geniculate cells are just born and the cortical layer IV cells, the principle destination of geniculate axons, are still not generated. According to the results from studies on placentals, the geniculate axons accumulate underneath the cortical plate after their arrival in the telencephalon and do not enter the cortical plate in substantial numbers until neurons of the target layers assume their final appropriate position (Rakic, '76; Lund and Mustari, '77; Shatz and Luskin, '86).

In rat, the thalamo-cortical axons enter the visual part of telencephalon by as early as E18. However, they remain accumulated beneath the cortical plate for several days and enter into the bottom layer of the cortex only when layer VI neurons assume their final position at the time of birth. Thalamic axons do not invade layer IV substantially until P8, after cortical cells comprising the layer IV complete their migration (Lund and Mustari, '77).

In monkey, even though the information regarding the earliest time of arrival of invading geniculate axons in the telencephalon is not available, it is clear that the geniculate axons are kept in waiting in the subplate zone more than one month after they are first detected in telencephalon by E78 until they enter layers IV and VI in detectable amounts by E124, well after neurons comprising these two layers have completed their migration (Rakic, '76, '77c)
In cat, the spatiotemporal relationship between innervating geniculocortical axons and their cortical cells has been examined in more detail and is found to be similar to that described in rat and monkey. After ocular injection of tritiated proline, the transneuronal label is first present in the occipital pole of the telencephalon by E42. The geniculate axons aggregate in the subplate zone for more than three weeks. They only invade into layer IV substantially by birth, several days after neurons comprising layer IV have completed their migration to the appropriate position (Shatz and Luskin, '86).

Why geniculate axons have to wait underneath the cortical plate for a considerable period of time during development is currently unknown. According to preceding reports, the geniculate axons enter the telencephalon at a stage when the formation of the visual cortex is just at its beginning and, in particular, when their target layers are not yet formed. As a resolution to such mismatch in timing, the geniculate axons accumulate in the subplate zone containing transient neuron-like cells (Chun et al., '87) and may recognize them as temporary target cells and even make synaptic connections with them (Shatz et al., '88).

After entering the cortical plate, the geniculate axons are initially distributed uniformly in layers VI, V and IV (Rakic, '76; Lund and Mustari, '77; Shatz and Luskin, '86; Peduzzi, '88). As maturation proceeds, they become gradually segregated into their appropriate cortical layers at later developmental stages. In monkey, the bilaminar concentration of transneuronal label indicating the dense termination of geniculate axons over sublaminae IVa and IVb only becomes visualized by E144 (Rakic, '76). In cat and rat it is after birth that substantial aggregation of geniculate fibres become more pronounced in layer IV (LeVay et al., '78; Lund and Mustari, '77), while in ferret, the adultlike pattern of geniculate axon termination in layer I, IV and VI is not achieved until approximately a month after birth (Peduzzi, '88).

The geniculate axons terminating in layer IV are further segregated into eyespecific ocular dominance columns at later stages in some species. In cat, this
segregation starts about three weeks after birth and is nearly complete by postnatal day 40 (LeVay et al., '78). In monkey, the segregation becomes detectable in late prenatal stages (Rakic, '76; Rakic, '77c) and assumes its mature pattern by about three weeks after birth (Hubel et al., '77; LeVay et al., '80).

In areal distribution of geniculocortical axons in the telencephalon during development, from an autoradiographic transneuronal study in monkey (Rakic, '76), it is observed that the transneuronal label in the cortical plate stops abruptly at the border between areas 17/18 by P124. Nonetheless, at this stage a detectable amount of transneuronal label is also present in area 18, where the geniculate projection is supposed to be absent. It is possible that while a fair degree of areal specificity is already present when geniculate axons just enter the cortical plate, there may be some exuberant geniculate axons in area 18.

**Formation Of The Corticogeniculate Projection**

Corticogeniculate axons start to innervate the diencephalon almost at the same stage as the innervation of the geniculocortical axons into the telencephalon in placentals. In monkey, following a cortical injection of tritiated proline, the autoradiographic label can be seen in magnocellular layers by E70. By E84, label also starts to accumulate in parvocellular layers (Shatz and Rakic, '81). In cat, the early nerve degeneration study shows that the corticogeniculate projection is already present in the LGNd by E48 (Anker, '77). On the other hand, it was recently shown that, after application of tritiated leucine or the fluorescent dye (DiI) in the presumptive visual cortex, the cortical axons reach the thalamus by as early as E39, but do not enter the LGNd until E59 (McConnell and Shatz, '88). In rat, the corticogeniculate fibres were reported to reach the diencephalon by about E20 and enter the LGNd by E23 (Thong and Dreher, unpublished observations, from Dreher and Robinson, '88b).
Development Of Cortical Connections With Pulvinar-LP Complex

Connections between the primary visual cortex and the pulvinar-LP complex are also composed by reciprocal afferent and efferent projections. Little information on the formation of afferent projections from the pulvinar-LP complex to the visual cortex is available, perhaps for technical reasons.

Cortical injection of tritiated proline or degeneration studies after cortical lesions make the description of the formation of efferent projections from the visual cortex to the pulvinar-LP complex during development possible. In monkey, the pulvinar receives a distinct projection from the visual cortex by E72. The cortical projection becomes substantial in the still undifferentiated pulvinar by E83-4. By E95-96, the corticopulvinar projection has achieved basic topographic order, as indicated by sharply edged and appropriately located label with respect to cortical injection site (Shatz and Rakic, '81). In cat, the first time that the degeneration is present in the lateral posterior following a cortical lesion is E45 (Anker, '77), though autoradiographically the cortical projection can be detected in the thalamus as early as E39 (McConnell and Shatz, '88).

It seems that cortical axons enter the geniculate and LP-pulvinar at a similar stage during development, despite the fact that they originate from different cortical layers which are generated and differentiate at different stages during development in a given species.

Development Of The Cortical Connection With SC

To trace the anatomical evidence for the onset of the corticotectal projection early during development, the cortical degeneration (Anker, '77), cortical injection of autoradiographic active amino acids (Shatz and Rakic, '81; McConnell and Shatz, '88) and injection of WGA-HRP in the superior colliculus (Thong and Dreher, '86) have been employed in a number of studies on representative placentals.
In monkey, it has been shown that the cortical axons arrive in the SC by E78. They largely remain in the SO. By E86, they are concentrated in substantial numbers in the SO, but are still insignificant in the SGS even at the anterior edge of the SC. By E96, substantial label is present in the SGS and SZ, in addition to the SO, and is well-defined in regions roughly topographically appropriate with that of cortical injection sites (Shatz and Rakic, '81).

In cat, the degeneration study showed that the cortical projection starts to accumulate in the SC by E48 (Anker, '77), while an autoradiographic study did not detect corticocortical axons in the SC until E52 (McConnell and Shatz, '88).

In rat, the onset of the corticocortical projection was demonstrated by retrograde labelling in the telencephalon following a collicular injection of WGA-HRP (Thong and Dreher, '86). Cortical projections start to enter the SC postnatally around P3-4 and become substantial after P5. It is argued that the high degree of laminar specificity of origin of corticocortical projections in layer V is present during development.

It is obvious that the formation of corticocortical projections in any given species is delayed in timing significantly compared with the formation of the corticothalamic projections to the LGNd and LP-pulvinar.

Development Of Interhemispheric Connections

Timing Of Formation Of Interhemispheric Connections

During normal development, the interhemispheric connections between visual cortices is the last to form among the cortical afferent and efferent connections in a given species, presumably due to the relatively delayed generation, migration and differentiation of callosal cells which are mainly situated in the more superficial layer III in the cortex, and the relatively long distance for callosal axons to travel during development.
In monkey, callosal neurons are first labelled by E112, although there is evidence that callosal axons have crossed over the midline by E97 (Dehay et al., '88).

In cat, the callosal axons are seen beneath the cortical plate in opposite visual cortex by E52 and in the gray matter near the border between areas 17 and 18 by P3 (Innocenti, '81; McConnell and Shatz, '88).

In rat, the callosal axons arrive in the deepest part of layer VI by P4 and enter the gray matter significantly by P6 (Lund et al., '84).

Emergence Of The Regional Specificity Of Callosal Connections

The restricted distribution of callosal connections in adult visual cortex is not present early during development. Rather, both callosal cells and axons have a widespread distribution in visual cortex early in development (Innocenti, '81; Lund et al., '84; Dehay et al., '88). In new born cat and rat, callosal neurons are found distributed in the entire extent of the areas 17 and 18. The termination of callosal axons in these animals also have a wide distribution, but only in the white matter or deepest part of the layer VI (Lund et al., '84). In monkey, it is observed that callosal neurons are widespread in area 18 at E114 and only become defined in a restricted region as seen in adult, by birth (Dehay et al., '88).

It has been experimentally shown that callosal neurons in inappropriate regions can still remain alive at a late stage after their axons or collaterals are cut (Innocenti, '81). It is argued that the elimination of the transient callosal axons at early stages may be caused by their failure to invade into the gray matter of the opposite visual cortex, so that among the early widespread callosal axons, only those in the appropriate region are allowed to enter into the gray matter and persist in their presence into adulthood (Lund et al., '84).
The Aim of the Present Study

In the present study, it is intended to extend the anatomical description of the mammalian visual cortical connections and its normal development into one of the best studied Australian marsupials, the tammar wallaby.

In chapter 2, the afferent and efferent connections of the visual cortex with main subcortical structures and with the visual cortical areas of ipsilateral and contralateral cerebral cortices will be described. The main interest of this chapter is the areal and laminar disposition of these afferents and efferents within the cortical laminar organization.

In chapter 3, I will concentrate on the establishment of the timecourse of the formation of the cortical afferent and efferent connections with main subcortical visual structures and cortical areas in the ipsilateral and contralateral visual cortices in the tammar. The interspecies comparison of the present results with those from studies on some representative placental mammals reveals that the tammar has a remarkably different time course in the formation of the visual pathways from representative placental mammals.

The spatiotemporal distribution of axons and cells subserving afferent and efferent visual cortical connections within the telencephalon during normal development is dealt with in chapter 4. The relationship between the formation of the laminar specificity of these axons and cells seen in the mature animal and the ontogeny of the cortical laminar organization during development is described.

Finally, in chapter 5, the interpretation and implication of the present results will be summarized and some lines of future researches closely related with this study are suggested.
Chapter 2. Afferent and Efferent Connections of the Visual Cortex in the Tammar Wallaby

Introduction

The afferent and efferent connections of mammalian visual cortex possess certain distinct features. First, the massive cortical afferents and efferents between the visual cortex and subcortical visual centres and opposite visual cortex are wired according to a rather stereotyped plan basically common to all the mammalian species. Second, these connections express a high degree of specificity at several levels. For example, in both subcortical nuclei and cortical areas, the axons and cells subserving these connections are organized according to a precise topographic order (topographic specificity). Moreover, in the cortex, both afferent axons and efferent cells from and to the subcortical and intercortical structures terminate and originate only in certain cortical areas or certain parts of the cortical areas (areal or regional specificities), as well as in certain cortical layers (laminar specificities).

In comparison to the situation in placentals where the anatomical features characterizing the mammalian visual cortical connections have been already well documented by decades of extensive studies in a number of representative placental mammals (see Jones, '85; Alan and Jones, '85 for review), considerably less is known about the visual cortical connections in marsupials from a limited number of studies. The cellular organization of cortical area 17 in marsupials has been described previously, showing that the typical six-layered cellular organization characterizing the striate cortex in placental mammals shares features with both American (opossum: Benevento and Ebner, '71a,b) and Australian marsupials (possum: Heath and Jones, '71; Haight et al., '80; wallaby: Mayner, '85). Although a knowledge of the nature of the retinotopic representations in the cortex is lacking, at least three visual cortical areas, the striate cortex, the surrounding peristriate cortex and more rostrally, the
posterior parietal cortex, have been identified in marsupials on the basis of their cytoarchitectonics and their connections with the dorsal lateral geniculate nucleus (LGNd) and the lateral posterior nucleus (LP) (possum: Haight et al., '80; opossum: Benevento and Ebner, '71b; Coleman et al., '77). In addition, there have been reports mentioning the presence of the characteristic distribution pattern of interhemispheric connections restricted to the striate/peristriate border region (Heath and Jones, '71; Crewther et al., '84) and the existence of intrahemispheric connections between the striate cortex and a number of extrastriate visual areas (Benevento and Ebner, '71a; Crewther et al., '84). In tammar wallaby, apart from a cytoarchitectonic description of area 17 and area 18, previous work on the cerebral cortex so far has largely concentrated on the relationships of the sensorimotor cortex with the thalamus (Mayner, '85).

The present study concerns the anatomical characteristics of the visual cortical connections in tammar wallaby. It is of general interest to know, for example, whether the organizational features of the visual cortex in this species are similar to those observed in placentals, given the fact that the Australian marsupials have taken a totally separate evolutionary course from placental mammals for more than 100 million years (Tyndale-Biscoe, '73). For example, do the areal and laminar distribution of the afferents and efferents in the cortex in the tammar resemble that in placentals and if so, to which representative group of placental mammals, is the resemblance most obvious? Apart from this, the present results are also required as the fundamental description of the mature pattern of the afferent and efferent connections of the visual cortex for the following developmental study on the formation of these connections during normal development in this species.

Injections of horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP) were made in the primary visual cortex, the LGNd, and the LP. The labelled terminals and labelled cells were analysed after anterograde and retrograde transport in regions and structures with respect to injection sites. Our results suggest
that, despite the isolation from the main mammalian evolutionary line for such a long time, the tammar has developed a visual cortex constructed on a scheme very similar to what has been described in placental mammals. With the operational advantages of using this animal in developmental studies, this similarity suggests the relevance of using this experimental model to ask types of questions which may be universal during the development of the mammalian visual cortex.

**Materials and Methods**

**Animals**

Ten adult males weighing between 3.5-6.0 kg were used in this study. They were obtained from a breeding colony in Canberra, Australia.

**Anaesthesia**

Initial anaesthesia was with an intramuscular injection of a mixture of ketamine (20mg/per kg body weight) and xylazine (2%, 0.4ml in each mixture). Within 10 minutes of receiving the intramuscular ketamine/xylazine injection, the animal became lightly anaesthetized. Then Surital (sodium thiamylal, Parke-Davis, 4% in saline) was injected as necessary via the tail vein to maintain the anaesthesia during the following operation. Each additional topping-up injection of Surital was 0.5ml and was usually effective for about 20 minutes.

**Surgery and HRP Injection**

After anaesthesia the head of the animal was immobilized in an electrophysiological recording set-up. The hair was shaved and the skin cut to expose
the skull. After exposure of the brain by drilling holes at the desired sites on the skull, WGA-HRP dissolved in distilled water, at a concentration of 2%, was injected either into the primary visual cortex or the LGNd/LP by a needle attached to a 0.5µl Hamilton syringe driven by a micromanipulator. The volume of each injection ranged between 0.05-0.15µl in the cortex and between 0.02-0.05µl in the thalamus.

Up to 3-4 individual WGA-HRP injections were made in each cortical injection case, in the attempt to cover the entire area 17, whereas in thalamic injection experiments, only a single WGA-HRP injection was made in each case. In the cortical HRP injection series, it was relatively easy to ensure that the HRP injections were made in the primary visual cortex under direct visual control, as area 17 in the tammar is large occupying the dorsal and dorsolateral aspects of the caudal occipital cortex.

In the thalamic HRP injection series, the stereotaxic coordinates of the LGNd found in a previous electrophysiological study (Wye-Dvorak, '85) were used to place the injection in the desired sites in the thalamus. To approach the LGNd, injections were placed 12mm below the cortical surface at a site 9mm caudal to bregma and 9mm lateral to the midsagittal on the dorsal surface of the brain. To approach the LP (in animal 88-WA5), the injecting needle was moved 1.5mm closer to the sagittal than in the LGNd injection case. Animals received an injection in only one side of the thalamus, except in one case the other side was also injected. To prevent the involvement of the cortex in HRP uptake in thalamic injection series, the tip of the injecting needle was carefully cleaned before penetrating through the cortex to the desired thalamic injection sites.

After injection, the holes on the skull were filled with Gelfoam and the skin sutured. Triple antibiotic powder (Coopers, Australia) was applied to the sutured skin. Animals were allowed to survive for 48 hours in indoor pens and were checked regularly.
Histochemistry

At the end of the survival period, animals were deeply anaesthetised by an overdose injection of Nembutal (sodium pentobarbitone) or, in later experiments, of a mixture of ketamine and xylazine in an amount approximately twice as much as used in the prior HRP injections. The chest was opened and the heart exposed. Prior to the perfusion, 0.1ml of heparin and 1.0ml of 1% sodium nitrite were injected directly into the left ventricle to facilitate the removal of blood. This was followed by a wash of 500ml of saline (0.9% NaCl) through the ascending aorta. Then 1,500ml of phosphate buffered (0.1M, pH7.4) perfusant containing 1% paraformaldehyde and 1.25% glutaraldehyde was used as a fixative, followed by a wash of 1,000ml of 10% sucrose solution in 0.1M phosphate buffer pH7.4. The brain was then dissected and soaked in 20% phosphate buffered sucrose solution overnight at 4°C.

Before being cut on the following day, the brain was embedded in a gelatin-albumin solution containing 30% albumin and 0.5% gelatin in phosphate buffer (0.1M, pH7.6) containing 20% sucrose. The solution was hardened by the addition of 25% glutaraldehyde in an amount of 5-10% of the volume of the gelatin-albumin solution. With the base of the brain as the horizontal plane, frozen sections in three series were cut coronally at 60µm. Two series were processed histochemically for the demonstration of the HRP product, with one of them counterstained with 0.025% thionin. The third series was stained with 0.025% thionin only, for the description of the cytoarchitectonics of the visual cortex and the visual thalamus.

Tetramethylbenzidine (TMB) was used as the chromagen in the histochemical processing to demonstrate the anterogradely and retrogradely transported HRP. The procedure described by Mesulam ('82) was followed. After being cut, sections were collected in 0.1M phosphate buffer, pH7.4. To start the histochemical processing, the sections were first rinsed in six changes of distilled water (10-15 seconds each), then placed in an incubation solution made just before the commencement of the incubation.
by mixing 292.5ml of solution A containing 300mg of sodium nitroferricyanide (Sigma) and 15ml of acetate buffer (0.2M, pH3.3) in 277.5ml distilled water, with solution B containing 15 mg 3,3',5,5'-tetramethylbenzidine (TMB, Sigma) dissolved in 7.5ml 100% ethanol by moderate heating (37-40° C). After 20 minutes of incubation at room temperature, 2-3ml of hydrogen peroxide solution, depending on the intensity of the reaction, at a concentration of 0.3% in distilled water was added to the incubation solution to initiate the enzymatic reaction which lasted another 20 minutes at room temperature. This was followed by six rinses in diluted 0.2M acetate buffer, pH3.3 (5ml buffer plus 95ml distilled water) for approximately 5 minutes each at 4° C.

After the histochemical processing, the sections were mounted immediately on to glass slides subbed with gelatin and chrome-alum and left to air-dry in the dark. The slides, either counterstained with 0.025% thionin or not, were then dehydrated in 0.2M acetate buffer, pH3.3 (10 seconds), 70% ethanol (10 seconds), 95% ethanol (10 seconds) and 100% ethanol (twice, 10 seconds each). Ethanols were diluted with 0.2M acetate buffer, pH3.3. It is recommended not to leave sections with HRP label longer than 10 seconds in each rinse in ethanol, as the HRP product is soluble in the ethanol (Mesulam, '82). After dehydration, the sections were cleared in two changes of xylene (3 minutes each). Sections were then coverslipped with D.P.X. neutral mounting medium (AJAX, Australia) and allowed to air-dry before observation under the microscope. The HRP labelling was examined in both bright and dark field.

**Results**

The following results are collected from 10 adult males which received WGA-HRP injections either in the visual cortex (four animals) or in the visual thalamus (six animals). The figures illustrating these results and tables I and II are placed together at the end of the chapter.
The cellular organization of the visual cortex

**The visual cortical lamination.** In tammar wallaby, this was first described by Mayner ('85, '89b) on the observation of Nissl-stained preparations. The thickness of the cortex in area 17 in tammar is about 2mm (2,000µm). On the basis of cytoarchitectonics, the typical cellular lamination pattern of mammalian visual cortex is present in tammar in that the whole thickness of the visual cortex is divided into six distinct cellular layers (Figs. 1 & 2).

Layer I. This is situated most superficially in the visual cortex and is about 150-200µm in thickness in both area 17 (Fig. 2A) and 18 (Fig. 2B). This layer is best characterized by the very low density of the cell bodies with different sizes from small to medium, and with moderate staining quality. While many cells are oriented parallel to the surface of the visual cortex, cells with different orientations are seen on occasion.

Layer II. This layer is only 50-100µm in thickness. Characterized by remarkably high packing and staining qualities not seen in the placental mammals, this layer can be easily distinguished from underlying layer III on the basis of cytoarchitectonics. Cells in this layer are sized from medium to large and shaped from round to pyramidal.

Layer III. This layer is the thickest layer in area 17 (750-800µm). In Mayner’s description, this layer includes two parts with certain cytoarchitectonic differences. The upper part has lower cell density and cells in this part are medium sized, moderately stained pyramidal cells. The lower part, on the other hand, is more densely packed and deeply stained, and is perhaps best contrasted to the upper part by the presence of some very large, deeply stained pyramidal cells, as well as those medium-sized pyramidal cells found in the upper part.

Beneath this two-part cellular parcellation of layer III, is a loosely packed cellular zone containing both small granule cells and medium-sized pyramidal cells.
This zone is clearly seen between the layer III cellular parcellation and layer IV granule cell zone. There was some hesitation in Mayner’s description as to which of the layers III and IV this zone should belong (Mayner, '85, '89a). In the present study, this zone is included in layer III which thus comprises three sublayers: 1). sublayer IIIa, cytoarchitectonically corresponding to Mayner’s upper part of layer III, contains no significant amount of LGNd afferents (Figs. 8-10); 2). sublayer IIIb is the lower part of the layer III cellular condensation with a moderate concentration of LGNd afferents (Figs. 8-10); 3) . sublayer IIIc is the low cell density zone with a little, if any, density of termination of LGNd axons (Figs. 8-10).

Layer IV. With a thickness similar to layers V and VI (250-300µm), this layer is densely and homogeneously packed with small-sized, and deeply stained granule cells, which allowed area 17 to be clearly distinguished.

Layer V. This layer can be easily distinguished from adjacent layers IV and VI cytoarchitecturally by its reduced cell density. It has the second lowest density of cells in the neocortex. Another prominent feature is the presence of some very large deeply stained pyramidal cells in the upper part of the layer. There are also many small to medium-sized round or pyramidal cells with moderate to deep staining qualities in both the upper and lower part of the layer V. The thickness of the layer is about 350µm.

Layer VI. This layer is about 250-300µm in thickness and is mainly occupied by medium-sized, pyramidal shaped and deeply stained cells. In Mayner’s description, this layer may be further divided into two parts according to the cellular packing quality: the upper part is more densely packed than the lower part. The above features of the cellular pattern of lamination made the borders of area 17 obvious.

**The laminar organization in area 18.** Although it still follows the fundamental six-layered scheme for the cortical cellular construction, the cytoarchitectonics of area 18 (Fig. 2B) is remarkably different from area 17. First, the overall thickness of the cortex in area 18 is significantly reduced to about 1,200µm in
comparison to area 17’s 2,000µm. This is contributed by the reduction of the thickness in several individual layers, but most significantly by the reduction of the thickness in layer III. For example, the thickness of layer III is reduced from 750-800µm in area 17 to 250-300µm in area 18, accounting for almost 60% of the total reduction in cortical thickness in area 18. Layers IV, V and VI in area 18 are also reduced in thickness by about 50-100µm, whereas layers I and II are not. In addition, while most of the cytoarchitectonic features in area 17 are preserved, area 18 differs from area 17 in certain aspects of sublaminar construction. For example, the large pyramidal cells in the low part of layer 3 in area 17 are not present in area 18; furthermore, the loosely packed cellular zone at the bottom of layer III, layer IIIc, is not visible in area 18.

**Laminar organization in the ventral temporal area (V-TP).** The dorsal part of this region is identical in cellular lamination to that seen in area 18 and was hard to separate from the neighbouring area 18 on the basis of cytoarchitectonics. However, more ventrally, layers V and VI were different in cytoarchitecture from area 18 and the dorsal part of the V-TP in that the cells tended to aggregated in rows parallel to the pial surface (Fig. 1 & 2C.).

**Identification of the visual cortical areas.** On the basis of cytoarchitectonics, Mayner (’85, ’89b) has identified area 17 in tammar wallaby. Surrounding area 17, is what Mayner previously called area 18 (Mayner, ’85) but later included as part of the ventral occipital area with the ventral temporal area (V-TP) (Mayner, ’89b). In the present study, by showing the presence of reciprocal connections between this area and the LGNd, this area is assumed to be the equivalent of area 18 in other species (see below).

The LGNd HRP injection also produced some label in the ventral temporal region ventral to area 18 and caudal to the temporal sulcus. This region was referred as the ventral temporal area (V-TP) and was assumed to be visually related (Mayner, ’85). The presence of the direct connections with the LGNd in this region (see below,
p60) suggests that the V-TP is also a visual area. Cortical HRP injections in area 17 also labelled V-TP (Fig. 6), further supporting the idea that V-TP is visually related.

Also, the present results show that, when the LP was involved in the HRP injection (see below), retrogradely labelled cells in a continuous band were found in a region rostral to the temporal sulcus (Fig. 11), which was called the dorsal temporal area (D-TP) previously by Mayner ('89a) and was assumed as the posterior part of the auditory cortex (Mayner, '85).

In summary, the present results provides the identification of area 17 and 18 by the criterion of distinctive cytoarchitectonics and this is further supported by the evidence of neuronal connectivity. Further, it is proved in the present study that the V-TP is directly connected with the LGNd and area 17. Thus the V-TP is identified as a cortical visual area by the criterion of neuroanatomical connectivity. Finally, the presence of retrogradely labelled cortico-LP cells in an area rostral to the V-TP suggests that this region may be also visual, rather than being related to auditory function. Further details of the interconnectivity are considered below.

Results of Cortical injections of WGA-HRP

The cortical HRP injection experiments in the present study were designed to investigate the afferent and efferent connections of the visual cortex with main subcortical visual structures and the opposite visual cortex. Four adult males were used in this series. In each case, three or four injections were made at randomly chosen loci within area 17, in an attempt to cover the entire area 17 with HRP. However, due to the limited spread of WGA-HRP, each individual HRP injection was rather defined, assisted by the fact that area 17 is almost lissencephalic and the distribution of the reaction-product surrounding each injection sites was only about 2mm in diameter (Fig. 6). As a result, only separate parts of area 17 were injected with HRP. This accordingly produced separate patches of HRP label in several
subcortical visual structures including the dorsal lateral geniculate nucleus (LGNd), the lateral posterior nucleus (LP), the superior colliculus (SC), and the ventral lateral geniculate nucleus (LGNv). In addition, the label was also found in area 18 and V-TP of the ipsilateral side and in the area 17/18 border region of the contralateral side.

**HRP label in the LGNd.** The morphological features of the tammar’s LGNd have been previously described by several independent studies (Sanderson et al., ’84; Wye-Dvorak, ’84; Mayner, ’85). On the basis of cytoarchitectonics, it can be divided into two segments: a lateral $\alpha$ segment with closely packed, deeply stained cells parcellated in highly distinct layers, and a medial $\beta$ segment with moderately packed and stained cells (Fig. 3B). Corresponding to the separate injections of WGA-HRP in area 17, patches of label were found in both $\alpha$ and $\beta$ segments of the LGNd (Fig. 3).

At higher magnification, in each patch of HRP label in the LGNd, both labelled terminals, appearing as granules of the HRP reaction product in the neuropil, and labelled cells, appearing as clusters of HRP reaction product contained within the cytoplasm of neurons, were identified (Fig. 4A), indicating the reciprocity of the cortico-LGNd connection.

**HRP label in the LP-pulvinar Complex.** The LP in the tammar can also be divided into two subdivisions on the basis of cytoarchitectonics: a lateral subdivision (LPl) medial to the $\beta$ segment of the LGNd, and a medial subdivision (LPm) mediodorsal to the LPl at the dorsal surface of the thalamus (Fig. 3B). Mayner (’85) argued for the presence of the pulvinar nucleus in this species, which is situated posteriorly to the LP and dorsomedially to the posterior part of the LGNd. In all other marsupials studied so far, this region is considered as the posterior part of the LP. Label produced by triple HRP injections in area 17 were found in all three subdivisions, although it is not clear whether this meant area 17 has separate connections with all these three subdivisions or different loci of area 17 are connected with different loci of the LP according to a retinotopic order. Label normally appeared
as patches in the LP-pulvinar, containing both anterogradely labelled axons and retrogradely labelled cells (Fig. 3A & 4B,C). Label in the LPM was sparse (not shown).

**HRP label in the SC.** The cellular construction of the SC in tammar follows the fundamental scheme for the anatomical organization of the mammalian SC. In thionin-stained preparation, distinct cellular laminae alternating between fiber-rich and cell-rich zones are recognized in the superficial part of the SC (Fig. 5B). Following the nomenclature used in all other mammalian species, the most superficial layer is the stratum zonale (SZ) with very low cell density. Beneath is the cell rich layer called the stratum griseum superficiale (SGS) and is composed of cells varying in size from small to large. Underneath the SGS is the stratum opticum (SO) which is again very low in cell density (Fig. 5B). Following the HRP injection in area 17, only anterograde labelling was found in the SC ipsilateral to the injected cortex. Corresponding to the defined multiple HRP injections in area 17, patches of anterograde HRP labelling were found in the superficial part of the SC. The patches of HRP labelling were rather sharply-edged, suggesting the topographic order in the corticocollicular projection in the SC (Fig. 5A). At high magnification, it was clear that the label was most densely distributed in the most superficial SZ and beneath in the SGS. In underlying SO, the label was rather faint (Fig. 5A). There was little sign of label in the collicular laminae beneath the SO.

**HRP label in the LGNv.** The ventral lateral geniculate nucleus (LGNv) is located medial to the optic tract and ventral to the LGNd. This nucleus is palely stained and low in cell density compared to the LGNd (Fig. 3B). In one of the cortical HRP injection cases, a small patch of very faint label was found in the dorsolateral corner of the nucleus. At high magnification, it appeared that only anterograde label was present in the LGNv.

**HRP label in the ipsilateral area 18 and V-TP.** After injections of WGA-HRP in area 17 patches of label containing both anterogradely labelled axons and retrogradely labelled cells were found in area 18 and V-TP respectively (Fig. 6). In
both area 18 and the V-TP, the anterogradely labelled axons largely terminated in layer IV and the lower part of layer III, whereas the retrogradely labelled cells were mainly distributed in layers III, IV, and V, with scattered cells in layer VI (Fig. 6). In both layers III and V, most of the corticocortical cells appeared to be large pyramidal cells, and in layer IV were the granule cells (Fig. 6).

**HRP label in the contralateral visual cortex.** Only in one case was label found in the contralateral visual cortex. This was when one of the HRP injections was very close to the area 17/18 border. The label in the contralateral cortex was situated in a similar mediolateral and caudorostral position near the area 17/18 border, symmetrical to the injection site. The retrogradely labelled cells were large pyramidal cells in layers III and V (Fig. 7). The anterograde labelling was very faint even with dark field optical microscopy.

In conclusion, the present results show that cortical area 17 in tammar is reciprocally connected with the ipsilateral LGNd, LP, cortical areas 18 and V-TP, and with the contralateral area 17/18 border region. In addition, area 17 sends axons to the SC.

**Results of Thalamic Injections of WGA-HRP**

Data were collected from six adult males. Initially, the aim of this experimental series was to place an HRP deposit in the LGNd then examine the pattern of the distribution of the geniculocortical axons and the corticogeniculate cells in cortical areas and in cortical layers. However, due to certain individual variations in the size of the brain, the locations of the HRP injections in the visual thalamus were not consistent in all cases. In addition, in one case the HRP deposit was deliberately put into the LP but may also have involved the β segment of the LGNd. As a result, there were basically two types of thalamic injection cases. In the first type, injections of the WGA-HRP exclusively involved the LGNd. In the second type, the focus of
the injection was located more medially and, to varying degrees, involved both the LGNd and LP. It seems likely judging from the relatively small regions of cortical label resulting from a relatively large injection site in the thalamus after reaction with TMB that the area of HRP uptake resulting in transport to the cortex is from a much smaller region than that shown by the TMB reaction product. It is possible that uptake occurs primarily only from regions covered with the most intense reaction product. In cases in which only one side of the thalamus was injected label was found only in the cortex ipsilateral to the injection site. Results yielded from injections of WGA-HRP in the LGNd and LP are described in following sections and summarized in table I.

**Cortical label after HRP injection in the LGNd.** There are two such cases with differing degrees of involvement of the $\beta$ segment of the LGNd in the injection site. While in both cases the $\alpha$ segment was densely covered with WGA-HRP (Figs. 8 & 10), in case animal 88-WA1, a large part of the $\beta$ segment was densely covered with HRP end product at the injection site (Fig. 10), whereas in case 88-WA5 the dense part of the injection site did not cover the $\beta$ segment and so it was less likely to be involved in HRP uptake (Fig. 8). As a result, some significant differences in the cortical HRP label between these two cases was observed.

In animal 88-WA5, with HRP uptake mainly occurring in the $\alpha$ segment and only limited if at all in the $\beta$ segment, the cortical label was almost entirely contained in the rostral part of area 17, about in the middle of its lateromedial extent (Fig. 8). Only very faint label was found in area 18 in a few sections. In area 17 anterogradely labelled axons were largely concentrated in layer IV and, less substantially, in layer VI. In dark field, anterograde labelling with very low concentration was also seen in layer I and the upper part of layer V. It was also clear that there was limited label in layer IIIb and some very faint label was also present in IIIc. The latter may have represented fibres of passage passing to more superficial layers (Fig. 9). Retrogradely labelled cells were exclusively located in layer VI in area 17 (Fig. 8 & 9). In area 18,
the anterogradely labelled axons were only present at detectable level in layer IV and the retrogradely labelled cells in layer VI.

In animal 88-WA1, on the other hand, with substantial HRP uptake in the β segment, as well as in the α segment of the LGNd, the label was more widely distributed in the cortex. Apart from the heavy presence in area 17, significant amounts of label were also seen in area 18, V-TP and in the medial cortex at this level (Fig. 10). This is in sharp contrast to 88-WA5 in which the injection was heavily concentrated in the α segment. Moreover, in area 17, apart from the very densely aggregated anterograde label in the layer IV and the moderately concentrated anterograde label in the layer VI, the anterograde label in layer I, sublayer IIIb appeared more substantial than that in case 88-WA5 (Fig. 8). However, label in layer I was still relatively sparse. In addition, sparse label was also scattered in the rest of the whole thickness of the cortex. The retrograde label, similar to that in case 88-WA5, was only found in the layer VI (Fig. 10). In comparison with that produced in case 88-WA5, both anterograde label in layer IV and retrograde label in layer VI in area 18 produced in this case appeared more substantial (Fig. 10). In V-TP, two separate patches of label were found ventrally (Fig. 10). Each of these contained both anterogradely labelled terminals in layer IV and retrogradely labelled cells in layer VI, though sometimes only either anterograde or retrograde labelling was present in one section.

In medial cortex, several patches of anterograde HRP label in layer IV and retrograde labelling in layer VI were found in some sections taken from the caudal pole of the occipital cortex. At the most caudal part of the occipital cortex, the more dorsally situated patch of labelled cells merged with the patch of labelled cells in layer VI of area 17 at the dorsolateral aspect of the occipital cortex, suggesting that label in area 17 may extend from the lateral surface to the medial surface of the occipital cortex by wrapping around the occipital pole. Similarly, from Figure 10, there is also
some suggestion that the patches of label in the more ventral part of the medial cortex may be the continuation of the label in area 18 and the V-TP.

Results from these two cases shows that the LGNd has direct connections with areas 17, 18 and the V-TP. Comparison of results between two cases suggests that 1). The $\alpha$ segment may be connected exclusively with area 17, whereas the $\beta$ segment is also connected with area 18 and V-TP; 2). In area 17, the results suggested that axons from the $\beta$ segment may terminate more densely in layer I and IIIb rather than layer IV. Injection including the $\beta$ but not the $\alpha$ segment confirmed this (see following section).

**Cortical label after HRP injection in the $\beta$ segment of the LGNd and lateral part of the LP.** There were four cases in this type of HRP injection in which the tip of the injecting needle was localized in the lateral margin or the lateral part of the LP (Fig. 11) and the HRP uptake involved both the $\beta$ segment of the LGNd and the lateral part of the LP.

In comparison with cases in which only the LGNd was injected with HRP, the cortical label produced in this case was remarkably different in the pattern of the laminar distribution of the anterograde and retrograde labelling in areas 17, 18 and V-TP.

In area 17, the anterograde HRP labelling was present in layer I, sublayer IIIb, and the upper part of the layer V (Fig. 11, 12, 13A & B), whereas the dense anterograde label seen in layer IV in LGNd HRP injection cases was not present. It was noticeable that the label in layers I and V in this case was greatly increased compared to the previous case in which only the LGNd was involved in the HRP uptake. In retrograde labelling, a band of very large pyramidal cells containing HRP product were found in the upper part of layer V, as well as numerous labelled cells of medium size and pyramidal shape in layer VI (Fig. 11).

Probably more remarkable was that in area 18 and particularly in the dorsomedial part of area 18, close to the border region with area 17, a tremendous
enhancement of the anterograde and retrograde label was present. The anterograde label in this region was substantial in all layers, but was particularly dense in layer I and IV (Fig. 11, 12, 13C & D). The retrograde label was also intensified very significantly, in particular in layer VI where there was a sharp increase in the number of labelled cells compared to neighbouring area 17 and V-TP (Fig. 12 & 13C & D).

The pattern of label in V-TP in this case was also different from that in LGNd injection cases. For example, apart from the anterograde label in layer IV and retrograde label in layer VI, retrogradely labelled large pyramidal cells in layer V, not seen in LGNd HRP injection cases, were found after this type of HRP injection. However, in layer V, retrogradely labelled cells was much less dense than those in layer VI (Fig. 11).

In medial cortex, retrogradely labelled cells were also found in layer V, as well as in layer VI. In one case with an injection covering most of the LGNd and LP, in sections taken from the caudal pole of the occipital cortex, the retrogradely labeled cells in layer V, along with those in layer VI, formed a continuous band extending all the way round from the lateral wall to the medial wall of the occipital cortex. It was noticeable that the density of labelled cells in layer V was lower than in layer VI. In another case with an injection site similar to 88-WA4 cells in layer V were less obvious in this region of the cortex but anterograde label was prominent in layer IV with retrogradely labelled cells in VI in two patches corresponding to the medial extensions of area 18 and V-TP (Fig. 14).

Another thing that was noticeable was that the cortical HRP label produced in the thalamic HRP injection cases involving LP seemed to extend more rostrally, in particular in the temporal region, than that produced in injection cases only involving the LGNd. For example, while the HRP label produced in LGNd injection cases was only seen in the relatively caudal part of the V-TP area (Fig. 10), the HRP label produced in the HRP injection case involving LP was also distributed in the more rostral part of V-TP (Fig. 11). Moreover, the region rostral to the temporal sulcus, the
previously assumed auditory cortical area (Mayner, '85), was also labelled. With the noticeable absence of the anterograde label, the label in these more rostrally situated areas appeared as a continuous band of retrogradely labelled cells in layer VI (Fig. 11).

In summary, in comparison with results from LGNd HRP injection cases, results of this case clearly show that cortico-LP connection are largely concentrated in area 18 where the axons from the LP terminate most densely in layers I and IV, and the cortical cells projecting to the LP are concentrated in layer V, and possibly in layer VI as well. Besides, it is clear that LP axons also terminate in layer I in area 17 and the V-TP, while cortical cells in layer V in area 17 and the V-TP project to the LP. Moreover, the present results suggest that the LP may also receive projections from layer VI cells in cortical areas situated in more rostral parts of the temporal and medial cortex.

Conclusions drawn from thalamic injections of WGA-HRP are summarized in Table II.

DISCUSSION

In the present experiment, WGA-HRP was injected either in the visual cortex, area 17, or in the dorsolateral thalamus in the tammar wallaby to investigate the afferent and efferent connections between visual cortex and the main subcortical visual structures, and the contralateral visual cortex. The anatomical organization of these connections at both cortical and thalamic levels are compared with those in other marsupials and in representative placentals.
The Laminar Organization of the Visual Cortex

There are two characteristic features in the laminar organization of area 17 in the tammar worth mentioning. First, layer II is remarkably distinct from underlying layer III cytoarchitectonically. In representative placental mammals, such a contrast in cytoarchitectonics is not present and the layer II/III border remains obscure (rat: Krieg, '46; grey squirrel: Kaas et al., '72b; cat: Lund et al., '79; macaque monkey: Lund, '88). Noticeably, this thin and cytoarchitectonically prominent layer II is also seen in possum (Haight et al., '80), although it is less obvious in American opossum (Benevento and Ebner, '71a,b).

Second, there is a zone of low cell density, termed layer IIIc in the present study, overlying the layer IV granule cell zone in area 17 but not 18. In primates, the zone in the equivalent position to this is the stria of Gennari and is a particularly prominent feature of the primate striate cortex. Its characteristic appearance is due to the presence of bundles of myelinated afferent axons. The presence of this zone of low cell densities was also previously noticed in possum (Haight et al., '80), but was not obvious in the American opossum (Benevento and Ebner, '71a,b). Whether in the wallaby, as in the primate, it contains bundles of myelinated afferents is not known. A myelin stain of the cortex would answer this.

From the previous description, in tammar, the border between layer III and layer IV was not clearly decided, as there was still certain hesitation as to which of layers III and IV the zone of low cell density should belong (Mayner, 85, '89b). In possum, with rather similar cytoarchitectonic features, this zone is included in layer III and layer IV only includes the granule cell zone (Haight, '80). In primates, this zone, the stria of Gennari, can be either included in layer III or in layer IV, depending on which layering scheme is adopted. For example, in owl monkey (Kaas et al., '76), in following Hassler and Wagner's layering scheme ('65), the stria of Gennari is included in layer III and is designated as sublayer IIIc, with the overlying cellular parcellation.
further divided into sublayers IIIA (without geniculate afferent termination) and IIIB (with geniculate afferent termination). Thus layer IV is composed only of the granule cell zone. In macaque monkey, on the other hand, the stria of Gennari is included in layer IV if following the more popularly used Brodmann’s layering scheme and is designated as sublayer IVb. Hassler and Wagner’s sublayer IIIb, with LGNd afferent termination, is thus designated as sublayer IVa, and the granule cell zone as sublayer IVc (Brodmann, '05; Polyak, '57; Lund, '73; Wiesel et al., '74; Hendrickson et al., '78). There are advantages and disadvantages for both layering schemes. While Hassler and Wagner’s appears to be more clearly related to the cortical cytoarchitectonics, Brodmann’s scheme allows for the major geniculate termination zone to be in layer IV as in non-primates. In tammar, with some apparent primate-like features, as indicated by the presence of a clearly defined zone of low cell density above the granule cell layer and of the similarity of the laminar distribution of the LGNd afferent termination in area 17 to that of the primate (see discussion below), it appears that either layering scheme can be adopted. To keep with the layering scheme used in other marsupials (Haight et al., '80), it was decided in the present study that in the tammar the zone of low cell density in area 17 is included in layer III. Thus, on the basis of slightly modified cytoarchitectonic description by Mayner ('85, '89a), with some information on the laminar pattern of the LGNd afferent termination in area 17 in hand, the tammar’s layers III and IV is described according to Hassler and Wagner’s layering scheme in that layer III is further divided into three sublayers: sublayer IIIa just underlying distinctive layer II; sublayer IIIb, identified by the presence of LGNd afferent termination; and the sublayer IIIc, the zone of low cell density overlying the granule cell zone. Layer IV in the tammar, therefore, only includes the granule cell zone.
Identification of Separate Visual Areas

On the basis of cytoarchitectonics, the cellular organization and the extent of areas 17 and 18 were identified previously in the tammar (Mayner, '85). The current results demonstrating reciprocal connections of these two areas with the LGNd and LP are supportive of the identification of areas 17 and 18 by the criterion of the cytoarchitecture. In addition, two other visual cortical areas have been identified. V-TP has direct reciprocal connections with the LGNd and the area in the more rostral part of the temporal region projects to the LP.

Although it was previously speculated that, given the cytoarchitectonic similarity to area 18, V-TP may be related to visual function in the tammar (Mayner, '85), the present discovery of direct reciprocal connections with the LGNd confirms that this region is a visual area.

In cat, there are several visual areas in the temporal cortex in a comparable position to that of tammar’s V-TP, including areas 20a, 21a, 21b (Rosenquist, '85, modified from Tusa et al., '81), and most of them are connected with area 17 (Symonds and Rosenquist, '84a). In owl monkey, this region contains three electrophysiologically identified associate areas including the visual area II (V2), the dorsolateral (DL), and middle temporal areas, and a few other visually responsive, but disorderly topographically organized areas (Allman, '81). In macaque monkey, this region seems to be mainly occupied by the lateral extention of areas V1 and V2, caudal to other identified visual areas situated more rostrally (Van Essen, '85). In other marsupial species, there is indirect evidence suggesting that the V-TP region may be a candidate for a visual area. For example, in Australian possum, the region with a similar location to V-TP in tammar, referred to as medial temporal (MT) and lateral temporal (LT) cortices, receives a projection from area 17 (Crewther et al., '84). In American opossum, it was electrophysiologically proved that this region is not related to auditory function (Lende, '63), and there is evidence that some areas in this region
are connected with the striate cortex (Benevento and Ebner, '71a), raising the possibility that this region might be visually related. In conclusion, it is required in marsupial species that an electrophysiological mapping study to be done to see whether there is any orderly retinotopic representation in this region.

It was also shown that areas on the medial edge of the cortex at the occipital pole have direct and reciprocal connections with the LGNd. It is most likely that these are the continuation of 17, 18 and V-TP wrapping around the caudal pole. In cat, in a similar region lies the medial extension of areas 17 and 18, and some other extrastriate visual areas (Tusa and Palmer, '80). In owl monkey, the similar location is also largely occupied by the medial extension of areas V1 and V2 (Allman, '81). In macaque monkey, the region also contains the medial extension of V1 and V2, and other visual areas (summarized by Van Essen, '85). It is currently unknown in other marsupial species whether this region is visually related, although Crewther et al. ('84) suggest that it may be in the possum as it sends a projection to the SC.

In addition, substantial numbers of cortico-LP cells are present in more rostral parts of the temporal cortex which in a previous study, was assumed to be the part of the auditory cortex (Mayner, '85). There is no evidence that LP-pulvinar is connected with the auditory cortex in both placental and marsupial mammals. Moreover, in cat, owl monkey and macaque monkey, it seems that the region in a similar rostrocaudal position is occupied by some identified visual areas (Rosenquist, '85, modified from Tusa et al., '81; Allman, '81; Van Essen, '85). In this context, the region previously thought as part of the auditory cortex, outlined by the temporal sulcus (Mayner, '85), is perhaps visual. Again, a retinotopic mapping study is necessary to confirm this.

In the absence of retinotopic mapping studies, the visual cortex in marsupials is often described as consisting of three cytoarchitectonically distinct regions including the striate cortex (area 17), the peristriate cortex (area 18), a narrow strip surrounding the striate cortex, and the posterior parietal cortex (possum: Heath and Jones, '71;
Haight et al., '80; opossum: Diamond and Utley, 63; Benevento and Ebner, '71a,b).
In the tammar the visual cortex is described anatomically as comprising areas 17, 18 and a ventral temporal area. However, given the present results on visual thalamocortical and intrahemispheric connections, and results on the intrahemispheric connections in opossum (Benevento and Ebner, '71a) and in possum (Crewther et al., '84), it is apparent that there may exist more visual areas with a wider extension in the cortex than previously thought.

Visual Cortical Connections with the LGNd

As shown by the presence of both anterogradely labelled terminals and retrogradely labelled cells in the LGNd following injections of WGA-HRP defined in area 17, these two structures are reciprocally connected. This is also demonstrated at the cortical level by the presence of the anterogradely labelled axons and retrogradely labelled cells in area 17 after the WGA-HRP injection in the LGNd. Such reciprocity has been found in all mammalian species.

It was shown in the present study that, apart from area 17, there are several other cortical areas situated in the ventral temporal and medial cortex which are reciprocally connected with the LGNd. The extent of the connections with the LGNd at the cortical level was previously described in a number of representative mammalian species including some marsupials. In primates, it is generally believed that the afferent and efferent connections between LGNd and the visual cortex exclusively terminate and originate in area 17 (Wiesel et al., '74; Lund et al., '75; Kaas et al., '76; Ogren and Hendrickson, '76; Hubel et al., '77; Hendrickson et al., '78; Graham, '82), although sparse reciprocal projections between the LGNd and area 18 were also reported (Wong-Riley, '76; Lin and Kaas, '77; Benevento and Yoshida, '81; Yukie and Iwai, '81). In contrast, in the cat and the mink, the LGNd as a whole seems widely connected with a number of visual and nonvisual cortical areas, as well as with
area 17 (Maciewize, '75; LeVay and Gilbert, '76; Hollander and Vanegas, '77; Raczkowski and Rosenquist, '80, '83; McConnell and LeVay, '86). In rat, the geniculocortical projections terminate heavily in area 17, and less significantly in areas 18 and 18a (Ribak and Peter, '75; Hughes, '77; Coleman and Clerici, '80; Perry, '80; Sefton et al., '81), while the corticogeniculate projections originate from areas 17, 18 and 18a (Sefton et al., '81). In marsupials, each of the three cytoarchitectonically distinct visual areas are connected with the LGNd in possum (Haight et al., '80), while in the opossum only striate cortex (area 17) seems to be connected with the LGNd (Coleman et al., '77). It appears that, with the widespread distribution of the afferent and efferent corticogeniculate connections beyond area 17 in the visual cortex, the areal extent of the LGNd-cortical connections in the visual cortex in tammar does not resemble that of primates, but that of non-primate placentals and other marsupials.

The respective contributions of the $\alpha$ and $\beta$ segments of the LGNd to the overall cortical extent of the descending cortico-LGNd connections in tammar may be deduced through the comparison of the results between selected cases of HRP injections. As mentioned earlier, when both $\alpha$ and $\beta$ segments are included in the uptake of HRP, apart from area 17, more cortical areas including area 18 and those in the V-TP are also labelled, whereas when the HRP uptake in the $\beta$ segment is much reduced or absent, the only HRP label found outside area 17 is in area 18 at a barely detectable level. Thus, it appears that in tammar, the $\alpha$ segment tends to connect primarily with area 17, whereas the $\beta$ segment tends to connect with a wider range of cortical areas, as well as area 17. A similar situation was also found in cat in which laminae A and A1, the equivalent of the $\alpha$ segment of the LGNd in tammar, are reciprocally connected with area 17 and 18 only, and the C laminar with area 17, 18 and 19 only, whereas the laminae C1-3 have connections with a wider range of cortical areas including not only areas 17, 18 and 19, but also several visual and nonvisual cortical areas (Maciewize, '75; LeVay and Gilbert, '76; Hollander and Vanegas, '77; Raczkowski and Rosenquist, '80, '83). A similar analysis on the
separate distribution from the individual segments of the LGNd in other marsupials is not available.

Similarly, the individual contribution of the α and β segments of the LGNd to the overall pattern of the laminar distribution of the LGNd afferents in the area 17 may be deduced. It is worth recalling that, with the apparent involvement of the β segment in HRP uptake, the anterogradely labelled LGNd axons in layer I and particularly sublayer IIIb in area 17 was substantial, whereas when the involvement of the β segment in HRP uptake was reduced, the anterograde label in layer I and the sublayer IIIb was accordingly reduced. Further, when the α segment, but not the β segment, was excluded from HRP uptake, the intensive anterograde label in layer IV was not present. Thus, it appears that in tammar, the axons from the α segment of the LGNd tend to terminate in layer IV, while axons from the β segment are concentrated in layer I and sublayer IIIb. In primates, the LGNd afferents terminate in four zones including layer I, sublayer IVa (or sublayer IIIb using the alternate layering scheme), and sublayers IVc, α and IVc,β. The afferents from LGNd parvocellular laminae (equivalent to the wallaby β segment) terminate moderately in layer I, sublayer IVa (or IIIb in lower primates) and predominantly in cortical sublayer IVc,β, whereas the afferents from LGNd magnocellular laminae (equivalent to the wallaby α segment) terminate predominantly in sublayer IVc,α (Hubel and Wiesel, '72; Wiesel et al., '74; Glendenning et al., '76; Kaas et al., '76; Hubel et al., '77; Hendrickson et al., '78; Carey et al., '79; Conley et al., '84)); In cat, the LGNd afferents terminate in layer I, the low part of layer III, layer IV, the upper part of layer V, and layer VI. Noticeably, in the absence of a well defined zone of low cell density above the granule cell of layer IV, the LGNd afferent termination in layer III, IV and V is continuous. Axons from laminae A and A1 terminate in sublayers IVa and IVb, and layer VI, while those from laminae C and C1-3 terminate in layer I, the low part of layer III and the upper part of layer V (LeVay and Gilbert, '76; Raczkowski and Rosenquist, '80; Conley, '88). In rat, the principal geniculocortical axons terminate in layer IV and the adjacent part of
layer III, and less significantly in layers I and VI (Ribak and Peter, '75; Hughes, '77; Coleman and Clerici, '80; Perry, '80; Sefton et al., '81). In opossum, an early degeneration study showed that the LGNd termination was largely concentrated in layer IV, with some expansion into the adjacent part of layer III, and layers I and VI as well (Benevento and Ebner, '71b). From this comparison, it appears that the overall laminar distribution of the LGNd afferent termination in area 17 tammar resembles that seen in primates, in that the LGNd afferent termination in layer IIIb of the wallaby and lower primates and layer IVa of higher primates is separated from the dense LGNd axon termination over the granule cell zone in both tammar and primates. In laminar distribution of axons from individual segments of the LGNd, the tammar seems to resemble the cat in the sense that axons from the more laterally situated and more laminated segment of the LGNd tend to terminate in the granule cell zone and layer VI, whereas axons from the more medially situated and less laminated segment tend to terminate in layer I and supra- and infragranular sublayers.

In other cortical areas in the wallaby, the geniculate axons appeared to terminate largely in layer IV and VI, while cortical cells projecting to the LGNd were situated in layer VI only. This is consistent with data in other mammalian species studied (Gilbert and Kelly, '75; LeVay and Gilbert, '76; Wong-Riley, '76; Hughes, '77; Lin and Kaas, '77; Sefton et al., '81).

Visual Cortical Connections with the LP

The Subdivisions of the LP-pulvinar in Tammar Wallaby. In a description of the thalamus of the tammar, Mayner subdivided the LP in this species into a lateral posterior subdivision and the pulvinar on the basis of cytoarchitectonics ('85). The pulvinar nucleus was not described in other marsupials. Instead, what Mayner called pulvinar was included in the LP in other marsupials. (opossum: Royce et al., '76; possum: Haight and Neylon, '78). The argument of the presence of the pulvinar
nucleus in tammar was based on the cytoarchitectonic distinction between this structure and the LP, and on the similarity of this structure in cytoarchitectonics to the cat’s pulvinar nucleus (Mayner, '85, '89b). However, in cat, only the lateral LP, but not the pulvinar nucleus, is connected with areas 17 and 18 (Raczkowski and Rosenquist, '83). The existence of the connection between the cytoarchitectonically identified "pulvinar nucleus" and cortical area 17, therefore, does not seem to support the identification of the pulvinar nucleus in this species by the criterion of the cytoarchitectonics. The reciprocal connections between area 17 and both the medial and lateral divisions of LP have also been described in the possum (Haight et al., '80) with striate cortex (area 17) more strongly connected with LPl than LPm. Labelling in LPm after area 17 injections was also sparser in the wallaby compared with labelling in LPl.

In area 17 of the tammar axons from LP mainly terminate in layer I, while the cortico-LP projections originate from those large pyramidal cells in the upper part of layer V. It was previously reported that in both higher and lower primates, the LP afferents principally terminate in layer I and less substantially in layer II and III in area 17 (Benevento and Rezak, '75; Glendenning et al., '75; Ogren and Hendrickson, '76, '77; Carey et al., '79). In cat, the LP axons terminate mainly in layer I, and less substantially in lower layer III and upper layer V in area 17 (Rosenquist et al., '74; Symonds et al., '81; Updyke, '81; Berson and Graybiel, '83; Raczkowski and Rosenquist, '83). In rat, the LP axons in area 17 also largely terminate in layer I (Hughes, '77; Herkenham, '80; Mason and Groos, '81). In all the representative mammals, the cortical axons from area 17 to the LP-pulvinar complex originate from layer V large pyramidal cells (monkey: Lund et al., '75; Ogren and Hendrickson, '77; Trojanowski and Jacobson, '77; cat: Kawamura et al., '74; rat: Mason and Groos, '81). Thus, the characteristic laminar specificities expressed by LP(pulvinar)-cortical axons and cortico-LP(pulvinar) cells in the striate cortex of representative placental mammals are also expressed in tammar.
There was a remarkable contribution of LP to the great concentration of thalamocortical connections within area 18 as evidenced by the robust anterograde label in layer I, the retrograde label in layer V, and the sharp increase of label in layer IV and VI which was only seen when the LP was involved in the HRP injection. It is thus clear that in area 18, axons from the LP are densely aggregated in layers I and IV, moderately concentrated in layers III and V. The cortical cells projecting to the LP are situated in layers V and layer VI.

This particular concentration of LP-cortical connections surrounding area 17, or a suggestion of it, is also observed in representative mammals. In monkey, it is obvious that LP-pulvinar axons and corticopulvinar cells are much more concentrated in extrastriate cortical areas surrounding the striate cortex (Trojanowski and Jacobson, '76; Ogren and Hendrickson, '77; Curcio and Harting, '78; Rezak and Benevento, '79). This is also evident in cat (Symonds et al., '81) and in rat (Hughes, '77; Herkenham, '80; Mason and Groos, '81).

Also, from the literature, it appears that the laminar distribution of the LP(pulvinar)-cortical axons and the cortico-LP(pulvinar) cells in extrastriate cortical areas is remarkably different from that in striate cortex in that axons from the LP-pulvinar complex are largely concentrated in layer IV, as well as in layer I. For example, in monkey, termination of pulvinar axons in layer IV and the adjacent part of layer III in extrastriate cortical areas have been reported in several studies (Benevento and Rezak, '75; Ogren and Hendrickson, '76, '77); In lower primate species, pulvinar axons also terminate in layer IV of the extrastriate cortical areas (Glendenning et al., '75; Carey et al., '79). The LP-cortical axons terminating in the extrastriate cortical areas are also aggregated in layer IV in rat (Hughes, '77; Herkenham, '80; Mason and Groos, '81). In cat's area 19, the heaviest LP axonal termination is also found in layer IV, although in other visual cortical areas LP axons heavily terminate in layer I (Rosenquist et al., '74; Hughes, '80; Miller et al., '80; Marcotte and Updyke, '81; Niimi et al., '81; Symonds et al., '81; Updyke, '81; Berson and Graybiel, '83;
Raczkowski and Rosenquist, '83). It is intriguing, however, that the laminar location of the cortical cells projecting to the LP-pulvinar in extrastriate cortical areas is not consistent among different mammalian species. In monkey (Trojanowski and Jacobson, '77) and cat (Kawamura et al., '74; Updyke, '81) it is the layer VI cells in extrastriate cortical areas that project to the LP-pulvinar complex, whereas in rat it is the layer V cells that send axons from extrastriate cortical areas to the LP-pulvinar complex (Mason and Groos, '81). In tammar, when the LP is involved in HRP uptake in more caudal parts of the V-TP and medial cortex both layer V and VI cells are labelled, while only layer VI cells are labelled in more rostral parts of the temporal and medial cortex. In conclusion, the laminar distribution of the cortico-LP cells in extrastriate cortical areas resembles neither the primates or cat and rat.

Finally, from the present results, it appears that, in tammar, while the cortico-LGNd connections tend to be concentrated in area 17 and the vicinity, the LP-cortical connection tend to be concentrated in 18 and more rostrally. This was also noticed in other animals (see Jones, '85 for review). In more rostral parts of the temporal region, the cortico-LP connections seemed to originate from layer VI only.

The Cortical Projection to the LGNv.

Present results shows that in the tammar, only a very weak cortical projection exists between area 17 and the LGNv. It is established in a number of placental mammals that the LGNv, or its less overt form in the primates, the pregeniculate nucleus, receives corticofugal fibers from area 17 and some other visual cortical areas. In monkey, the cortical projection to the pregeniculate nucleus comes from area 17 and certain extrastriate areas, and terminates in the external, non-retinal-recipient part of the nucleus (Ogren and Hendrickson, '76). In cat, the cortical projection to the LGNv seems to originate from all parts of areas 18 and 19, but only the posterior part of area 17, and terminates in the lateral part of the nucleus (Updyke, '77). In rat
(Nauta and Bucher, ’54), tree shrew (Abplanalp, ’70), and rabbit (Giolli et al., ’78; Hollander et al., ’79), the cortical projection arises from area 17 and the adjacent peristriate belt. In possum, the cortical projection to the LGNv was also seen after injection of autoradiographic tracer in striate and posterior parietal cortical areas (Haight et al., ’80).

The Cortical Projection to the SC

The fact that only anterograde labelling is present in the SC following a cortical HRP injection indicates that, as in other mammalian species, the tammar’s corticocollicular connection is composed of a corticofugal projection only. The HRP labelling in the SC was defined and sharply-edged with respect to a defined cortical HRP injection, suggesting that it is organized according to a topographic order. Unfortunately, the data available was not sufficient to describe the details of such topographic order. In placental mammals, the corticocollicular projection is topographically organized and in register with the retinocollicular projection in the SC.

Results from the cortical WGA-HRP injections defined in area 17 indicate that in adult tammar, area 17 is an important source of the corticocollicular projection. However, whether other cortical areas also supply axons to the superficial layers in the SC remains to be tested. In the literature, it is claimed that the cortical cells projecting to the superficial part of the SC are mainly from area 17, but also from areas 18 and 19, plus some other extrastriate cortical areas in both monkey (Graham, ’82; Fries and Distel, ’83; Fries, ’84) and cat (Hollander, ’74; Updyke, ’77; Segal et al., ’83; Segal and Beckstead, ’84). In rat, it is demonstrated that the corticocollicular cells are present in areas 17, 18 and 18a (Sefton et al., ’81; Olavarria and Van Sluyters, ’82). In another Australian marsupial, the possum, the corticocollicular projection originated from both striate and peristriate areas (Haight et al., ’80).
The cortical afferents mainly terminated in the most superficial laminae, SZ, and the underlying SGS. In the SO, the concentration of the corticocollicular axons was largely reduced. This pattern of the corticocollicular axonal termination is remarkably similar to those previously described in monkey (Graham, '82), cat (Segal et al., '84), rat (Olavarria and Van Sluyters, '82), and possum (Haight et al., '80). In all mammals studied, the corticocollicular cells are the large pyramidal cells in layer V (monkey: Fries and Distel, '83; cat: Hollender, '74; Updyke, '77; Segal and Beckstead, '84; rat: Sefton et al., '81; Olavarria and Van Sluyters, '82; possum: Haight et al., '80), except that Fries ('84) recently argued that in monkey’s striate cortex, some layer VI cells, as well as those in layer V, also project to the SC. On the other hand, as previously described, the retinocollicular axons largely terminate in SGS and SO, but are absent in the SZ in this species (Wye-Dvorak, '84), and in virtually all other mammals (monkey: Hubel et al., '75; cat: Graybiel, '75; Harting and Guillery, '76; rat: Wise and Lund, '76; Land and Lund, '79; Australian marsupials: Pearson et al., '76; Sanderson and Pearson, '77; Sanderson et al., '79).

Intrahemispheric Connections of Area 17

The present results demonstrate that area 17 of the tammar sends projections to layers III and IV of area 18 and to some cortical areas in the V-TP. In return, the cells in layers III, IV, V, and very few in layer VI of these areas send axons back to area 17. The intrahemispheric connections between area 17 and other cortical areas have been extensively studied in placentals. In rat, area 17 is reciprocally connected with the areas 18 and 18a (Montero et al., '73; Olavarria and Montero, '81), and certain areas in parietal and temporal cortex as well (Miller and Vogt, '84). It is known that axons from area 17 largely terminate in layers II and III in areas 18 and 18a, and cells in the same layers of areas 18 and 18a project to area 17 (Olavarria and Montero, '81). In cat, area 17 has extensive connections with other visual areas including areas 18, 19,
20a, 21a, 21b, and the posterior lateral lateral syprasylvian (PLLS), the posterior medial lateral syprasylvian (PMLS), and the anterior medial lateral syprasylvian (AMLS) (Symonds and Rosenquist, '84a,b). In areas 18, 19, 21b and PLLS, cells projecting to area 17 are situated in either layers II/III and, in less numbers, in V/VI; in areas 21a and PMLS, cells projecting to area 17 are mostly from layers V and VI, with some also from layers II and III; in areas 20a and AMLS, the cells projecting to area 17 are situated in layers V and VI only (Symonds and Rosenquist, '84a,b). In tree shrew, it was shown by a WGA-HRP study that area 17 is reciprocally connected with area 18, the temporal dorsal (TD) and the temporal posterior (TP) areas, and the posterior limbic cortex areas. Projections from area 17 terminate in layers II-V in area 18, with the heaviest in layer IV, and in layers II-IV in TD. Cortical cells projecting to area 17 are mostly situated in layer III in all cortical areas, but are also seen in layer V and, to a lesser extent, in layer VI (Sesma et al., '84). In monkey, area 17 has reciprocal connections with at least four associate areas including the V2, V3, MT, and V4 (see summarized version by Van Essen, '85). While projections from area 17 mainly terminate in IV and lower III, cells projecting to area 17 are localized in layers II/III and V/VI (Rockland and Pandya, '79; Tigges et al., '81; Lin et al., '82). The intrahemispheric connections in marsupials are the subject of a limited number of studies. In opossum, it was reported that the striate cortex (area 17) sends projections to layers I-IV of some extrastriate visual areas in temporal cortex (Benevento and Ebner, '71a). In possum, the striate cortex was found reciprocally connected with some cortical areas in the posterior parietal, the medial temporal, and the lateral temporal cortex (Crewther et al., '84). In addition, there was a suggestion that the striate and peristriate cortex were also reciprocally connected although this was obscured by the spread of label at the injection site (Crewther et al., '84). It appears that the axons from the striate cortex largely terminate in layers III and IV in extrastriate areas, and that the cells projecting back to the striate cortex are situated mostly in layers II and III, but also in layers V and VI (Crewther et al., '84).
By comparison, the most striking feature in the intrahemispheric connections of the tammar is that, apart from the cells in layers III, V and VI, layer IV granule cells are also projecting to area 17. This has not been mentioned much previously in either placentals or marsupials, except in the cat that a small proportion of intrahemispheric cells projecting to area 17 are situated in layer IV (Segraves and Rosenquist, '82a).

Interhemispheric Connections of Area 17

The fact that only those HRP injections located near the cytoarchitecturally distinct area 17/18 border produced label at a similar symmetrical location in the opposite area 17 suggests that the distribution of the intercortical connection in area 17 is restricted to the lateral margin next to the area 17/18 border. That the interhemispheric connection in area 17 is restricted to a limited region adjacent to the area 17/18 border is well-documented in monkey (Wong-Riley, '74; Winfield et al., '75; Kaas and Lin, '77), tree shrew (Sesma et al., '84), cat (Innocenti, '80; Segraves and Rosenquist, '82a,b), rat (Cusick and Lund, '81; Miller and Vogt, '84), and in both possum (Heath and Jones, '71; Crewther et al., '84) and opossum (Benevento and Ebner, '71a).

The present results show that in tammar, the cortical cells projecting to the opposite area 17 are distributed in both cortical layers III and V, with a more extensive distribution in layer III. In monkey, the callosal cells in area 17 are large pyramidal cells in layer III (Wong-Riley, '74; Winfield et al., '75; Kaas and Lin, '77). In cat, the callosal cells in area 17 are located mainly in the deeper part of the layer III and, less significantly, in the upper part of layer VI (Segraves and Rosenquist, '82a,b). In rat, the callosal cells in area 17 originate from layer II, III and V (Rothblat and Hayes, '82).

It is established that marsupials do not possess the corpus callosum (Owen, '1837), the massive fiber bridge conducting the intercortical connections between two
visual cortices in placental mammals. Its function of interocular transfer is replaced by the fasciculus aberrans and anterior commissure (Nelson and Lende, '65; Putnam et al., '68; Heath and Jones, '71; Robinson, '82). In placental mammals, while the intercortical connections between the two visual cortices are conducted via the corpus callosum, the anterior commissure also acts as an alternative pathway for the intercortical transfer in primates (Sullivan and Hamilton, '73; Butler, '79).

Concluding Remarks

The present results show that, despite being on a separate evolutionary course for more than 100 million years (Tyndale-Biscoe, '73), the visual cortical connections in the tammar are organized in a remarkably similar way to those in placentals. All essential anatomical features characterizing the mammalian visual cortex and visual cortical connections are seen in this species. With the operational advantages of using the pouch young animals of this species in developmental studies, this similarity suggests the relevance of using this experimental model to address types of questions concerning the formation of visual cortical connections, which may be universal to all mammalian species.
Figure 1.

Upper: A low power view of a thionin stained cross section cut through the occipital pole of an adult tammar wallaby. Note that the cellular lamination in area 17 is considerably wider than that in area 18 and the ventral temporal area (V-TP). It is characterized by a particularly wide layer III and the presence of a cell sparse zone (layer IIIc) above the granule cell zone, layer IV. Also note that at this part of the cerebral cortex these three areas are separated from each other by distinct sulcal features. For detailed cytoarchitectonic features of these three areas see figure 2. Arrows indicate borders between cortical areas.

Lower: A drawing of the lateral view of the cortex of the tammar showing the possible extent of areas 17, 18 and V-TP and of the auditory cortex on the basis of cytoarchitectonics (Reproduced from a figure by Mayner, '85). The vertical line indicates the rostrocaudal position of the section shown above.

Bar: Upper = 2mm; Lower = 5mm.
**Figure 2.** The cytoarchitectonics of the different cortical areas at the occipital pole of the cerebral cortex in an adult tammar.

A. Area 17. The six layered laminar organization is clearly present. Layer II is easily distinguished from layer III by its prominent cell density and staining quality. Layer III is very wide and comprises different sublayers according to cytoarchitecture. Particularly distinct is the cell sparse zone in the lowest part of the layer. Underlying layer IV is the most prominent layer by its densely packed and deeply stained granule cells. Layer V is easy to recognize by its lower cell density and some very large pyramidal cells in it. In layer VI cell density becomes higher and many of the cells in the layer are medium-sized pyramidal cells.

B. Area 18. The six layered arrangement is still distinct. Also layer II is similarly prominent as compared to area 17. However, the whole thickness of the gray matter is largely reduced compared to that in area 17, noticeably due to the reduction of layers III and IV in thickness. Also note the presence of the cell sparse zone overlying layer IV is not obvious in this area.

C. Area V-TP. Cytoarchitectonically, the superficial layers in this area are rather similar to those in area 18. The deeper layers, on the other hand, have a remarkably different cellular arrangement from that seen in area 18 in that cells in both layers V and VI are lined up in parallel rows.

Bar: in A, B and C = 500µm.
Figure 3. Low power photomicrographs of cross sections through the thalamus in the adult tammar.

A. HRP label in the LGNd and LP after multiple injections of WGA-HRP in area 17. Two patches of label are localized in the LGNd (large arrow heads). The dorsal one has a distribution in both alpha and beta segments. Two patches of HRP label, one dorsally and one ventrally, are also identified in the LPl (small arrow heads), whereas in the LPlm only sparsely distributed labelled cells are found (not shown).

B. The cytoarchitecture of the thalamus in an adjacent section from a series stained with thionin but not reacted for HRP. The $\alpha$ and $\beta$ segment can be identified by their locations and their cell packing density. The subdivisions LPl and LPlm can also be identified though the border between them is not clear-cut. Below the LGNd is the LGNv. This is no longer present in the adjacent section in the series shown in A.

Bar = 1mm.
Figure 4. High power photomicrographs showing anterograde labelled terminals of corticothalamic axons and retrogradely labelled thalamocortical cells in the thalamic structures following injections of WGA-HRP in area 17.

A. The anterogradely labelled terminals of corticogeniculate axons (small arrow head) and retrogradely labelled geniculocortical cells (large arrow head) in the LGNd.

B. The labelled terminals of cortical axons and labelled LP cells projecting to the visual cortex in the ventral part of the LPL.

C. The labelled terminals of cortical axons and labelled LP cells in the dorsal part of the LPL.

Bar = 100µm.
**Figure 5.** Low power photomicrographs of cross sections through the SC in the adult.

A. Dark field view of HRP label in the SC after injection of WGA-HRP in area 17. A sharp-edged zone of label is shown in the lateral part of the SC (arrow head) and is largely confined in the collicular laminae SZ, SGS and, with less intensity, SO.

B. Bright field view of an adjacent thionin stained section showing the cytoarchitecture of the SC. The SZ is the most superficial layer. Beneath it is the SGS, a zone of high cell density, except for its most superficial region where cortical input is sparse. The SO is a fibre rich zone containing optic axons.

Bar = 1mm.
Figure 6. The intracortical connections between area 17 and other cortical areas in the tammar.

Upper: Camera lucida drawings showing one of three injections of WGA-HRP made in area 17 in this case and the resulting label in areas 18 and V-TP of the ipsilateral side. The cross hatching represents regions with a high concentration of injected WGA-HRP. Large dots represent retrogradely labelled cells, and small dots represent anterogradely labelled terminals. Some fine lines represent labelled fibres. Sections are numbered from the caudal occipital pole. Note in section 22, in each of areas 18 and V-TP, two separate patches of label are present. In both areas 18 and V-TP, axons from area 17 terminate in layers III/IV, whereas cells projecting back to area 17 are primarily located in layers III and V/VI. In addition, in both areas 18 and V-TP small numbers of cells in layer IV are also found projecting to area 17.

A. Photomicrograph of a dark field view of HRP label in area 18 showing the laminar distribution of intrahemispheric axons and cells. Anterogradely labelled axons from area 17 terminate in layers III/IV. Some labelled axons can be seen in the lowest part of layer VI. Retrogradely labelled cells projecting to area 17 are mainly distributed in layers III (arrow) and, less obviously in this section, in V/VI.

B. Cytoarchitectonics of the intrahemispherically labelled region shown in A.

C. A magnified view of the laminar distribution of intracortical axons and cells in area 18. Note labelled cells in layer IV (arrow head), as well as in III (arrow head) and V.

Bar: in A and B = 200µm; in C = 100µm.
**Figure 7.** The intercortical connection between area 17 of the two sides.

Upper: Camera lucida drawings of cross sections with sites of cortical injections of WGA-HRP in area 17 and retrogradely labelled cells in area 17 of the contralateral cerebral hemisphere. Cross hatching represents regions with a high concentration of injected WGA-HRP, whereas dots represent retrogradely labelled cells. Dashed lines indicate the cytoarchitectonically recognized border between areas 17 and 18. Sections are numbered from the caudal occipital pole. Two of three injections in this case are indicated at this plane of sectioning, one situated medially and one laterally very close to the border between areas 17 and 18. The third is situated more caudally and relatively medially (see figure 6). Labelled cells are confined in a patch across the area 17/18 border, appearing in a location symmetrical to the injection close to area 17/18 border.

A. A dark field view of HRP label showing the distribution of retrogradely labelled interhemispheric cells in area 17 adjacent to the border region between areas 17 and 18. Cells are primarily distributed in layer III.

B. A bright field view of an adjacent cross section stained with thionin showing the cytoarchitectonics of the labelled region in A.

Bar: in A and B = 200µm.
Figure 8. Camera lucida drawings of the HRP label in the cortex and the injection site in the thalamus in case 88-WA5. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections.

Upper: The distribution of HRP label in the cerebral cortex. The small dots represent the anterogradely labelled terminals and large dots represent retrogradely labelled cells. Label is confined in area 17. LGNd afferents largely terminate over layer IV and VI, and also have a faint presence in some sections in layer I and sublayer IIIb. Cortical cells projecting to the LGNd are situated in layer VI.

Lower: The distribution of the injected WGA-HRP in the thalamus. The area with dense HRP products is represented by cross hatching, and the area with less dense HRP products by single hatching. The thick lines medial and ventral to the LGNd represents the labelled axons in the optic radiation. The dotted line in the LGNd represents the border between the alpha and beta segment of the nucleus. The drawings show that the area with dense HRP products is in the alpha segment of the LGNd, suggesting HRP uptake mainly occurs in the alpha segment.
Figure 9.

A. The dark field view of the cortical label in area 17 for case 88-WA5 following a single injection of WGA-HRP primarily located in the alpha segment of the LGNd. Camera lucida drawings of the cortical label and injection site are shown in figure 8. The anterogradely labelled terminals of geniculocortical axons are highly concentrated over the layer IV granule cell zone extending with very low density above and below this layer. Terminals of LGNd afferents are also present in sublayer IIIb, layer VI and, with a less intensity, in layer I. Also shown are the retrogradely labelled cells in the layer VI.

B. Bright field view of an adjacent thionin stained section showing the cytoarchitectonics of HRP labelled region in area 17.

Bar = 500µm.
88-WAI

Dorsal
Rostral

1 mm

2 mm
**Figure 10.** Camera lucida drawings of HRP label in the cerebral cortex (upper) and of the injection site in the thalamus (lower) in case 88-WA1. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections.

**Upper:** The distribution of HRP label in the occipital pole of the cerebral cortex. The small dots represent labelled terminals and large dots represent labelled cells. HRP label is present in area 17, 18 and V-TP. Labelled regions on the medial side of the cortex represent the continuation of areas 17, 18 and V-TP, wrapping round the occipital pole. As well as terminal label in IV and VI in area 17, there is also strong label in IIIb and sparse label in I. Retrogradely labelled cells are in VI. In areas 18 and V-TP anterograde label is confined to IV and VI.

**Lower:** The distribution of HRP products in the LGNd. Cross hatching represents areas with a high concentration of HRP products, whereas single hatching represents areas with a low concentration of HRP products. Thick lines represent labelled axons leaving the LGNd in the optic radiation. The dotted line in the LGNd represents the border between alpha and beta segments of the nucleus. The drawing shows that both alpha and beta segments contain high concentration of HRP products, indicating that HRP uptake occurs in both segments in this case.
Figure 11. Camera lucida drawings of cortical label (upper) and the site of the thalamic injection (lower) of WGA-HRP in case 88-WA4. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections.

Upper: The distribution of the HRP label in areas 17, 18 and V-TP. Small dots represent the labelled terminals. Large dots represent the labelled cells. Terminal label in area 17 is found in layer I, layer III, primarily in IIIb, the upper part of V, and VI. It is absent in IV. Retrogradely labelled cells are found in V and VI. In area 18, there is enhanced anterograde label in all layers, particularly in IV and I, with retrogradely labelled cells in V and VI. In V-TP retrogradely labelled cells are in V and VI and anterograde label is in IV.

Lower: The distribution of the injected HRP in the LGNd and LP. Cross hatching represents regions with a high concentration of HRP, whereas single hatching represents regions with a low concentration of HRP. The injected HRP is mainly concentrated in the beta segment of the LGNd and lateral part of the LP, suggesting that the HRP uptake mainly occurs in these regions, but not in the alpha segment of the LGNd in this case.
Figure 12. The photomicrographs of cross sections of the visual cortex demonstrating the area 17/18 border region in an animal with a similar injection site to that shown in figure 11, with injected HRP primarily in beta segment of the LGNd and the lateral part of the LP.

A. The dark field view of the HRP label in areas 17/18 border region following an injection of WGA-HRP covering the beta segment of the LGNd and the lateral part of the LP. Note the very much enhanced label in area 18, particularly of anterograde label in V, IV, III and I, and retrogradely labelled cells in VI. Also note the absence of anterograde label in IV in area 17. It is present in I, III, primarily IIIb, upper V and VI. Retrogradely labelled cells are in upper V and VI. See Fig. 13 for more detailed view of the laminar distribution of HRP label in areas 17 and 18 respectively.

B. The bright field view of an adjacent thionin stained section showing the cytoarchitectonics of the area 17/18 border region. The border between areas 17 and 18 is indicated by the start of narrowing of the thickness of layer III.

Bar = 1mm.
**Figure 13.** Higher power view of the cortical label in areas 17 and 18 shown in figure 12 resulting from a single injection of WGA-HRP covering the beta segment of the LGNd and the lateral part of the LP.

A. The dark field view of the HRP label in area 17. The thalamic afferent termination is mainly concentrated in layer I, sublayer IIIb and the upper part of the layer V. A sparser termination of thalamic axons is also seen in sublayer IIIa. Note the thalamic afferent termination is largely absent in layer IV and IIIc in this case.

B. The adjacent thionin stained section showing the cytoarchitectonics of the labelled region in area 17.

C. The dark field view of the HRP label in area 18. In regions adjacent to the area 17/18 border, the termination of thalamic afferents is substantial in all supragranular layers, but is particularly concentrated over layers I and IV. In underlying layer V, the anterogradely labelled afferents are also obvious. In the more ventral part of area 18 (right side of the field), the thalamic afferents termination in layer I is largely reduced and in layer III becomes insignificant. Retrogradely labelled cells are present in layers V and, more substantially, in layer VI.

D. The cytoarchitectonics of the labelled region of area 18 shown in C.

Bar: in A and B = 200µm and in C and D = 500µm.
Figure 14. Photomicrographs of cross sections taken from the medial wall of the occipital pole containing the medial extension of area 18 and V-TP.

A. The dark field view of the HRP label in the medial extension of areas 18 and V-TP following a thalamic injection of WGA-HRP covering both the beta segment of the LGNd and LPI. In both areas, the thalamic afferents primarily terminate in layer IV and labelled cells are confined in layer VI.

B. The cytoarchitectonics of the region with HRP label shown in A.

Bar = 500µm.
# TABLE I. SUMMARY OF RESULTS OF THALAMIC HRP INJECTIONS

<table>
<thead>
<tr>
<th>Animals</th>
<th>88-WA1</th>
<th>88-WA4</th>
<th>88-WA5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of possible HRP uptake</td>
<td>In both α and β segments of the LGNd</td>
<td>In β segment of the LGNd, and lateral part of the LP</td>
<td>Largely in α segment of the LGNd, possibly in β segment as well</td>
</tr>
<tr>
<td>Areas with anterograde HRP label</td>
<td>Mainly in 17, also in 18 and the V-TP</td>
<td>Very dense in area 18, also detected in 17</td>
<td>Concentrated in area 17, very faint in 18</td>
</tr>
<tr>
<td>Layers with anterograde HRP label</td>
<td>In area 17: in IV mainly, also in IIIb and VI, faintly in I, IIIc and V. In 18 and V-TP: largely in IV.</td>
<td>In area 18 very dense in I and IV, but also substantial in III, V and VI. In 17 mainly in I, IIIb and V.</td>
<td>In area 17 in IV mainly, but also in IIIb in a few sections. In area 18 very faint in IV.</td>
</tr>
<tr>
<td>Areas with retrograde HRP label</td>
<td>Similar to the anterograde HRP label</td>
<td>Similar to the anterograde HRP label, with more rostral extent in temporal cortex</td>
<td>Similar to the anterograde HRP label.</td>
</tr>
<tr>
<td>Layers with retrograde HRP label</td>
<td>Only in VI in all areas.</td>
<td>In both V and VI, with larger number in VI. More rostrally in the temporal area only in VI</td>
<td>Only in VI in both areas 17 and 18.</td>
</tr>
</tbody>
</table>
Table II. Principle Laminar Distribution of Thalamocortical Afferents and Corticothalamic Cells in Visual Cortical Areas

<table>
<thead>
<tr>
<th>Cortical areas</th>
<th>17</th>
<th>18</th>
<th>V-TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar distribution of afferents from α segment</td>
<td>Dense in IV, moderate in VI</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>of the LGNd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminar distribution of afferents from β segment</td>
<td>Moderately in I, IIIb and upper V</td>
<td>Moderately in IV</td>
<td>Moderately in IV</td>
</tr>
<tr>
<td>of the LGNd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminar distribution of afferents from lateral part</td>
<td>Largely in I, possibly in IIIb and V?</td>
<td>Dense in I, IV, moderate in III and V</td>
<td>Moderately in IV?</td>
</tr>
<tr>
<td>of LP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminar distribution of cells projecting</td>
<td>Only in VI</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>to α segment of LGNd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminar distribution of cells projecting</td>
<td>Only in VI</td>
<td>Only in VI</td>
<td>Only in VI</td>
</tr>
<tr>
<td>to β segment of LGNd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminar distribution of cells projecting</td>
<td>In V, may be in VI too?</td>
<td>In V and VI</td>
<td>In V and VI?</td>
</tr>
<tr>
<td>to the LP</td>
<td></td>
<td></td>
<td>only in VI in rostral part</td>
</tr>
</tbody>
</table>

Table II. Principle Laminar Distribution of Thalamocortical Afferents and Corticothalamic Cells in Visual Cortical Areas

Cortical areas 17 18 V-TP

<table>
<thead>
<tr>
<th>Cortical areas</th>
<th>17</th>
<th>18</th>
<th>V-TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar distribution of afferents from α segment of the LGNd</td>
<td>Dense in IV, moderate in VI</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Laminar distribution of afferents from β segment of the LGNd</td>
<td>Moderately in I, IIIb and upper V</td>
<td>Moderately in IV</td>
<td>Moderately in IV</td>
</tr>
<tr>
<td>Laminar distribution of afferents from lateral part of LP</td>
<td>Largely in I, possibly in IIIb and V?</td>
<td>Dense in I, IV, moderate in III and V</td>
<td>Moderately in IV?</td>
</tr>
<tr>
<td>Laminar distribution of cells projecting to α segment of LGNd</td>
<td>Only in VI</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Laminar distribution of cells projecting to β segment of LGNd</td>
<td>Only in VI</td>
<td>Only in VI</td>
<td>Only in VI</td>
</tr>
<tr>
<td>Laminar distribution of cells projecting to the LP</td>
<td>In V, may be in VI too?</td>
<td>In V and VI</td>
<td>In V and VI? only in VI in rostral part</td>
</tr>
</tbody>
</table>
Chapter 3. The Time Course for the Formation of the Visual Cortical Pathways in Tammar Wallaby During Development

Introduction

The early development of primary visual pathways from the retina has been well described in both placentals (Rakic '77c; Bunt et al. '83; Shatz '83; Williams and Chalupa '82; Cucchiaro and Guillery, '84; Godement et al., '84; Shatz and Sretaven, '86; Shatz et al., '88; Sretaven et al., '88) and marsupials (Cavalcante and Rocha-Miranda '78; Sanderson et al. '82; Wye-Dvorak '84; Harman and Beazley '86). Less is known about the development of the visual cortical connections with other visual centres (Rakic '76; Anker '77; Lund and Mustari '77; Shatz and Rakic '81; Shatz and Luskin '86). In the case of placental mammals, this is largely due to the difficulties of experimental manipulation as the early part of the development takes place in utero. The use of developing marsupials may provide a solution to this technical difficulty since much of the development occurs after birth when more accessibility is allowed in experimentation. An important aspect of this accessibility, for example, is the opportunity for electrophysiological as well as anatomical analysis of visual pathway development, which to date in placentals has only been achieved by an in vitro approach (Shatz and Kirkwood, '84).

From the limited published works, it is established that LGNd axons start to innervate the telencephalon in about the middle of the period from the beginning of the gestation to eye opening (Rakic, '76; Anker, '77; Lund and Mustari, '77; Shatz and Luskin, '86). Shortly after the ascending geniculocortical axons reach the visual cortex, the descending corticogeniculate axons start to project to the LGNd (Anker, '77; Shatz and Rakic, '81). Noticeably though, with the different origins of cortical layers which form at different stages of development, the corticogeniculate projection from layer VI and the corticocollicular projection from layer V reach their targets at
almost the same time (Anker, '77; Shatz and Rakic, '81). In addition, being the last to form among the visual cortical connections, the interhemispheric connections between visual cortices are found to have a widespread distribution early in development and the mature pattern of the restricted termination and the origin of the interhemispheric connections in the region of the area 17/18 border is only achieved by progressive retraction of the axons from other parts of the visual cortex (Innocenti, '81; Lund et al., '84; Dehay et al., '88). On collected data in representative placental mammals, it is suggested that, in terms of the relative timing of the occurrence of the developmental events, the similar time course is followed in all representative mammals in the formation of the visual cortical connections (Dreher and Robinson, '88b).

Previous studies in placental mammals have largely relied on transneuronal transport of tritiated amino acids (Rakic, '76; Shatz and Rakic, '81; Shatz and Luskin, '86) or silver staining of degenerating axons and terminals following thalamic lesions (Anker, '77; Lund and Mustari, '77). Both approaches have certain technical disadvantages in showing the earliest innervating axons in the target structures. For example, in studies using transneuronal transport of radioactive substances, it was suggested that the background label in the young animals is especially high (LeVay et al., '78) thus reducing largely the sensitivity of detection of axons, particularly in small amounts, at the early stages during development.

In this study, the more sensitive tracing substrate, WGA-HRP was used to extend the anatomical description of the development of the visual system in marsupials. From a previous description given in Chapter 2, it is known that the neuronal organization of visual cortical connections in the tammar is very similar to the representative placental mammals. It is also known, however, that the tammar, as a marsupial species, has a remarkably different mode of reproduction from that in placentals. From a previous study on the time course of the formation of primary visual pathways in this species (Wye-Dvorak, '84), it appears that the innervation of
the retinal axons into the LGNd and SC occurs much earlier than that in placentals in terms of relative timing. Thus, it is of interest to know whether, at the cortical level, this precedence in the formation of the visual pathway in the tammar is also present.

**Materials and Methods**

**Animals.**

Twenty four pouch young (PY) tammar wallabies (Macropus eugenii) aged from 12 to 122 days postnatal (12d to 122d) were obtained from a breeding colony. The tammar is a seasonal breeding animal with the first birth usually occurring in late January to early February. Up to June, PY were produced by removal of the existing one in the pouch, resulting in the birth of another PY after 26-28 days (Renfree and Tyndale-Biscoe '78). Between June and August, birth of a new PY was induced 26-28 days after a single injection of 5mg/kg of bromocriptine (Sandoz, Australia) (Tyndale-Biscoe and Hinds '84). PY younger than 41d were of known birth date, while the age of older PY was determined from a chart of head lengths of animals of known age (Poole, personal communication).

**HRP injections**

PY at 48d and younger were anaesthetized by hypothermia and those aged from 56d onwards were anaesthetized by intramuscular injection of ketamine and xylazine with the dosage of injection of ketamine varying according to the age. In PY younger than 100d the dose was 1mg/100g body weight, in PY around 100 d 2mg/100g, and for PY older than 100d 3mg/100g. In all cases, the dose of xylazine was 0.2mg/100g body weight.
WGA-HRP (Sigma) was injected in the presumptive visual part of the telencephalon, which was assumed to be in a similar rostrocaudal and dorsolateral location as in the adult. In PY between 12 and 20 days the injection of the WGA-HRP was conducted by pipettes (about 50µm in diameter) coated with dried WGA-HRP. This was made immediately before the injection by the preparation of a highly concentrated WGA-HRP solution (50%) which was left to air dry until the solution reached a gelatinous state. The tip of the pipette was then touched into the solution so that it was coated by the HRP solution. After minutes, the coating of the HRP on the surface of the tip of the pipettes was dry again. The cortex was exposed, the pipette inserted just below the pial surface using a micromanipulator and left in place for approximately 2 minutes. Deposits in the superior colliculus were made in the same way. In PY 29 to 48 days, glass micropipettes about 50µm in diameter were filled by capillary action with a solution of 1% WGA-HRP in distilled water, then inserted into the cortex and left in place for about 2 minutes to deposit the HRP by diffusion. In older PY, injections of either 1% WGA-HRP or 30% free HRP (Sigma, Type VI) in distilled water were made using a Hamilton syringe. The volume injected ranged from 0.01 to 0.05µl depending on age. In PY of 86 days and older, multiple injections were made to cover as much of the visual cortex as possible. In one case (122d), detergent-soaked HRP-chips were inserted into several sites in the visual cortex instead. The detergent-soaked HRP-chips were prepared by dissolving free HRP (Sigma, type IV) in a 5% aqueous detergent solution (Nonidet P-40) and dried into a solid mass which was then fragmented into small chips of convenient size (Escher et al., '83). In addition, to reexamine when the retinal axons invade the LP, the intraocular injection of 2% of WGA-HRP was made in a PY aged 17 days. Animals were kept in a humid incubator at 37° C for 12-28 hours depending on age.
Histochemistry.

At the end of the survival period, animals were deeply anaesthetized either by hypothermia (48d and younger) or, in cases of older PY, by an overdose of intraperitoneal injection of sodium pentobarbitone. Prior to the perfusion, 0.05-0.20ml of heparin and 0.05-0.20 ml of 1% sodium nitrite, depending on the age of animal, were injected in the left ventricle of the heart. The perfusion was initiated by 5 minutes of wash through the ascending aorta with phosphate buffer solution (0.1M, pH7.4), followed by 1% formaldehyde/1.25% glutaraldehyde in 0.1M phosphate buffered solution at pH 7.4 for 30 minutes. A wash of 0.1M phosphate buffered solution containing 10% sucrose followed for a further 30 minutes. The position of the injection sites on the visual cortex after dissection were drawn using a camera lucida attachment. Brains were kept in 0.1M phosphate buffer containing 20% sucrose at 4° C overnight. They were then immersed in gelatin-albumin solution for 20 minutes and then embedded in gelatin-albumin hardened with glutaraldehyde (for detail see Chapter 2). With the base of the brain as horizontal plane, two series of frozen sections were cut coronally at 30µm for PY under 56d and at 40µm for older PY. Both series were processed with the tetramethyl benzidine method (for details see Chapter 2) with one series stained with 0.025% thionin, while the other remained unstained.

Results

Data were collected from 23 PY divided into three experimental series. Seventeen (17) PY were in the cortical injections series (between 12d-122d), 5 in the collicular injection series (between 41-81d), and only 1 in the intraocular injection case.
As in adults, the presumptive primary visual cortex can be determined on the basis of cytoarchitectonics in PY older than 81 days, when the future layer IV can be distinguished so that the difference between areas 17 and 18 in the thickness of the layer III can be appreciated. In younger PY, the presumptive primary visual cortex is assumed to be in a geographically similar location to adults in the caudal pole of the telencephalon.

The onset of a given connection was determined by the time when anterograde and/or retrograde labelling was first detected after an injection of WGA-HRP. Anterograde HRP labelling was identified as dust-like granules of histochemical reaction product in the neuropil, whereas retrograde labelling was identified as clusters of granules of reaction product contained within the cytoplasm of cell bodies (see previous chapter). A brief description of cytoarchitectural features of the diencephalon and the mesencephalon during development is also given where appropriate in the following sections. Figures illustrating the results are placed together at the end of the chapter.

Formation of Thalamocortical Connections

This was examined in PY aged between 12-85d after cortical injections of WGA-HRP. The establishment of the thalamocortical projections from the LGNd and the LP to the ipsilateral visual cortex was determined by identification of retrogradely labelled cell bodies in these thalamic nuclei, whereas the descending corticothalamic projections to the LGNd and LP were determined by the presence of anterogradely labelled terminals in these thalamic regions. At no stage was any convincing evidence found of label in the thalamus contralateral to the injected cortex. The formation of the projections from LGNd and LP to the visual cortex and of the cortical descending projections to these nuclei at different stages during development will be described in a chronological order.
12 days. There were two experimental cases at this age. In each case single WGA-HRP deposit was made in the telencephalon, one sited dorsolaterally and the other laterally in the caudal pole of the telencephalon, the presumptive visual cortex. In the diencephalon, the LGNd and LP were not identifiable on the basis of cytoarchitectonics at this stage. They were assumed to occupy the similar position in the dorsolateral part of the diencephalon as that in PY of slightly older ages where they were cytoarchitectonically recognizable. After 12-14 hours of survival followed by histochemical processing, no label was found in the diencephalon at this stage after a careful search.

15-17 days. Two PY, one at 15d and the other at 16d, each received a single WGA-HRP deposit in the dorsolateral part of the presumptive visual cortex. The size of the injection site, as indicated by the HRP reaction product after histochemical processing, was about 1mm in diameter (Fig. 15A). The diencephalon at this stage was still extremely immature and was characterized by homogeneously packed undifferentiated cells. The LGNd and LP were very difficult to identify by cytoarchitectonic criteria (Fig. 15B). However, the distribution of the anterograde labelling following an intraocular injection of WGA-HRP in a 17d PY indicates that the LGNd and LP are located along the lateral edge of the diencephalon (Fig. 15D) and both have begun to receive retinal innervation by this age.

The HRP labelling was first found in the lateral edge of the diencephalon following the placement of WGA-HRP deposit in the telencephalon at this stage. The label was mostly contained in a thin zone which extended ventrodorsally, probably covering both the LGNd and LP (Fig. 15A). Even in dark field, the label was very sparse, indicating that establishment of the corticothalamic connection is just at its beginning. Under high magnification in bright field, it appeared that the granules of HRP products were contained within the cell bodies, although there is difficulty to confirm this at this stage as the staining quality of cells is poor, sections are thick
relative to the size of cells, and the filling of the reaction products within the cell bodies is very incomplete (Fig. 15C).

20 days. At this stage, it became possible to separate the LGNd and LP together from their neighbouring structures by the histological difference that the cells in the LGNd and LP are more densely packed and deeply stained, and both nuclei were just starting to become cytoarchitectonically separable from each other (Fig. 16B).

In this case, with a size of about 1.1mm in diameter, the single WGA-HRP placement was located rather laterally in the dorsolateral part of the caudal telencephalon, with an extension to more ventral parts of the lateral wall of the telencephalon (Fig. 16A). Label was detected in a number of structures. The label in the laterodorsal region of diencephalon, most noticeably, was very much enhanced compared to that found at 15-16d (Fig. 16A). It was distributed widely in a region covering most of the LGNd and LP, with a greater density in the dorsal part of the LGNd. Under high magnification, it was relatively easy to identify granules of HRP products contained within cell bodies (Fig. 16C), as not only the proportion of the labelled cells, but also the filling of HRP products in each labelled cell, were increased at this stage compared to 15-16d. There was no sign of anterograde HRP labelling at this stage.

HRP labelling was also present in the optic radiation in both the telencephalon and diencephalon. In the diencephalon, the labelled optic radiation could be traced ventrodorsally at the horn of the third ventricle, via a route in a relatively cell sparse region just medial to the ventral lateral geniculate nucleus (LGNv), to the dorsolateral edge of the diencephalon, where it is merged with the labelled LGNd and LP.

Label was also found in a non-visual structure in the diencephalon. In the caudal part of the diencephalon, the dorsolateral part of the medial geniculate nucleus (MG), a principle auditory thalamic structure, was heavily labelled. It appeared that only retrogradely labelled cells were present in the nucleus at this age.
29-43 days. There were four experimental cases covering this age range, 29d, 36d, 41d and 43d. During this period, the diencephalon was undergoing a rapid change in its size, shape and differentiation. The LGNd and LP were becoming increasingly distinguishable on the basis of cytoarchitectonics during this period (Figs. 17&18) and by 41d the LGNd and LP can be easily recognized from the surrounding structures (Fig. 18). However, cells in both nuclei were still homogeneously distributed so that the identification of the lamination in the LGNd and of the subdivision in the LP is not possible on the basis of cytoarchitectonics.

The HRP injection in all these cases were circumscribed (about 1.5mm in diameter), but with different locations in the telencephalon. As a result, HRP label in different parts of the LGNd and LP was produced (Figs. 17-19). For example, in a 36d PY, a single HRP deposit placed in the lateral part of the presumptive visual cortex produced HRP label mainly confined in the dorsal third of the LGNd (Figs. 17&19). In a 41d PY, a single HRP deposit placed in the medial edge of the presumptive visual cortex produced a patch of HRP label in the ventral part of the LGNd (Figs. 18&19). It was noticed that by as early as 36d, border between labelled and unlabelled cell groups was rather sharp, indicating that a certain degree of topographic precision may be acquired by geniculocortical cells at this stage (Figs. 17&19). This topographic precision of origin of geniculocortical projections was more obvious by 41d (Figs. 18&19).

The density of filling of the retrogradely transported HRP molecules within the cell bodies was also undergoing an increase during this period and, by 43d, most of the cytoplasm of labelled cells was filled (Fig. 18C). However, there was still no sign of convincing anterograde labelling at this stage (Fig. 18C).

48 days. In this case, a large part of the presumptive visual cortex was covered by HRP injection. Extensive label was observed in the LGNd, LP, and the MG as well. Most noticeably, considerable amounts of particles of HRP products were first seen between cell bodies in both LGNd (Fig. 18D) and LP (not shown) at this age.
71 days. By this stage, the cell density in the medial most part of the LGNd began to reduce and there appeared a certain suggestion of the emerging cellular lamination (Fig. 20B).

The WGA-HRP deposit was placed rather dorsomedially in the presumptive visual cortex in this case and the injection site was about 0.8-1.0mm in size. Two well defined patches of label were found in the thalamus, one in the LP and the other in the LGNd. In both nuclei, as shown in Figure 20A&B, the label appeared as wedge-shaped and sharply edged columns extended inwards from the superficial surface to the most medial part of the nuclei. At high magnification, the retrogradely labelled cells were now almost completely filled with HRP products (Fig. 20C). The anterograde label is increasing between the labelled cells at this stage (Fig. 20C), but the pattern of a homogeneous background of dust of HRP products against retrogradely labelled cells seen in the mature animals was still not formed.

85-86 days. At this stage, the lamination in the LGNd becomes more distinguishable.

In this group, one 85d PY received a single HRP injection in the medial part of area 17 (Fig. 21), while the other PY at 86d received two HRP injections localized medially and laterally in the rostral part of the area 17. Injection sites in all cases appeared as defined patches of HRP reaction product with 0.9-2.0mm in diameter. In both animals, label in the LGNd and LP was rather similar to the adult pattern. First the label was well sharply edged and defined in the nucleus (Fig. 21). Second, the cytoplasm of retrogradely labelled cells was completely filled with granules of HRP products (Fig. 20D). Third, the anterograde labelling was now similar to the adult pattern in that a homogeneous distribution of granules of HRP products in substantial amounts was present (Fig. 20D).

Thus, by 85 days, the thalamocortical connection appeared already to resemble the mature pattern.
Formation of the Corticocollicular Projection

This was examined in two experimental series. In one series, the same as used to examine the formation of the thalamocortical connections, animals received a cortical injection of HRP to find out at what stage the anterograde labelling is first present in the superficial layers of the SC. In the other series, animals received an injection of HRP in the SC to test when the cortical cells projecting to the SC were labelled.

**Cortical injections.** All cases in this study were examined for anterograde HRP labelling in the mesencephalon. The first arrival of corticocollicular axons, was not detected until 71d. In the dark field, some scattered labelled fibres were first seen in the deep layers of the most rostrolateral part of the SC (Fig. 22A). In the superficial layers of the SC, however, no anterograde labelling was present at this stage.

By 81d, the superficial layers of the SC, the SZ, the SGS and the SO can be first identified on the basis of cytoarchitectonics (Fig. 22C.), although not as distinctly as in the adult. At this time, a patch of anterograde labelling of high density was observed in the caudal part of the SC after a single injection of WGA-HRP in the lateral part of the area 17 (Fig. 22B). Anterograde labelling was distributed heavily in all three superficial layers with the heaviest distribution found in the most superficial layer, the SZ. The collicular label was reasonably defined though not sharply edged and appeared more widespread through the depth of the SC than in the adult. For example, faint label was also observed in the deeper layers under SO and was present throughout the SGS whereas in the adult it is largely absent from the most superficial part of the SGS.

**Collicular injections.** To localize the cells projecting to the SC in the cortex at early stages of the formation of the corticocollicular projection and to confirm the timing from the cortical injection series, a single deposit of HRP was placed in the SC of animals aged 41, 51, 68, 75 and 81 days. This was aimed at the middle of the
rostrocaudal and mediolateral extent of the SC. An attempt was made to limit the HRP application within the most superficial layers. However, the spread of HRP into the deeper layers was obvious in all cases.

Among all cases in this experimental series, labelling of cortical cells was only found in the case of 81d animal, confirming the results from cases using cortical injections of WGA-HRP. Substantial amounts of retrogradely labelled cells were found in the medial part of the area 17 (Fig. 23). These were the pyramidal cells situated in future layer V (Fig. 23B).

Considerable numbers of labelled cells were also found in other cortical areas including regions in the temporal and parietal cortex. The auditory cortex may be also labelled due to the involvement of deeper layers of the SC in this case, although this is difficult to judge due to the lack of information on the exact location of the auditory cortex in either the adult or the pouch young in this species. In these regions, retrogradely labelled cells were similarly contained in layer V with a pyramidal-like appearance.

Formation of Intrahemispheric Connections

This was examined in the cortical HRP injection cases. The first time that anterograde and retrograde label was found in the ipsilateral cortex was 99 days (Fig. 24). This is when the lower part of layer III, as well as layers IV, V and VI, had already been formed (Fig. 24B). The label was present in area 18 and the V-TP area of the caudal part of the cortex. In both areas, anterogradely labelled terminals were largely concentrated in layers V and VI and the underlying subplate (Fig. 24A&B), rather than in layers III and IV, as seen in the adult (see Chapter 2). Besides, more axons were labelled in the SP in the region than regions without intracortical HRP label (Fig. 24). The laminar distribution of retrogradely labelled cells was different in area 18 and the V-TP area in that while they were only obvious in the layers V and VI
in the V-TP area, they were also scattered in layer IV and the forming layer III in area 18 (Fig. 24).

Formation of Interhemispheric Connections

In cases which received cortical injections of WGA-HRP, four age groups (81-86d, 99d, 111d and 122d) were chosen from the series for the following description.

81-86 days. There were three experimental cases in this group, 81d, 85d and 86d. In all cases, the injection site was close to the margin of the area 17. These labelled fibres were within the optic radiation in the cortex and became dispersed from it at the horn of the third ventricle, where they continue to traverse further down via the capsule exterior (CE) and enter the anterior commissure (CA). However, the labelled processes could be hardly detected further beyond in the contralateral CE and CA.

99 days. The PY of this age received a dense, well defined injection of WGA-HRP in the rostral part of area 17, about 1.5mm from the 17/18 border. The injection was about 1.2mm in diameter. Labelled fibres in small numbers were found in the opposite visual cortex. They were scattered in both areas 17 and 18, and extended from the 17/18 border region as far as the medial edge of area 17, but largely limited in the rostral half of the area. These labelled fibres were only detected within the white matter under developing cortex (Fig. 25).

111 days. In this case, three HRP injections were made in the area 17, one localized in the middle of the mediolateral extension of the caudal part of area 17 and the other two localized in the rostral part of area 17, with one in the medial edge of area 17 and the other adjacent to the 17/18 border. Both anterogradely labelled terminals and retrogradely labelled cells were found in the opposite visual cortex. In anterograde labelling, sparse fibres were only seen in the vicinity of the 17/18
border and were only detectable in layer V (Fig. 26). The small number of retrogradely labelled cells were found in layers III (Fig. 26A&B). These cells were scattered widely well into the medial part of the area 17 (Fig. 26) and were large pyramidal cells.

**122 days.** At this age, a massive HRP administration covering much of the areas 17 and 18 was produced by detergent combined free HRP deposit placed in about the middle of the area 17 (Fig. 27). This yielded extensive contralateral cortical labelling of the cells of origin of the interhemispheric connection between areas 17 of the two sides. The retrograde labelling was distributed in both areas 17 and 18. In area 17, labelled cells were present in both layers III and V, with a different distribution. In layer III, these intercortical cells were distributed widely in both mediolateral and rostrocaudal direction, in a region covering virtually the whole of the area 17. In layer V, however, these intercortical cells had a much more limited distribution. Mediolaterally, they were only present in a restricted region medial to the 17/18 border (Fig. 27). Rostrocaudally, they were also present in a more limited extension than in layer III (Fig. 27). In area 18, only layer III cells were labelled. In a region more lateroventral to area 18, small numbers of labelled cells were found in layer III in a few sections (not shown). The anterograde labelling, on the other hand, was not obvious in this case.

**Discussion**

**Methodological Consideration**

In this study, WGA-HRP was used to examine the formation of cortical afferents and efferents. In all cortical injection cases, as indicated by the presence of anterograde and retrograde label in the LGNd and LP, all HRP placements aimed at the presumptive visual cortex were successful, except in one case in a 20d PY, the
WGA-HRP deposit was placed more lateral so that, apart from the lateral part of the visual cortex, the dorsal part of the future auditory cortex seemed also involved in the HRP uptake, as evidenced by labelled cells in the MG, a principle auditory thalamic nucleus. In collicular injection cases, while the superficial layers were successfully administered with HRP, the deeper layers may be also involved in HRP uptake. However, it is not likely that the involvement of the deeper layers could mislead the interpretation of the earliest timing of the formation of the corticocollicular projection, which is also decided by the result from cortical injections of HRP.

**Time of the Formation of Retinal Projection to the Contralateral LP**

In a previous study using anterograde transport of tritiated proline, it was established that the earliest time that retinal axons reach the contralateral LP was 31 days, about 3 weeks after the innervation of the retinal axons into the contralateral LGNd (Wye-Dvorak, '84). This timing is questioned by the present results from cortical HRP injection cases. Axons from the LP have been shown reaching the future visual cortex by as early as 15 days, at the same time when axons from the LGNd reach the visual cortex. Furthermore, the histogenesis of both nuclei seem to occur concordantly during development. This led to the requirement to reexamine the earliest time of the innervation of the retinal axons into the LP.

Using the HRP technique which is more sensitive in the detection of the earliest axons in the target structures, the present results show that by as early as 17 days, substantial numbers of retinal axons have already arrived in the LP. Thus the innervation of retinal axons into the LP occurs at a much earlier stage than previously thought. It seems likely that retinal axons may enter both LGNd and LP at a similar stage during development in the tammar.
Time Course of the Formation of Visual Cortical Afferents and Efferents

The findings of the study presented in this chapter are summarized in figure 28. From the present results, it was found that a limited number of axons from some of the LGNd and LP neurons have already reached visual cortex by 15 days though both diencephalon and telencephalon are still at the beginning of their histogenesis. By 20 days, the thalamocortical projection becomes substantial, as indicated by the presence of large number of labelled cells in both LGNd and LP. The corticothalamic axons, however, do not start to project to the LGNd and LP until about 30 days later at 48d. By 71 days, there is some indication of the first arrival of a limited number of corticocollicular axons in the vicinity of the future SC, though they have not yet innervated the target structure significantly. By 81 days, as results from both cortical and collicular HRP injection cases suggest, the corticocollicular projection has been formed. The interhemispheric axons from area 17 begin to accumulate in the opposite visual cortex by 99 days, though they still have not invaded the cortical layers. Meanwhile, the intrahemispheric axons from area 17 have accumulated in area 18 and the V-TP in substantial amounts, but have not invaded layers III and IV, their appropriate destination in the adult, and axons projecting back to area 17 largely from layers V and VI of these regions have also reached their target area. By 111 days, cortical cells projecting to the opposite visual cortex are first labelled, indicating that the reciprocal intercortical connection between area 17 of both sides is formed.

The comparison of these results with those found in the placental mammals shows that the tammar has a remarkably different time course in the formation of the visual cortical connections from those seen in some representative placental mammals. First, the relative timing of the establishment of the thalamocortical projection in tammar is much earlier than that of placental mammals. For example, in tammar, the thalamic axons start to invade the visual cortex at 15 days postnatal, about 41-43 days
after conception and 120 days before the eye opening which occurs around 140 days after birth. In macaque monkey, the earliest time that has been examined is embryonic day 78 (E78), 78 days after conception and 45 days before eye opening and the geniculate axons at this time were already in the visual cortex (Rakic, '76). In cat it was reported that the geniculocortical projection begins to enter the telencephalon by E35 and can be found in the presumptive visual cortex by E42, 42 days after the conception and 30 days before the eye opening (Shatz and Luskin, '86). In rat, thalamic axons began to invade the visual area by E18, 18 days after conception and 18 days before the eye opening (Lund and Mustari, '77). Thus, while the formation of the thalamocortical projection in the tammar starts in the first quarter of the whole duration of the period between conception and eye opening, a period termed the caecal period (Dreher and Robinson, '88a), it does not begin until the second half of this period in placentals. This precedence is still very obvious by the stage of the formation of the corticogeniculate projection which occurs in the tammar before the end of the first half of the caecal period, whereas in representative placental mammals this takes place by the end of two thirds of the caecal period (macaque monkey: Shatz and Rakic, '81; cat: Anker, '77; rat: Dreher and Robinson, '88b).

There are several possible factors which may explain this remarkable difference between the tammar and the placentals in timing of the formation of the thalamocortical connections. One possibility is the use of different techniques. The direct application of the WGA-HRP in the cortex may improve the sensitivity of the detection of the earliest thalamocortical connections in a number of ways. First, in the case using HRP, the formation of the thalamocortical connection can be detected as soon as it is established, whereas in the case using transneuronal transport of autoradioactivity the detection of the formation of the thalamocortical connection must also rely on the maturation of the retinogeniculate synapses as well. It is possible that the transneuronal transport at the synapses between retinal axons and geniculate cells may not be allowed until considerably later than the formation of the thalamocortical
projection, thus the detection of the formation of the geniculocortical projection by use of autoradioactivity could be retarded. This may explain why the geniculocortical axons can not be detected until 10 days (or 14% of caecal period, in comparison with 3% of caecal period in the rat and 6% of caecal period in the tammar) after the first detection of the retinogeniculate axons. This may also explain why in cat and macaque monkey the detection of the geniculocortical projection is considerably later than in the rat, in which the formation of the thalamocortical projection was detected by nerve degeneration following lesions in the thalamic nuclei (Lund and Mustari, '77). Also, use of the HRP as a tracer of neuronal connection avoids the problem of the general background label faced in studies using transneuronal autoradiography. This general background label largely reduces the sensitivity of the technique in detecting the real label, particularly in the case using developing animals, as the background label is even higher and the earliest terminal label can be overshadowed (LeVay et al., '78).

However, it is unlikely that the different sensitivity between the HRP technique and the autoradiographic technique alone can possibly account for such remarkable precedence by the tammar compared to the placentals in the formation of the geniculocortical projection. Interspecies difference might also contribute to this. For example, in another species of wallaby (quokka), the generation of the LGNd starts before 20% of the caecal period, whereas in the placentals, the generation of the future LGNd cells is yet to start until 29-33% of the caecal period (rat: Mustari et al., '79; cat: Hickey and Hitchcock, 84; Hitchcock et al., '84; monkey: Rakic, '77b,c). Similarly, at a cortical level, in the tammar the generation of cortical cells starts before embryonic day 25 (E25), or 15% of the caecal period (Reynolds et al., '85). In the placentals, on the other hand, the cortical neurogenesis has not started until 31-33% of the caecal period (rat: Raedler and Raedler, '78; cat: Luskin and Shatz, '85a,b; monkey: Kostovic and Rakic, '80). This precedence by the tammar is also obvious in the formation of the retinogeniculate projection [23% of the caecal period in the
tammar (Wye-Dvorak, '84) vs 44% of the caecal period in the rat (Bunt et al., '83) and the cat (Shatz, '83). Thus, the earlier formation of the geniculocortical projection appears as one of the many facts reflecting the overall advanced ontogenesis of the central nervous system in the tammar at early stages of development.

Second, by comparison, the formation of the descending cortical projections to the LGNd and SC is remarkably delayed after the formation of the geniculocortical projection. For example, in the tammar, the formation of the descending corticothalamic projection is 30 days (19% of the caecal period) later than the formation of the ascending thalamocortical projection, whereas this is only 6 days (9% of the caecal period) in the cat and 5 days (14% of the caecal period) in the rat. This is more obvious in the formation of the corticocollicular projection. In placentals, this occurs almost homochronically with the formation of the corticogeniculate projection. In the tammar, however, the formation of the corticocollicular is 36 days later than the formation of the corticogeniculate projection.

Because of the delayed formation of the descending cortical projections to the LGNd and SC, the lead in timing by the tammar in the formation of the cortical connections is lost by the time when the corticocollicular projection is arising. In tammar, the cortical axons arrive in the SC by 81 days, about two thirds of the whole duration of the caecal period, a timing remarkably similar to the formation of the corticocollicular projection in the placentals. This synchrony between the tammar and placentals is maintained into later stages, when the interhemispheric connection is formed.

Thus, in comparison with the placentals, it appears that the overall process of the formation of the cortical visual pathways can be divided into two phases according to the rate of the development. The first phase occurs in the first half of the caecal period, during which the formation of the geniculocortical and the corticogeniculate projections is prior to that in placentals by 20% of the caecal period or more. The second phase is in the second half of the caecal period and is characterized by the
prolonged delay in the formation of the corticocollicular projection, which makes the initially advanced time course in the tammar become synchronized with that in the placentals.

In contrast to the differences in the relative timing of the formation of the visual pathways, the sequential order in which connections at different levels through the visual pathway are formed is similar in the tammar and placental mammals. For instance, in both marsupials and placentals the developmental elements in the formation of the visual pathway occur according to a similar hierarchical order. The connections between the periphery and primary visual cortex are formed earlier (e.g. the retinogeniculate and retinocollicular projections) and those at higher levels in the brain are formed later (e.g. the intrahemispheric and interhemispheric connections).

The hierarchical order of onset of projections originated from different cortical layers is worth further discussion in relation to the generation of cortical layers. It is established that in both the placentals and the tammar the projections originating from different layers are formed at different stages, in that those from the deeper layers such as the corticogeniculate projection from layer VI, are formed earlier, while those from the more superficial layers, such as the interhemispheric projection mainly from layer III, are formed later. This is probably not surprising, in the context that the neurons comprising the deeper layers precede those for more superficial layers in generation, migration and differentiation (Angevine and Sidman, '61; Bruckner et al., '76; Crossland and Uchwat, '82; Hicks and D'Amato, '68; Lund and Mustari, '77; Luskin and Shatz, '85b; Rakic, '74,'77a; Reynolds et al., '85; Shimada and Langman, '70).

The Temporal Relationship Between the Formation of the Retinogeniculate Projection and the Geniculocortical Projection

From the previous study it was established that retinal axons first reach the LGNd 12 days after birth (Wye-Dvorak, '84), just three days prior to the first arrival
of the geniculate axons in the future visual cortex. This close matching in timing between the formation of the retinogeniculate and geniculocortical projections is also obvious in rat (Lund and Mustari, '77; Bunt et al., '83). In the cat, the matching in timing between the formation of the retinogeniculate (E32) (Shatz, '83) and geniculocortical (E42) (Shatz and Luskin, '86) projections is not so obvious. However, this may be due to the difficulty in the determination of the earliest arrival of the geniculate axons as the earliest detection of geniculocortical axons in the telencephalon has to rely on the sufficient maturation of the synaptic connections to allow the significant amount of transneuronal transport of labelled amino acids. In the macaque, the temporal relationship between the formation of the retinogeniculate projection and the formation of the geniculocortical projection is not possible to judge as both may have already formed quite a time before E78, the youngest age studied (Rakic, '76). Thus, it is possible that the geniculate cells may start the outgrowth of their axons while they begin to receive the input from the retina.

The present results also suggest that there is a certain degree of topographic specificity existing in the geniculocortical projection at a stage as early as 29 days. The precision in topographic order is well developed by 42 days and similar to the adult by 71 days, as indicated by the sharpness of the band of label traversing across all LGNd laminae. This timing is somewhat similar to the development of the topographical precision in the retinogeniculate projection (Marotte, '89).

These observations suggest that the formation and reshaping of the retinogeniculate projection and geniculocortical projection may occur simultaneously in the tammar. Whether this is suggesting the independence of the two processes or a chance for the possible interactions between retinal axons and geniculate cells in the LGNd during the ontogeny of the retinogeniculocortical pathway is not known. It has been previously reported that, in the absence of retinal inputs, the topographic order of geniculocortical projections still can be formed in anophthalmic mice although with less precision (Kaiserman-Abramof et al., '80). However, whether this is also the case
during normal development in animals without genetic abnormality, and whether the
timing of the outgrowth of the geniculate axons is regulated by the retinal input during
normal development is yet to be examined.

The formation of the topographic precision of the origin of the geniculocortical
projection in the LGNd has also been the subject in a recent study using WGA-HRP
retrograde tracing technique in the hamster (Naegele et al., '88). After cortical
injection of HRP in animals at various ages, the distribution of retrogradely labelled
geniculate cells are found to undergo a progressive refinement to achieve the mature
pattern of the topographic specificity. While this process is similar to what is revealed
in the tammar in the present study, it is intriguing that the topographic precision of the
geniculocortical projection becomes obvious by 36d, about 40% of the caecal period,
in the tammar, but it is not until p5-6, about 70% of the caecal period, that the similar
degree of topographic precision is achieved in the hamster.

The Formation of Intrahemispheric Connections

The present results show that in the tammar, at the initial stages of the formation
of the intrahemispheric connections, axons from area 17 have a remarkably different
laminar distribution in areas 18 and the V-TP from that in adult. In adult, the axons
from area 17 largely terminate in layers III and IV and cells projecting to area 17 are
mainly distributed in layers III, V and VI (Chapter 2). The accumulation of
intracortical axons in deeper parts of the visual cortex may be related to the fact that
when intrahemispheric connections were first established layer III had still not
completed its formation. However, it is not clear why axons from area 17 should wait
in layer VI and the subplate, as layer IV, one of their appropriate terminations, has
already formed and become innervated by axons from thalamus at this stage (Chapter
4).
The present results also show that at this stage the cells projecting back to area 17 only have a limited distribution in layer III in areas 18 and V-TP, presumably also due to the delayed formation of layer III at this stage. Many of the cells destined for this layer might still have not sent their axons to area 17 as they are still relatively immature. Alternatively, cells designated for the future layer III might have already sent their axons into the target area, but they themselves are still positioned in the deeper layers on their way migrating to the layer III.

The Formation of Interhemispheric Connections

The present results demonstrate that in the tammar, cortical cells projecting to the opposite visual cortex have a widespread distribution in extent in area 17 and a large part of area 18 at the early stage (122 days) of the formation of the interhemispheric connections. This has been also described previously in a number of placental mammals. In the new born cat and rat, callosal neurons are found not to be restricted to the 17/18 areas border as in the adult, but are distributed in the entire extent of the areas 17 and 18. In fetal macaque monkeys, the distribution of callosal neurons in area 18 shows the common sequence of an early widespread distribution followed by regression whereas in area 17 the callosal cells are largely absent both in fetus and in adults (Dehay et al., '88; Kennedy et al., '86). The termination of callosal axons in newborn cats and rats also have a widespread distribution, but only in the white matter and lowest part of the layer VI. In the gray matter, they are only present in substantial numbers in the restricted region near the 17/18 border (Innocenti, '81; Lund et al., '84).

It is believed that the widespread callosal cells in regions other than the area 17/18 border zone which give rise to the callosal connection in adult, remain alive at a later stage and they become acallosal by the elimination of their callosal axons (Innocenti, '81) and/or the loss of axons or collaterals, as reported in the case of
transitory bilateral projections to ipsilateral and contralateral visual areas from the auditory cortex in kittens (Innocenti, '84).

The elimination of the transient callosal axons at early stages seems to be caused by their failure to innervate into the gray matter of the opposite visual cortex (Lund et al., '84). Therefore, the appropriate synaptic connection with target cells might be one of the essential conditions for the survival of callosal axons which are able to innervate into the cortical layers and, presumably, make synaptic connections with neurons in the restricted region near the area 17/18 border.
Figure 15. Photomicrographs of cross sections of diencephalon in a PY at 15d (A, B and C) and a PY at 17d (D).

A. Dark field view of HRP label (arrow) at the dorsolateral edge of the diencephalon after WGA-HRP deposit was placed in the presumptive visual part of the telencephalon in a PY at 15d. The label is faint and is distributed in a region probably covering both LGNd and LP. Inset shows a dorsal view of the cortex with the injection site covered by HRP indicated by cross hatching. This was reconstructed from sections. The circle within the cross hatching marks the site of injection.

B: Cytoarchitectonics of the lateral part of the diencephalon at 15d. The future LGNd and LP are largely undifferentiated at this stage and appear as a relatively densely packed and deeply stained cellular entity.

C: High power view of the labelled region in A. Only a small proportion of the cells are labelled with very few granules of HRP products contained within each labelled cell (arrow heads).

D. Dark field view of thalamic HRP label after an intraocular injection of WGA-HRP in the contralateral eye in a PY at 17d. Labelled retinal axons heavily innervate the LGNv. In the LGNd, however, the retinal axon termination is less substantial and only obvious in the middle part of the nucleus dorsoventrally. In the LP, the retinal input termination is also substantial, thus suggesting the LP has already become largely innervated by retinal axons at this stage.

Bar: in A, B and D = 100µm; in C = 10µm.
Figure 16. Photomicrographs of cross sections showing HRP label in the dorsolateral diencephalon and optic radiation after a cortical HRP deposit in a 20d PY.

A: Dark field view of HRP label in the diencephalon in a 20d PY which received a cortical injection of WGA-HRP. The label has become greatly intensified in both LGNd and LP compared to that seen at 15d. In addition, some HRP label is seen in the optic radiation medial to the future LGNv. Inset shows a dorsal view of the cortex with the site covered by HRP indicated by cross hatching. This was reconstructed from sections. The circle within the cross hatching marks the site of injection.

B: Cytoarchitectonics of the diencephalon at 20d. LGNd and LP are still not differentiated, but can be distinguished from the future LGNv by a zone of relatively low cell density. The optic radiation becomes visible, providing some indication of the medial border of the LGNd at this stage.

C: The magnified view of HRP labelled region in the LGNd shown in A. Note both proportions of the labelled cells and the number of HRP products contained within each labelled cell body are largely increased, contributing to the increased intensity of the LGNd/LP label at this stage.

Bar: in A and B = 100µm; in C = 10µm.
Figure 17. Photomicrographs of cross sections of LGNd and LP with HRP label in a PY at 36d after an injection of HRP in visual cortex. The site of injection and a series of camera lucida drawings through the LGNd and LP is shown in figure 19.

A. Dark field view of HRP label in the LGNd and LP. The arrow head represents the presumptive border between LGNd and LP. Following a defined placement of WGA-HRP in the relatively caudolateral part of the visual cortex, the HRP label in the LGNd is confined in the dorsal part of the nucleus. Label in the LP (arrow) is also confined in a patch.

B. Bright field view showing the cytoarchitectonic features of LGNd and LP at this stage. The arrow head points to the possible border between LGNd and LP. Both nuclei are still highly undifferentiated.

Bar = 100µm.
Figure 18. Photomicrographs showing thalamic HRP label resulting from a cortical injection of WGA-HRP in PY at 41d, 43d and 48d.

A. Dark field view of the HRP label in the LGNd and LP in a 41d PY following a defined placement of WGA-HRP in the relatively rostromedial part of the visual cortex. The HRP label in the LGNd is confined in the ventral part of the nucleus (arrow head). In the LP, HRP label is also confined in a patch (arrow). The injection site and a series of camera lucida drawings through the LGNd and LP is shown in figure 19.

B. Cytoarchitectonics of the LGNd and LP at 41d. LGNd is still a homogeneously packed cellular entity, although its ventral and medial border is distinct at this stage.

C. High power view of the label in the LGNd of a 43d PY after receiving a cortical injection of WGA-HRP. Note much of the cytoplasm of labelled cells is filled with the HRP products at this stage. However, there is still no sign of the presence of anterogradely labelled terminals of descending corticogeniculate axons by this age.

D. The high power view of the label in the LGNd in a 48d PY following a cortical injection of WGA-HRP. Labelled cells become completely filled with HRP products. The most important feature is, however, the first presence of the anterogradely labelled terminals (arrow) of corticogeniculate axons between the cell bodies.

Bar: in A and B = 200µm; in C and D = 10µm.
36d

LATERAL

CAUDAL

Dorsal

Rostral

1 mm

41d

LATERAL

CAUDAL

Dorsal

Rostral

1 mm

1 mm

LGNd
LP
Figure 19. Camera lucida drawings showing the distribution of thalamic HRP label and cortical injection site in PY at 36d and 41d. In both sets of drawings, cross hatching in the cortex represents areas with dense injected HRP, whereas in the thalamus, dots represent the retrogradely labelled cells resulting from cortical injection of HRP.

36d: The HRP injection is located relatively caudolaterally over the visual cortex and is mainly confined in the upper layers of the developing cortex. In the diencephalon, labelled cells are mainly confined in the dorsal part of the LGNd and, on a few occasions, in the ventral part of the LP.

41d: The cortical injection of HRP is located relatively rostromedially, and injected HRP is distributed in the whole depth of the cortex. Labelled cells are found in both LGNd and LP. In LGNd, they are mainly confined in the ventral part of the nucleus.

Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections.
Figure 20. Photomicrographs of HRP label in the LGNd at 71d (A, B and C) and 85d (D).

A. The bright field view of a cross section without thionin stain showing the HRP label in the LP and LGNd after a defined placement of WGA-HRP in a relatively rostromedial part of the visual cortex. Note the border between labelled and unlabelled regions becomes rather sharp at this stage.

B. The cytoarchitectonics of the LGNd at 71d. Cell density in the medial most part of the nucleus, where the beta segment will emerge, has started to reduce.

C: The high power view of the HRP label in the LGNd. Apart from retrogradely labelled cells, anterogradely labelled terminals (arrow) of corticogeniculate axons become substantial in the neuropil at this stage.

D: A high power view of the HRP label in the LGNd at 85d. The anterograde labelling begins to resemble the mature pattern, appearing as a homogenous distribution of granules of HRP products between retrogradely labelled cells.

Bar: in A and B = 200µm; in C and D = 50µm.
Figure 21. Camera lucida drawings showing the site of cortical injection of HRP and the distribution of resulting thalamic HRP label at 85d. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections.

Upper: The distribution of the injected HRP in the visual cortex. The cross hatching represents the area with dense HRP products. The left shows a cross section taken in the centre of the injection site, indicating the distribution of the injected WGA-HRP in the depth, as well as in the mediolateral extent of the visual cortex. The right is the dorsal view of the visual cortex showing the location and extent of the injected HRP over the visual cortex.

Below: Series of camera lucida drawings showing the distribution of anterogradely labelled corticothalamic terminals and retrogradely labelled thalamocortical cells in the LGNd and LP. Large dots represent labelled cells and small dots represent labelled terminals. Anterograde and retrograde label is present in substantial amounts in both LGNd and LP. A faint presence of anterograde and retrograde label is also found in the medial most part of the LP. In the LGNd, the label is very defined in the ventral part of the nucleus.
Figure 22. Photomicrographs showing the HRP label and cytoarchitectonics in the SC in PY at 71d (A) and 81d (B and C) after a single defined placement of WGA-HRP in the visual cortex.

A. 71d. Magnified dark field view of labelled fibres (small arrow heads) in the deep layers at the rostrolateral margin of the SC. The large arrow head represents the lateral border of the SC.

B: 81d. Low power dark field view of the HRP label in the SC. The anterograde labelling is mainly contained in the SZ, SGS and the SO. Faint label is also seen under the SO (arrow).

C. 81d. Low power bright field view of an adjacent thionin stained cross section showing cytoarchitectonic features of the SC at this stage. The collicular lamination has emerged so that the SO, SGS and SZ become identified.

Bar: in A = 100µm; in B and C = 200µm.
**Figure 23:** Photomicrographs of cross sections of the visual cortex at 81d in a PY which received a collicular placement of WGA-HRP.

A: Dark field view of retrogradely labelled cells in the medial part of the visual cortex. These cells are positioned in layer V.

B: Thionin-stained section adjacent to A, showing the cytoarchitecture of the visual cortex at this stage. Layers V and VI have become distinct on the basis of cytoarchitecture. For a detailed description of the cytoarchitectonics of the visual cortex at this stage, see Figure 31 in chapter 4.

Bar = 200µm.
Figure 24. Intrahemispheric connections at 99d in the tammar. Sections in the series are numbered from the caudal occipital pole.

Upper: Series of camera lucida drawings showing the first appearance of the HRP label in area 18 and V-TP after a placement of WGA-HRP in area 17 of the ipsilateral visual cortex. The cross hatching in section 52 represents the region with a dense distribution of injected HRP. Large dots represent retrogradely labelled cells. Small dots represent anterogradely labelled terminals, and fine lines represent labelled axons. Cells are mainly distributed in layer V, except on rare occasions they are also seen in layer III in area 18. Axons from area 17 are primarily concentrated in layers V and VI and the underlying SP. The presence of labelled axons in the SP is obviously greater than in neighbouring regions. Dashed line across the cortex represents the border between areas 17 and 18.

A. A dark field view of a cross section showing the laminar distribution of anterogradely labelled terminals and axons and retrogradely labelled cells in area 18. Labelled cells are primarily in layer V, whereas in layer III, only scattered labelled cells are seen. Anterogradely labelled terminals are sparsely present primarily in layer V.

B. A bright field view of an adjacent thionin stained cross section demonstrating the laminar features of the labelled region in A.

Bar: A and B = 100µm.
Figure 25. Photomicrograph showing a dark field view of an example of anterogradely labelled interhemispheric axons (arrow head) in the white matter of the visual cortex following a placement of WGA-HRP in the rostrolateral part of area 17 in the contralateral visual cortex in a PY of 99d.

Bar = 100µm.
Figure 26. Interhemispheric connections at 111d in the tammar.

Upper: Camera lucida drawings showing sites of cortical HRP injections and label in the contralateral visual cortex. In this case, three defined injections of WGA-HRP were made in area 17, but only one of them was located close to area 17/18 border. Cross hatching in section 19 and 70 (upper corner of right side) represents regions with dense injected HRP. Dots in section 26 and 27 represent retrogradely labelled interhemispheric cells while fine lines represent interhemispheric axons. The interhemispheric cells have a widespread distribution at this stage, although interhemispheric axons, on the contrary, have a much restricted distribution adjacent to the area 17/18 border. Sections in the camera lucida drawing series are numbered from the caudal occipital pole.

A: The photomicrograph of the dark field view showing retrogradely labelled interhemispheric cells (arrows) in layer III in area 17.

B. The photomicrograph of an adjacent section stained with thionin showing the cytoarchitecture of the labelled region in A.

Bar = 100µm.
**Figure 27.** Interhemispheric connections at 122d in the tammar. Sections in the camera lucida drawing series are numbered from the caudal occipital pole.

Upper: Camera lucida drawings showing the site of an extensive cortical implant of detergent soaked-HRP chips and the resulting retrograde label in the contralateral visual cortex. At the injection site, the cross hatching represents the region with a high concentration of HRP, and the single hatching represents the region with a low concentration HRP. Dots represent retrogradely labelled cells. There is a widespread distribution of interhemispheric cells at this stage, particularly in layer III. In layer V, labelled cells are present in the vicinity of area 17/18 border. In area 18, the interhemispheric cells have a limited distribution in layer III.

Below: Dark field photomicrograph of a cross section with retrogradely labelled cells showing the laminar distribution of the interhemispheric cells in the border region of areas 17 and 18. The arrow head indicates the cytoarchitectonic border between areas 17 and 18. In area 17, retrogradely labelled interhemispheric cells are present in both layers III and V (arrows), whereas in area 18, they are primarily confined in layer III with a minimal presence in layer V.

Bar = 100µm.
Figure 28. A diagramatic summary of the time course for the formation of visual pathways in the tammar wallaby. In the diagram, each pathway is symbolized by either a solid line or broken line (only in the cases of retinal pathways), with the circle representing the cell body and the fork representing terminals. The time of the formation of each particular pathway is marked close to the line representing that pathway in number of days after birth.

The data on the time of formation of retinal projections to the contralateral LGNd and SC is from Wye-Dvorak (’84). The time of the formation of the retinal projection to the contralateral LP was reexamined in the present study and 17d was the earliest time examined. Given the fact that the retinal axons accumulated in the LP appeared more substantial than in the LGNd at this age (see figure 15), it is possible that retinal axons invade the LP at the same time that they invade the LGNd and SC.
Chapter 4. The Developmental Distribution of Axons and Cell Bodies Subserving the Afferent and Efferent Connections in the Wallaby Visual Cortex

Introduction

The anatomical description of the establishment of geniculocortical connections during development comes from studies on prenatal and neonatal placental mammals (Rakic, '76, '77c, '81; Anker, '77; Lund and Mustari, '77; Hollander et al., '79; Distel and Hollander, '80; Shatz and Rakic, '81; Shatz and Luskin, '86; McConnell and Shatz, '88; Peduzzi, '88).

The geniculate axons enter the telencephalon at a very early stage during development when their target structures in the cortex are still at the very beginning of their formation. For example, at the time when geniculate axons enter the cortex, neurons comprising the future cortical layer IV, the principle destination of the geniculate axons in adult, are just born, and have still not completed their migration to their appropriate location in the cortical plate, and those comprising the future layer VI are still highly undifferentiated (Berry and Rogers, '65; Rakic, '74, '76, 77c; Lund and Mustari, '77; Raedler and Raedler, '78; Kostovic and Rakic, '80; Luskin and Shatz, '85a,b; Shatz and Luskin, '86). As a result, the geniculate axons do not enter the cortical plate, from which the future cortical laminae will arise, but wait and accumulate in the underlying adjacent subplate zone (Rakic, '76; Lund and Mustari, '77; Shatz and Luskin, '86; Peduzzi, '88) containing the earliest born cortical cells (Luskin and Shatz, '85a,b) with neuron-like immunoreactivities (Chun et al., '87). It is postulated that these transient subplate cells may serve as the temporary targeting cells for the relatively early arriving geniculate axons (Shatz et al., '88).

The present experiment series was set to follow the experiments described in the last chapter. In previous experiments, by injecting the visual cortex with WGA-HRP
and labelling the cortical and subcortical structures in pouch young animals between 12 days (12d) and 122 days (122d) postnatal, a timecourse for the formation of the afferent and efferent connections to and from the visual cortex was established. Apart from confirming the previous results, this study concerns the laminar distribution of the axons and cells subserving the reciprocal corticogeniculate and cortico-LP connections during normal development, with particular interests in: whether in the tammar, as described in placental mammals, the geniculocortical axons have to wait for a considerably long period underneath the future cortex before they start to innervate the cortical layers at a relatively late stage and when and how the characteristic laminar specificity of the thalamocortical afferent termination and the corticothalamic efferents seen in mature visual cortex is achieved during development.

WGA-HRP has been applied directly in the visual part of the diencephalon, with, in most of our cases, a coverage of both LGNd and LP. Following anterograde and retrograde transportation, the disposition of thalamocortical axons and corticothalamic cells in the telencephalon at various stages during development are charted together with the cytoarchitectonics of the telencephalon.

**Materials and Methods**

**Animals**

Twenty-three PY animals aged from 22d to 118d were used in this study. They were obtained from a breeding colony in Canberra. The protocols for production of pouch young of desired age and for determination of age were previously described elsewhere (Chapter 3).
Surgery And WGA-HRP Injection

Animals were anaesthetized either by cooling in PY of 45d or younger or by injection of a mixture of ketamine and xylazine in older animals. The dose of xylazine (0.2mg per 100g body weight) was the same in all age groups, while the amount of ketamine varied according to the age of PY. In PY between 54d and 82d the dose injected was 1mg/100g body weight, and in PY at 99d and 118d it was 2mg/100g body weight. One injection of mixed ketamine/xylazine was sufficient for the experimental operation. The animal’s head was then immobilized on a holder with the chin levelled on the horizontal surface. The skull was opened either by cutting a triangular flap of bone when the bone was still soft before 35d or by drilling a hole at the desired sites. In animals of 35d and younger, when the telencephalon had still not grown dorsocaudally enough to cover the diencephalon, the presumptive LGNd and LP could be visualized on the dorsolateral aspect of the diencephalon after exposure of the brain. The administration of WGA-HRP was conducted by placing WGA-HRP coated pipettes (about 50µm in diameter) directly into the LGNd and LP. This was prepared just before the injection by making a highly concentrated solution of WGA-HRP, then immediately before it dried, placing the tip of the pipette into the solution so that the WGA-HRP could be stuck onto the tip of the pipettes. The detergent soaked-HRP chips (see chapter 3) were placed directly in the thalamus and were applied in some PY between 25d and 45d. The results of these cases with thalamic injection of detergent-HRP were used to compare results from cases with injections of WGA-HRP. In PY at 45d and older, the LGNd was approached vertically by needle attached to a microsyringe filled with 2% WGA-HRP solution penetrating through the telencephalon. The coordinates of the LGNd were different in each age group because of the different brain sizes and were obtained from measurements in formalin-fixed brain specimens of similar ages. The tip of needle was carefully cleaned before being
driven into the brain by a micromanipulator to minimize the possible involvement of structures passed by the injecting needle. The amount of WGA-HRP in each case using needle injection varied according to age from 0.015-0.050µl, in the hope that defined but condensed HRP uptake regions in the visual thalamus could be produced in PY of various ages.

Animals were kept in an incubator at 37° C for 12-28 hours depending on age.

Histology

At the end of the survival period, animals were deeply anaesthetized by cooling or an overdose of a mixture of ketamine/xylazine. Heparin and 1% sodium nitrite solution were injected into the left heart ventricle immediately before the perfusion was started with phosphate buffer (0.1M, pH 7.4) through the ascending aorta. This was followed by a perfusant containing 1% paraformaldehyde/1.25% glutaraldehyde in 0.1M phosphate buffer (pH7.4) for about 30 minutes. Following this was a wash of phosphate buffered 10% sucrose solution for another 30 minutes. Brains were then dissected and taken into phosphate buffered 20% sucrose solution for storage at 4°C overnight. On the following day the brain was embedded in a gelatin/albumen mixture as previously described (Chapter 2, Methods). With the thickness of 30µm in animals of 35d and younger, of 40µm in animals between 45d and 82d, and of 50µm in animals of 99d and 118d, frozen sections were cut in two series and processed for HRP by using the TMB method as previously described (Chapter 2, Methods). One series was counterstained with 0.025% thionin to appreciate the cytoarchitectonic features of the diencephalon and telencephalon at the various stages studied. Camera lucida drawings were made to demonstrate the distribution of the anterogradely labelled thalamocortical axons and the retrogradely labelled corticothalamic cells in relation to the formation of the visual cortex during development.
Results

The figures illustrating the following results are grouped together at the end of this chapter.

Formation of the Cortical Lamination

The following description on the formation of the cortical laminar organization is based on the observation in presumptive visual cortex in PY aged between 12d and 118d. The visual cortex can be easily recognized from neighbouring areas in PY older than 82 days, when layer IV is cytoarchitectonically distinct and the forming layer III overlying it is thicker in area 17 than in area 18 (see Chapter 2, figure 1). In younger PY, the visual cortex is assumed to be in a similar position in the dorsal and dorso-lateral part of the caudal pole, to that area confirmed as visual by the previous study in which WGA-HRP was injected in the cortical region and the LGNd and LP were labelled (see Chapter 3). The laminar organization of area 17 and 18 in the adult has been fully described in Chapter 2.

The terminology used in our description follows that designed by the Boulder Committee ('70), modified by Kostovic and Mollivar ('74). According to them the developing telencephalon has four major zones. The ventricular zone (VZ) adjacent to the ventricle is the region of neurogenesis. Above the VZ is the subventricular zone (SV). Overlying the SV is the intermediate zone (IMZ), the future white matter of the cortex. On top of IMZ is the cortical plate (CP). The CP consists of a concentration of cells which have migrated from the VZ and will form the future layers of the visual cortex. Besides, there is a dorsal most zone, the marginal zone (MZ). Kostovic and Mollivar ('74) have further subdivided the IMZ into an upper region with a lower density of more mature looking cells, the subplate (SP), and a lower region containing axon bundles.
12 days. At this stage, four fundamental zones are already present running parallel to the pial surface of the telencephalon (Fig. 29A). At the base of the telencephalon adjacent to the ventricle, the VZ is the most predominant zone in the telencephalon at this stage occupying about two thirds of the whole thickness of the telencephalon. Cells in this zone, with prominent nuclei oval in shape, are closely aligned in radial columns. Some mitotic figures can be seen in the zone. The SVZ is not yet formed at this stage. Above the VZ is the IMZ which at this stage contains some scattered pale staining cells which are probably migrating towards the CP. The CP is now only 2-3 cell in depth. Cells in the CP are deeply stained but still not yet as densely packed as at late stages, indicating that the CP is just at the beginning of its formation. Cells in the CP are mostly round in shape. Overlying the CP is the marginal zone. Cells in this zone are small and very sparsely distributed.

From 16 to 36 days. During this period, the transformation of the cytoarchitectonics of the telencephalon is characterized by the continuing growth of the CP and underlying IMZ and SVZ. Two cytoarchitecturally distinct sublayers are becoming apparent in the CP by 16 days, in particular in the more lateral part of the telencephalon (Fig. 29B). In the upper part of the CP, cells become extremely densely packed from 16d and, probably because of this, become oval or even spindle like shaped. Thus this sublayer, as also described in other species, is termed the cell compact zone. The cell compact zone during this period grows from 3-4 cells to 5-6 cells in thickness, and will not increase dramatically throughout the course of development. In Nissl-stained preparations, the cells in this at all stages during development have an immature look (Figs. 29-31). Underlying the cell compact zone is the lower part of the CP with less densely packed cells. With moderately dark staining, cells in the zone are rounder than those in the cell compact zone (Figs. 29B-D). It is becoming obvious from 20d that cells in the low part of the CP are aligned in loosely packed rows parallel to the cortical surface (Figs. 29C&D). This unique feature of the cellular organization will persist into late stages of development until the
mature pattern of the cortical lamination emerges. In contrast to the limited growth of
the cell compact zone in thickness from this stage on, the lower part of the CP will
increase its thickness very rapidly at subsequent stages to form most of the future
visual cortex. By 36d, the entire thickness of the CP starts to exceed that of the VZ.
Also noticeable at 36d is that a cell sparse cleft is emerging underneath the cell
compact zone (Fig. 29D). With its relative position moving downwards with the
addition of the new born cells on its top, this cell sparse cleft will persist into late
stages (Fig. 30) until it disappears by 85d.

There is relatively little change in cytoarchitectonics within the IMZ during this
period, apart from its growth in thickness. By 16d, the SVZ on top of the VZ becomes
distinct. Round in shape with moderate staining quality, cells in this zone are also
closely packed, though not as much as those in the cell compact zone and underlying
VZ (Fig. 29B). The VZ during this period remains the most prominent zone in the
telencephalon, despite the fact that its proportion of the whole depth of the
telencephalon is increasingly reduced due to the rapid growth of other zones. The MZ
also increased its depth substantially during this period and cells within it appear to be
more mature looking than those in other zones

42-76 days. During this period, the most dramatic changes occur in the lower
part of the CP just underneath the cell compact zone. First, as the result of a
continuing increase in thickness, the CP becomes the thickest structure in the
telencephalon. From 42d onwards, if put together with the MZ, the future cortical
layer 1, the CP occupies approximately half of the whole depth of the telencephalon
(Fig. 30). Second, a cell sparse cleft, which is first seen at 36d underneath the cell
compact zone, becomes increasingly obvious during this period. At the beginning of
this time period it is first seen at more rostral and lateral levels in the cortex and
becomes apparent more caudally later. This cell sparse cleft persists at the subsequent
times observed at 48d, 56d (Fig. 30B), 65d (Fig. 30C) and 76d (Fig. 31A). It becomes
more and more apparent from 56d that this cell sparse cleft separates the low part of
the CP into two subdivisions with different cytoarchitectonic features (Fig. 30B&C). By 76d, this cell sparse cleft is localized between cytoarchitectonically and connectionally identified cortical layers V and VI (Fig. 31A). Third, there are some obvious cytoarchitectonic changes during this period. For example, by 56d, it is recognized that cells underneath the cell sparse cleft are small, round, and less densely packed, while those above the cell sparse cleft are larger, and more densely packed (Fig. 30B). Furthermore, while it appears that cells above the cell sparse cleft are still organized in parallel rows, there is no suggestion of this cellular organization below the cell sparse cleft. By 65d, though the parallel alignment of cells in the subdivision above the cell sparse cleft seems also to have disappeared, the difference in cytological features between the two subdivisions becomes more obvious (Fig. 30C). Another noticeable change is the formation of a new layer of cells underneath the cell compact zone by 76d. Cells in this newly formed layer are almost equally densely packed and deeply stained with those in the cell compact zone, but are apparently smaller and granule like in shape. This layer starts to become separated from the cell compact zone by an intervening layer of less densely packed cells of larger soma size (Fig. 31A).

The IMZ during this period can be cytoarchitecturally divided into two zones: a relatively cell-free zone just beneath the CP, the SP, and an underlying cell-rich zone of palely stained cells. With little change in cytoarchitectonics, both the VZ and SVZ are reducing in thickness sharply during this period, while the marginal zone, on the other hand, approximately doubles its thickness during this period (Fig. 30). From 48 days, the cells in the MZ become considerably mature looking.

82 days. By this stage, the layer of granule cells first seen at 76d is positioned further down away from the cell compact zone. This is presumably the future layer IV. Underneath this granule cell zone, layers V and VI can be identified now on the basis of cytoarchitectonics, as cells in both layers start to acquire some cytological features characteristic of the adult (Fig. 31B). For example, some cells in the future
layer V begin to possess basic features typical of large pyramidal cells, and some of those in the future layer VI have become more like the mature pyramidal cells of layer VI. The results on the distribution of retrogradely labelled cells at this stage (Fig. 43&44, see following section) are consistent with the cytoarchitectonic identification of the future layers IV, V and VI at this time. Above and beneath the future layer IV granule cell zone, cell density is noticeably reduced compared to the neighbouring layers.

There is little change in the IMZ at this stage from the previous stage. While the SVZ is still present as a thin concentration of undifferentiated cells, the VZ has virtually disappeared (Fig. 31B).

**99 days.** At this stage, layer IV, V and VI have undergone further maturation both cytoarchitecturally and cytologically (Fig. 31C). Between the cell compact zone and layer IV, the future layer III is forming and cells in this intervening layer are acquiring their cytological features. In the IMZ, the cell density is largely reduced compared to the previous stage. More ventrally, the characteristic SVZ is not identifiable on the basis of cytoarchitectonics at this stage, although a concentration of cells is still present in the region adjacent to the ventricular surface.

**118 days.** At this stage, the cell compact zone has reduced its thickness to 2 cells. From the first glimpse, it seems that all cortical layers have acquired the mature pattern (compare with adult, figure2-Chapter 2) in their cellular organization (Fig. 31D). However, there are still signs of immaturity of the visual cortex at this stage. For example, cells comprising the cell compact zone are still largely undifferentiated. Moreover, there are still large numbers of cells in the future white matter whereas in the adult cells are extremely sparse. Nonetheless, much of the characteristic laminar organization typical of the mammalian primary visual cortex is formed by this age in the tammar.
Developmental Distribution Of Thalamocortical Axons and Corticothalamic cell bodies in the Visual Cortex

Data were collected in 11 PY in which the visual thalamus was administered successfully with either WGA-HRP or detergent HRP. In most of the cases, the thalamic injections covered both LGNd and LP, but in a few cases it spread into neighbouring diencephalic structures as well (see below).

The anterograde label, in the form of granules of HRP products in the neuropil, was interpreted as the labelled thalamic axons innervating into the telencephalon, while the retrograde label, in the form of clusters of granules of HRP products within the neuronal cytoplasm, was interpreted as the cortical cells projecting to the thalamic region receiving the HRP injection. Without any reliable cytoarchitectonic characteristics in PY younger than 82d, the region destined for future visual cortex was localized in the dorsal and dorsolateral part of caudal pole of the telencephalon, since HRP injections in this region in animals of the same range of ages retrogradely labelled the LGNd and LP (see Chapter 3).

The PY aged 22d was chosen as the youngest in this experimental series for the reason that this is about the earliest time that a reasonable amount of retrograde labelling was found in presumptive LGNd and LP after cortical injections of WGA-HRP (Chapter 3). The oldest age examined was 118d, when the mature pattern of cortical lamination is emerging.

22 days. After histochemical processing much of the lateral diencephalon contained dense reaction products, including both the LGNd and LP in this case. Part of the medial wall of the telencephalon was also labelled from the injection site, although this was largely limited to the most medial part and was not likely to contribute significantly to the robust cortical anterograde labelling which could be followed from the injection site in the diencephalon along the thalamic optic radiation into the telencephalon. Labelled axons only reached the most rostral part of the
presumptive visual cortex. The thalamic axons had the greatest concentration in the optic radiation in the IMZ, but were also substantially distributed across the lower part of the CP (Fig. 32). At high magnification, some labelled axons could be seen entering the lowest part of the cell compact zone of the CP. However, much of this subdivision and overlying MZ were free of anterograde labelling.

**25-28 days.** In this age group, the detergent HRP was used in the LGNd and LP injection. The injection was focused in the dorsolateral part of the caudal diencephalon covering both LGNd and LP, with some spread to the medial wall of the telencephalon. Anterograde labelling was first seen in the marginal zone at 25d and became more dense by 28d (Fig. 33). It was noticed that the anterograde label in the MZ in this case was even higher than that seen at later stages, e.g., 35d and 45d (Fig. 35&36). The reason for this was not clear, although it seemed unlikely that this was contributed by the spread of HRP from the injection site as there was a clear gap between this and the transported label. Sparse label also appeared in the cell compact zone of the CP in this case, but was much less significant in comparison to label in the overlying MZ and the underlying region of the CP (Fig. 33A).

**35 to 65 days.** There are four ages in this group: 35d (Fig. 34), 45d, 54d (Fig. 38) and 65d. After histochemical processing, it was obvious that the HRP administration in the cases of 35d, 45d and 54d animals was extensive. As a result, while both LGNd and LP were involved in the HRP uptake in these cases, some other thalamic structures were also involved (see below). For example, in all these three cases, the dorsomedial part of the ventral basal nucleus medial to the LGNd was covered by intensive HRP reaction product. Furthermore, in the case of 45d animal, the HRP injection also involved the optic radiation leaving the thalamus and the surrounding nuclei. In the case of 65d animal, the injection was focused in the lateral margin of the LP and was much less intensive and extensive than those in other cases in this group.
The Anterograde Labelling. During this period, the distribution of anterogradely labelled thalamic axons in the telencephalon was characterized by several features. First, throughout this period, in regions where there was intense anterograde labelling it was densely and uniformly distributed in much of the CP, leaving only the cell compact zone uninnervated, and in the cell-sparse part of the IMZ, the SP (Fig. 35, 36, 39&40). In the deeper part of the IMZ, with reduced intensity, labelled bundles of axons could also seen (Fig. 34, 35, 36&38). In each case with a successful thalamic HRP deposit, the densest concentration of labelled thalamic axons can be expected in a region covering the SP and the entire region with less densely packed cells in the CP (Fig. 35, 36, 39&40). Second, there is a progressive enhancement of the termination of the thalamocortical axons during this period in the MZ. For example, at 35d, only sparse labelling is detectable in the MZ (Fig. 35C). By 45d, the MZ labelling is obvious when observed in the dark field (Fig. 36) and by 54d, label in MZ becomes very substantial (Fig. 40). Third, at any stage observed, no substantial labelling was found within the cell compact zone except the lowest part adjacent to the underlying less densely packed cellular zone (Fig. 35C), although in dark field the HRP labelling is at detectable levels (Fig. 36C). At high magnification, labelled fibres passing through this region of the CP can be seen occasionally (Fig. 41A).

In the cases of 35d (Fig. 34), 45d and 54d (Fig. 38), almost the whole of the presumptive LGNd and LP were covered by HRP injection, and the areal distribution of anterograde label was extensive. By 35 days anterograde label could be followed from the thalamic radiation into the visual cortex where it was concentrated dorsally. It could now be followed right to the caudal pole of the cortex (Fig. 34). At 45d and 54d, the anterograde labelling ended abruptly at the dorso-lateral aspect of the telencephalon (Fig. 38&39).

The Retrograde Labelling. The convincing evidence of characteristic retrograde label was first obtained at 45 days. In vertical distribution, the retrogradely labelled
cells were largely defined in two bands. One band was in the cell compact zone of the CP. In a given region with HRP label, it seemed that a large proportion of the cells were labelled in the cell compact zone (Fig. 36). These labelled cells were actually the occupants of the cell compact zone and, therefore, had the characteristic olive or spindle like shapes. Another band of labelled cells was found in the lower part of the CP underneath the cell sparse cleft (Fig. 37). At high magnification, these cells appeared to be less densely labelled and more spindle-like in shape than those in the cell compact zone (Fig. 37C).

Largely due to the relatively extensive HRP injection in the thalamus in this case, the retrogradely labelled cells have a wide areal distribution. Labelled cells in the cell compact zone were distributed widely in a region extending rostrocaudally from the caudal pole of the telencephalon to the presumptive somatosensory cortex and mediolaterally from the centre of presumptive area 17 at the caudal pole to the ventrolateral part of the telencephalon at the rostral end of the label (Fig. 37). In more ventrolateral regions these are cells deeper in the cell compact zone than further dorsally (Fig. 37). On the other hand, labelled cells in the lower part of the CP did not extend to the caudal pole. They were only present in more rostral and ventrolateral regions and their distribution appeared to be correlated with the presence of the cell sparse cleft in the CP. In the more dorsal and caudal parts of the cortex this was absent and so was this lower group of labelled cells. Figure 37A&B shows these labelled cell in lateral cortex ending at approximately the same point as the cell sparse cleft disappears. In the caudal part of the telencephalon, while labelled cells situated dorsally are clearly within the presumptive area 17, those in the more ventrolateral part of the telencephalon are probably in the future area 18 and V-TP visual areas (Chapter 2). More rostrally, labelled cells situated more dorsally may be in the somatosensory cortex, whereas those situated more ventrolaterally may be in the auditory cortex.

The retrograde labelling at 54d differed from that seen at 45d in both the distribution in the depth of the CP and the distribution in the extent of the
telencephalon. In the depth of the CP, the position of the labelled cells in the upper part of the CP had shifted downwards beneath the cell compact zone, presumably being displaced inward by newly arrived cells now forming the cell compact zone (Fig. 41). The position of labelled cells in the lower part of the CP was relatively unchanged (Fig. 39&41). In areal distribution, the retrograde label was not as extensive as that seen in the 45d animal, presumably due to the more defined HRP injection in the thalamus (Fig. 38). For example, labelled cells beneath the cell compact zone seemed to be primarily present in the presumptive area 17 and in an area rostral to it, possibly the future somatosensory cortex. The distribution of labelled cells in the lower part of the CP were apparently limited in the caudal part of the telencephalon. While not so obvious dorsally in what is clearly the future area 17, they were mostly situated more laterally, in an area which probably includes area 18 and V-TP (see Chapter 2.).

At 65 days, due to lower intensity of the HRP injection in the thalamus in this case, the label in the telencephalon was relatively faint. Regionally, the retrograde label was only detectable in presumptive area 17 (Fig. 42A&B). In the depth of the CP, the labelled cells above the cell sparse cleft were displaced further down (Fig. 42A&B), while those under the cell sparse cleft were not obvious in this case possibly due to the fact that the LGNd in this case was minimally involved in the HRP uptake. Label in the MZ was strong presumably due to the major involvement of LP in the injections.

76 days. In this case, the injection of WGA-HRP was focused in the dorsolateral thalamus covering both the LGNd and LP, and possibly the neighbouring medial geniculate nucleus. As a result, the retrograde labelling was distributed in area 17 and areas ventral to it, which included area 18 and V-TP, while more rostrally, the retrogradely labelled cells may be in the future auditory cortex. Two bands of labelled cells could now be seen clearly within the cytoarchitectonically identifiable layers V and VI at this stage (Fig. 42C&D).
82 days. In this case, a single WGA-HRP injection was made into the dorsolateral thalamus and covered both the LGNd and LP (Fig. 43).

The Anterograde Labelling. Coincident with the dramatic cytoarchitectural change witnessed at this stage, that is, the histological emergence of the future layer IV, V and VI, the initially uniformly distributed HRP labelling in the CP first showed variations in density. Six bands of label parallel to the pial surface could be recognized (Fig. 44A&B). The upper most band was in the MZ; the second covered a region from the deepest part of the cell compact zone down to the top of granule cell zone of the future layer IV; the third which was less dense was over the granule cell zone; the fourth was over the future layer V and the fifth was over the future layer VI; and finally, a thin band of anterograde labelling was in the underlying optic radiation. At this stage, while retrogradely labelled cells had a similar laminar distribution to that in mature animals (see below), the anterogradely labelled axons were still distributed in the visual cortex in a quite different mode from those in mature animals in two ways. First, the geniculocortical axons were still not yet accumulated most predominantly in layer IV as in the mature cortex. Rather, they seemed to be most dense in layer VI. Second, thalamocortical axons were more widespread throughout the depth of the cortex than in the adult. Labelled axons were also present in a large part of the emerging layer III and the whole of layer V, whereas in the adult, they are mostly concentrated in a restricted part of layer III and the upper part of layer V (Chapter 2). In areal distribution, apart from in area 17, there was a remarkably intensified termination of thalamocortical axons in area 18 at the area 17/18 border, which can be cytoarchitectonically identified at this stage (Fig. 43&44C&D). In this region, with the most intensive concentration over the future layers I and IV, anterograde labelling was present in substantial amounts throughout all of the MZ, CP, and SP, but was insignificant in the cell compact zone though at a detectable level. The anterogradely labelled axons extended for a short distance in area ventral to area
18, but were absent further ventrally despite the presence of large numbers of retrogradely labelled cells in these areas (Fig. 43).

The Retrograde Label. The labelled cells were aggregated densely and exclusively in layer V and VI which were easily identified by cytoarchitecture at this stage (Fig. 44). The individual labelled cells had started to acquire the mature cytological features of pyramidal cells.

The areal distribution of labelled cells in both layers V and VI was wide (Fig. 43). They extended in a continuous fashion from the medial part of area 17 lateroventrally to the more ventral part of the lateral aspect of the cortex covering area 18 and the V-TP, both proved to be connected with the LGNd in the adult (Chapter 2). Rostrocaudally, retrograde label seemed to reach the rostral margin of area 17. The density of the layer VI retrograde labelling varied in different cortical areas. Like the anterograde labelling, the retrograde labelling in area 18 at the 17/18 border region was enhanced (Fig. 44C&D).

99 days. In this case, the WGA-HRP injection was primarily focused in the LGNd. HRP reaction products were intensive in both α and β segments, suggesting both were sites of uptake. HRP uptake may also have occurred at the lateral margin of the LP, since a limited number of retrogradely labelled cells were present in the upper part of layer V (see below. Fig. 45A).

The Anterograde Labelling. As Figure 45 shows, at this stage the heaviest aggregation of geniculate axons was over the granule cell zone, layer IV, while a lighter presence of these was found in layer VI. In dark field, label was also observed over the upper part of layer I, the forming layer III, and layer V (Fig. 45C). In areal distribution, the anterograde labelling was only found in area 17 and stopped abruptly at the cytoarchitecturally identified area 17/18 border.

The Retrograde Labelling. Regionally, the retrogradely labelled cells, like the anterogradely labelled axons, were also only present in a defined region in the lateral part of the caudal visual cortex and stopped rather sharply at the border of areas 17/18.
Noticeably, however, the continuous band of retrogradely labelled cells had a wider regional distribution than the anterogradely labelled axons within the area 17 probably because retrograde labelling is easier to demonstrate than anterograde labelling. The labelled cells were mainly distributed in cortical layer VI projecting to the LGNd. In layer V, retrograde labelling was so sparse that only a few large pyramidal cells were labelled (Fig. 45A&B), presumably due to the fact that the LP was minimally involved in the HRP administration in this case.

118 days. In this case, the WGA-HRP injection was localized in a similar part of the thalamus to that in the 82d animal in that both LGNd and LP were covered. As a result, the anterograde labelling was present in both area 17 and area 18 region (Fig. 46). In area 17, the laminar distribution of the anterogradely labelled thalamocortical axons had basically assumed its mature pattern at this stage. As shown in figure 46A&B, the thalamocortical axons were heavily concentrated over layers I, IV and VI, with a less dense termination over the lower part of layer III and upper part of layer V. In area 18 in the border region adjacent to area 17, thalamic axons were very densely concentrated in virtually all cortical layers with the most substantial distribution found in layers I, III and IV (Fig. 46C&D).

The Retrograde Labelling. The retrograde labelling in this case was very much the same as that observed in the 82d animal in the laminar and areal distribution pattern.

In laminar distribution, the retrogradely labelled cells were only present in layers V and VI, with labelled cells now having mature cytological features in both layers (Fig. 46).

In areal distribution, as in the 82d animal, the labelled cells extended widely. Apart from in most parts of area 17, labelled cells were also found in area 18 (Fig. 46C) and V-TP in the more lateroventral part of the caudal cortex. Again, retrogradely labelled cells in layer VI formed a wide band in area 17, but were more densely and
intensively aggregated in area 18 (Fig. 46C), whereas in V-TP, the width of labelled cells was much reduced.

In summary, anterogradely labelled thalamic axons were found densely and evenly distributed in much of the entire depth of the CP from 22d to 65d. By 82d, with the first appearance of the future layers IV, V and VI, the thalamic axons seemed more concentrated over these forming layers. By 99d, when layer IV and VI start to resemble the mature pattern, the LGNd afferent termination becomes identical to that seen in the adult. In contrast to the widespread distribution of anterogradely labelled thalamocortical axons in the telencephalon at the early stages, the retrogradely labelled corticothalamic cells were defined in two bands after HRP injections covered both the LGNd and LP from when they were first seen at 45d. Finally, perhaps due to the widespread administration of the HRP in the thalamus, areas in the presumptive somatosensory and auditory cortex were also labelled.

Discussion

The present study was designed to examine the developmental distribution, during the ontogeny of the telencephalon, of axons and cells subserving the afferent and efferent corticothalamic connections with the LGNd and LP. It was hoped that the direct application of WGA-HRP in the LGNd and/or LP and its anterograde transport would provide a more sensitive method than those used in previous studies (Rakic, '76; Anker, '77; Lund and Mustari, '77; Shatz and Luskin, '86) to appreciate the spatial relationship between the formation of the visual thalamocortical connections and the maturation of the cortical cellular lamination. Also its retrograde transport would provide information on the developmental distribution of cells of origin of descending connections from the cortex. This approach was possible in our case by using the pouch young of the tammar where access to the thalamus was relatively
easy, as the formation of visual thalamocortical and corticothalamic connections occurs after birth (Chapter 3).

Formation of the Cortical Lamination

The nomenclature of developing telencephalic structures revised by the Boulder Committee ('70) and modified by Kostovic and Mollivar ('74) is followed in this study. In comparison with a previous description on the cytoarchitectonics of the developing telencephalon in the tammar (Reynolds et al., '85), the layering scheme used in this study is different, particularly in the definition of the CP and the SP. While Reynolds et al. ('85) suggested that the CP contained only the equivalent of what is called the cell compact zone in this study, and the entire region of less densely packed cells was the SP, both are regarded as the CP in this study for two reasons. First, on the basis of cytoarchitectonics, according to Kostovic and Mollivar ('74), the SP is very low in cell density and is situated in the upper part of the IMZ just overlying the optic radiation. As figure 30 shows, this is also clear in the PY of the tammar. Although by using the autoradiographic methods, the SP in the cat has recently been considered, by the criterion of cellular fate, to extend slightly further dorsally into the base of the CP (Luskin and Shatz, '85a), it is obvious that the SP only occupies the lowest part of what was previously thought as the base of the CP. It is highly unlikely, therefore, that the whole of the less densely packed zone in the tammar during development could be the SP. Second, according to the definition of the CP, at least most of the less densely packed zone should be considered as the cortical plate. It is generally agreed that the CP comprises cells designated for the future cortical layers. Autoradiographic studies in placental mammals (Kostovic and Rakic, '80; Luskin and Shatz, '86) have already shown that much of the region below the cell compact zone will become part of the cortex, as occupants there are identified as layers V and VI cells in the adult. With the lack of the similar kind of data in the
tammar, it is still unclear at the moment if and how much of the lower part of the less densely packed zone could be considered as the SP on the basis of cellular fate. However, from the present study, the cells projecting to the LGNd and LP, which in the adult are situated in cortical layers V and VI, were all clearly localized in the less densely packed zone from very early in development. To this end, it appears that in the tammar, a large part, if not all, of the less densely packed zone is part of the CP, certainly from 45 days onwards when labelled cells can be identified within it.

In a tritiated thymidine autoradiographic study, it has been reported that the cortical neurogenesis in the tammar also follows the inside-out pattern common to other mammals (Lund and Mustari, '77; Rakic, '77c; Luskin and Shatz, '85a,b) in that the neurons comprising the deeper cortical layers are born earlier than those comprising the more superficial layers (Reynolds et al., '85). Unfortunately, the study only covered a very limited range in age and no conclusions could be made with regard to the timing of the birth of cortical neurons comprising particular cortical layers. However, there are some transient landmarks and cytoarchitectonic features in the telencephalon during development, which can be used to predict when overall cortical neurogenesis and cell migration might end, and when some of the cortical layers might be formed. For example, the presence of the VZ can be taken as an indicator of ongoing neurogenesis. In our preparations, the histologically characteristic VZ became indistinct by 85d, suggesting that the generation of cortical cells was ending around this stage. Similarly, the ongoing process of cortical cell migration could be inferred by the presence of the cell compact zone of the CP. It was observed that the cell compact zone in the tammar became reduced to 2 cells in thickness and started to resemble layer II in the adult by 118d, thus the overall migration of the cortical cells is probably largely over by this stage. It was also noticed that there was a cell sparse cleft in the less densely packed zone of the CP. It first emerged in more lateral and rostral parts of the telencephalon beneath the cell compact zone by 35d and became apparent at subsequent stages from 42d to 85d. This
zone separates the low part of the CP into two divisions and seems eventually to vanish between layers V and VI. It is thus possible that the subdivision beneath this cell sparse cleft may become layer VI, whereas the subdivision overlying this cell sparse cleft may become layer V. In this light, it suggests that the layer VI cells may largely complete their migration by about 35d, since the cell sparse cleft emerges at the upper part of the CP at this stage. Another distinct landmark in the telencephalon from 75d is the first appearance of the granule cell zone of the future layer IV characterized by the densely and homogeneously packed small sized granule cells. This zone becomes separated from the cell compact zone by 75d. This suggests that the future layer V forms between 35 and several days earlier than 75d, and the future layer IV forms before 75d. However, it should be pointed out that this description is based solely on cytoarchitectonic observation and lacks sufficient accuracy required for fine analysis of the generation, migration and differentiation of the cortical neurons destined for each particular layer during the ontogeny of the visual cortex. To do so, a systematic autoradiographic analysis is required.

It is obvious from our observation that there are some remarkable interspecies differences in cytoarchitectonics of the telencephalon between marsupials and placentals. For example, during early stages, the cell compact zone of the CP of the marsupials is only about 4-6 cell in thickness and occupies relatively a small proportion of the entire depth of the CP, whereas in placentals, from the author's observation on published photographs, a large proportion of the entire depth of the CP is very densely packed with deeply stained undifferentiated cells (Lund and Mustari, '77; Luskin and Shatz, '85a, b; Rakic, '74, '88). This may be explained by the different dynamics of neuronal proliferation and migration in the telencephalon during the formation of the cortical layers. It was noticed that the cortical formation in the tammar takes a much longer period to accomplish than in placental animals of similar size. For example, in the tammar it is about 110 days between the first arrival of the earliest born cells to their destination in the CP (Reynolds et al., '85) and the
emergence of the six layered laminar organization, whereas in the cat this is only about 50 days (Luskin and Shatz, '85b).

Development of the Thalamocortical Projections

The present results show that by 22 days, substantial amounts of thalamocortical axons have innervated the telencephalon, thus further confirming the timecourse for the formation of the geniculocortical and LP-cortical projections, which was established by previous experiments using cortical injection of WGA-HRP and retrograde labelling of the LGNd and LP neurons (Chapter 3). As mentioned earlier, the formation of the geniculocortical and the LP-cortical projections are remarkably earlier than that seen in a number of placentals (Chapter 3, also see the summary diagram in Chapter 5). The likely factors which may contribute to this are discussed elsewhere (Chapter 3, discussion).

The comparison with what has been described in placentals (Lund and Mustari, '77; Rakic, '77c; Shatz and Luskin, '86) shows that the developmental pattern of the terminal distribution in the visual cortex in the tammar is remarkably different. First, the distribution of thalamic axons in the CP/SP region is particularly wide in the tammar. It is clear that, in the tammar, at each stage during the period from 22d to 82d, thalamic axons are distributed densely and widely in the CP/SP region, leaving only the thin cell compact zone of the CP not innervated by substantial amounts of thalamic axons. In placentals, in contrast, the axons from the LGNd are still largely restricted in the SP and lowest part of the CP plate (Lund and Mustari, '77; Rakic, '77c; Shatz and Luskin, '86). This remarkable contrast in the termination pattern of the thalamic axons in the telencephalon can be best appreciated by comparing an 82d PY wallaby and a E52 fetal cat, as they are at a similar developmental stage in that the future layers IV, V, and VI have become cytoarchitectonically identifiable. In the wallaby, the thalamic axons are present in each of the layers IV, V, and VI in
substantial amounts. In cat, however, the majority of the geniculocortical axons are still accumulated in the SP and the lowest part of the layer VI (Shatz and Luskin, ’86).

There are several possible factors which may contribute to the different modes of ingrowth of thalamocortical axons into the future cortical layers between the wallaby and the placental mammals. One factor could be technical. As already pointed out earlier (Chapter 3), the direct application of WGA-HRP followed by the anterograde transport enables the detection of the earliest axons innervating the target structures more sensitively than the transneuronal transport of the autoradiographic activity or nerve degeneration in a number of ways. In the particular situation of detecting the ingrowth of the thalamic axons in the CP, similarly, by the direct application of HRP in the visual thalamus, large amounts of the tracer enzyme can be taken up in the region of injection and transported directly into the target cortical structures, whereas in the situation of transneuronal transportation, the amount of label substance transported into relay cells in the LGNd is largely limited at the synaptic junctions between retinal axons and geniculate cells projecting to the visual cortex. It is estimated that only 1-3% of the tracer amino acids taken up and transported by retinal ganglion cells can successfully pass synaptic junctions and be taken up by geniculate cells (Grafstein, ’71). It is very likely that in young animals, when the synaptic connections are still not fully mature, such a coefficient of the synaptic transfer through the retino-geniculo-cortical system could be further reduced. Furthermore, by use of HRP in tracing the neuronal connections, one can also avoid the problem of the general background label which, in the case of transneuronal autoradiographic transportation, can be caused by the autoradiographic processing for the demonstration of the labelled axons. This general background label largely reduces the sensitivity of the technique in detecting the real label, particularly in the case using developing animals, as the background label is even higher so that the earliest terminal label could be overshadowed (LeVay et al., ’78). However, whether and how much the difference in techniques accounts for the difference in results seen
in the tammar and the placental mammals is open to questioning until the same method is used in a placental.

A certain degree of interspecies difference may also underlie the different spatiotemporal mode of ingrowth of the thalamic axons into the CP/SP zones between placentals and marsupials during development. As mentioned earlier, there is a distinct difference in the cellular construction of the CP between marsupials and placentals. In marsupials, throughout the development, in most parts of the CP the cell density is moderate, only in a thin band on the top of the CP are cells very densely packed. While thalamocortical axons are dense in the less densely packed zone, their presence in the overlying cell compact zone is virtually absent. In placentals, on the other hand, most parts of the CP are very densely packed with cells during development except at late stages when the density of the cells in the CP is reduced and the cytoarchitectonically characteristic lamination emerges. From the literature, it appears that in placentals the thalamic axons begin to grow into more superficial part of the CP only when the cell density in this part of the CP is largely reduced and the volume of the neuropil increases (Lund and Mustari, '77; Shatz and Luskin, '86). For example, in cat, before embryonic day 55, almost the entire depth of the CP is very high in cell density and the innervating geniculocortical axons seemed only to be accumulated in the lower density SP and the lowest part of the layer VI. It is not until the cell density is largely reduced by birth that significant amounts of geniculocortical axons become accumulated in layer IV (Shatz and Luskin, '86). Thus it seems in both marsupials and placentals that the cell density may somehow determine or regulate the ingrowth of the afferent axons into the CP. For example, perhaps for a simple physical reason, the ingrowing thalamocortical axons are just not able to penetrate into the very densely packed cells. Also possible is that there might be certain inhibitory factors in the cell compact zone which might prevent the ingrowth of the thalamocortical axons. It has been reported that cell density can influence certain growth factor-induced cellular mitogenesis such that the cell growth is arrested in
dense culture (Paulsson et al., '88). In this light, it is possible that the ingrowth of the thalamocortical axons into the CP during development may be induced by certain growth factors in the telencephalon and that such inductive effects by growth factors may be regulated by the cell density of the CP. It was of concern that transneuronal transportation of WGA-HRP may have occurred in our case, thus contributing to the wide spread of terminal label in the CP. WGA-HRP has been used as a transneuronal tracer in a number of studies (Gerfen et al., '82; Itaya and Van Hoesen, '82; Peduzzi, '88). However, it is not likely to play a role in our case for a number of reasons. First, the animals in our cases are left to survive for only 12-28 hours after WGA-HRP application. Longer survival times than this have been used for transneuronal transportation. Second, there is no apparent transneuronal pathway known at this stage. Further there was no diminution of label in upper parts of the CP which would be predicted if this label was resulting from transneuronal transport. Finally, free HRP rather than WGA-HRP gave a similar distribution.

As early as 45 days anterograde label was found to stop abruptly laterally in the visual cortex reminiscent of the distribution of label seen at the 18/V-TP border region in some adult cases and the 17/18 border at 99 days. This suggests that these borders may exist long before they can be recognized cytoarchitecturally and that thalamocortical axons are distributed in a similar fashion very early in development with respect to these borders as they are in the adult. Certainly by 82 days, approximately two months prior to eye opening, when the 17/18 border can first be recognized cytoarchitecturally the areal distribution of label within areas 17, 18 and V-TP was similar to that seen after similar injections in adults.
Disposition of the Corticothalamic Cells During Development

The earliest time that convincing evidence of retrogradely labelled cells in the telencephalon became available was 45d, when two bands of labelled cells were found in the low part of the CP and in the cell compact zone. Judged on the observations made on these two bands of labelled cells at subsequent times, it seemed likely that the cells aggregated in the low part of the CP eventually become the layer VI cells projecting to the LGNd, and those first seen in the cell compact zone become continuously replaced downwards by later migrating cells and eventually become situated in the upper part of layer V. From these observations it appears that cortical axons from both future layers V and VI have reached their thalamic target structures by 45d. This is consistent with the previous finding that cortical axons begin to innervate the LGNd and LP in substantial amounts between 42 to 48 days (Chapter 3). Thus the present results confirm the timing of the formation of the descending cortical projections to the LGNd and LP revealed in the previous cortical WGA-HRP injection study (Chapter 3). The two bands of labelled cells were only seen initially in more rostral and lateral parts of visual cortex whereas at later times they extended caudally and dorsally suggesting that rostrolateral regions of cortex were more mature. Thus, the single band of labelled cells within the cell compact zone further caudally at 45 days probably represents later developing cells which will eventually be situated in layer VI. A rostrolateral to caudomedial gradient of maturation in the developing rat cortex was also noted by Lund and Mustari ('77).

It is also clear that, throughout development, unlike the anterogradely labelled axons, the distribution of the retrogradely labelled cells throughout the depth of the CP is rather defined in the bands corresponding to the appropriate cortical layers in the future. Thus it appears that only those cortical cells located in the appropriate cortical layers are able to project to their appropriate thalamic target structures. This suggests
that the origin of the corticothalamic projection has a high degree of laminar specificity from the beginning of its formation.

On the other hand, it is not possible to determine in detail from the present results if and how the regional or areal specificities have been achieved by corticothalamic cells in the telencephalon, because of the large sizes of the HRP injections in the diencephalon. As already mentioned earlier, in most of the cases, the HRP administration was widespread and covered both the LGNd and LP and in the youngest animals involved other thalamic structures as well. In the case of interhemispheric connections, it has been well-documented in placentals that the callosal cells have a widespread distribution in the visual cortex early during development. The mature pattern of the limited distribution of callosal cells in the cortical areas is achieved at later stages by axonal elimination (Innocenti, '81; Lund et al., '84; Dehay et al., '88). Such a widespread distribution of callosally projecting cortical cells during development is also observed in the tammar following an injection of WGA-HRP in the opposite visual cortex (Chapter 3).

Correlation Between the Formation of the Laminar Organization and the Segregation of Thalamic Axons in the Cortex

The present results show that, in tammar, the formation of the cortical laminar organization and the establishment of the laminar-specific distribution of the afferent termination occurs concurrently during development. Between 22d to 67d, when the CP is still highly undifferentiated, the thalamic axons are distributed uniformly in the entirety of the CP except the thin cell compact zone. By 82d, as the first signs of laminar organization become distinguishable, the initially uniformly distributed thalamic axons in the CP develop into three bands overlying the future cortical layers IV, V, and VI. By 99d, as layers IV and VI, the principle destination of the geniculocortical projection, have acquired their fundamental cytoarchitectonic
characteristics, the laminar distribution of the geniculocortical axons becomes virtually identical to the mature pattern.

The concurrence of the formation of the cortical laminar organization and the segregation of the thalamic axons into their appropriate cortical layers suggests possible interactions between the two developmental events. For example, is the formation of the cortical anatomical organization dependent on thalamic input? This has been examined in the cat’s somatosensory system (Wise and Jones, '78). After deletion of the ventrobasal nucleus, the principle thalamic structure in the somatosensory system, the basic plan of the six-layered cortical laminar organization in the somatosensory cortex remains unchanged, although there are changes in several secondary features, such as a reduction in the overall thickness of the cortex, and of the thickness of each individual cortical layer, a reduction of the cell staining quality, and a reduction of the cell density, particularly in layer IV (Wise and Jones, '78). This suggests that thalamic input alone is not sufficient to determine the overall construction of the cortical lamination. Some other factors such as the innervation of axons of cortical origin, the growth of dendrites of local neurons, and the growth of neuropil, are also involved. On the other hand, in monkey’s LGNd, the early, prenatal removal of the retinal input from one eye does prevent the formation of the cellular lamination (Rakic, '77d). It is possible, conversely, that the establishment of the laminar organization may facilitate the segregation of the thalamic axons into their appropriate target layers. For example, it is likely that only those thalamocortical axons terminating in appropriate cortical layers are retained whereas those terminating in inappropriate layers might be eliminated. In the retinogeniculate system, retinal axons are distributed over a wider region of the LGNd early during development and only those terminating in appropriate laminae are retained in adulthood (Rakic, '77c; Calvacante and Rocha-Miranda, '78; So et al., '78, '84; Bunt et al., '83; Shatz, '83; Wye-Dvorak, '84; Harman and Beazley, '86). In inappropriate laminae, retinal axon terminations are largely removed by elimination of side branches (Sretevan and Shatz,
'84; Shatz and Sretevan, '86). In this light, it is likely that the segregation of the thalamic axons into the appropriate target cortical layers at later stages may also involve the retraction of early widespread collaterals or side branches of thalamic axons in inappropriate cortical layers.

A recent description of the development of geniculocortical arbors in the hamster (Naegele et al., '88) has shown that widespread collaterals distributed radially in the cortex are withdrawn later in development and that many short branches present early in development are indeed absent later. However, the elaboration of axonal arbors from simple branches also occurs, suggesting that elaboration in appropriate layers may also play an important role in the development of laminar distribution of thalamic afferents in the visual cortex.
Figure 29. The cytoarchitectonics of the telencephalon of the pouch young tammar wallaby aged between 12 to 36 days postnatal.

A. 12 days. At this stage the VZ (arrow head) is the most prominent in the telencephalon. The MZ (arrow) is very thin. Beneath is the CP just at the beginning of its formation. The IMZ contains very few cells.

B. 16 days. The superficial subdivision of the CP begins to form the characteristic cell compact zone which at this stage comprises most of the CP. Beneath this zone there is a suggestion of the emergence of the less densely packed subdivision of the CP. The overlying MZ (arrow) is more obvious. The IMZ is still low in cell density. The SVZ becomes apparent at this stage overlying the VZ (arrow head), which now has reduced much in thickness.

C. 29 days. The presence of two subdivisions of the CP: the cell compact zone (small arrow head) and the less densely packed zone become apparent. The cell density in the lower part of the IMZ has increased and subdivision in the upper part of the IMZ, the SP, first becomes apparent. SVZ has reduced its thickness compared to that at 16d, while there is little change in the VZ (large arrow head).

D. 36 days. A cell sparse cleft is first seen emerging beneath the cell compact zone. Beneath is the less densely packed zone of the CP which now, together with the cell compact zone, becomes the most prominent part of the telencephalon. The IMZ is now differentiated into two distinct subdivisions: an upper subdivision with very low cell density, and a lower subdivision with high cell density. The SP is situated in the upper subdivision of the IMZ. SVZ and VZ become proportionally less significant than at previous stages.

Bar: in A, B and C = 150µm; in D = 100µm.
**Figure 30.** The cytoarchitectonics of the telencephalon in the tammar wallaby at 42d, 56d and 65d.

A. 42d. The cell sparse cleft is displaced slightly further downwards into the less densely packed part of the CP compared to that at the 36d. The lining up of cells in rows parallel to the pial surface is still very obvious in the low part of the CP. The SP at this stage is beneath the CP comprising the upper part of the IMZ. The rest of the IMZ is occupied by cells in moderate density. The arrow points to the cell compact zone of the CP.

B. 56d. The MZ has become more mature looking, the CP is still similar to previous ages in its thickness. In other parts of the CP, the cell sparse cleft has further moved down. In the region below it, the cellular alignment in rows parallel to the pial surface seems to have disappeared and the cell density becomes reduced in particular in the lowest part. However, this cellular feature is still obvious in the region between the cell sparse cleft and the cell compact zone. In the IMZ, the region with very low cell density becomes expanded. The VZ seems further reduced in thickness by this age.

C. 65d. The MZ becomes very mature looking cytoarchitectonically. The cell compact zone is indicated by an arrow. The cell sparse cleft is still visible and some cytological difference between cells below and above it may be seen in that cells above it look larger than cells below it. In the region above the cell cleft zone, the parallel arrangement of cells has virtually disappeared. The part of the CP below the cell sparse cleft is similar to that seen at 56d in cytoarchitectonics. So are the IMZ, SVZ and VZ.

Bar = 200µm.
Figure 31. Photomicrographs illustrating the cytoarchitectonics of the developing visual cortex from 76d to 118d.

A. 76 days. First sign of the formation of layers V and VI is visualized under the CP in which layer IV is still forming. Above these the cell compact zone and the MZ have undergone little change from 65d. Underlying the IMZ, the SVZ and VZ together become further reduced in thickness.

B. 82 days. By this age the lower part of the CP seen in A is positioned further down away from the cell compact zone and forms layer IV. Underneath this cells in layers V and VI become more mature looking. There is little change in other parts of the developing visual cortex, except the VZ at the bottom seems to have virtually disappeared.

C. 99 days. By this age cells in layers IV, V and VI become more mature. Above these layer III is forming and cells in it are also acquiring their features. The MZ is further increased in its thickness. In the IMZ below layer VI, cell density in its lower part becomes reduced. A characteristic SVZ is not seen at this stage.

D. 118 days. By this age the visual cortex has achieved its basic mature pattern. The cell compact zone has reduced in its thickness and begins to resemble layer II in the adult (see figure 2 in Chapter two). In layer III a cell sparse zone in the lowest part becomes obvious. In the white matter, cell density has been largely reduced so that it looks similar to that in the adult (see figure 2 in chapter two).

Bar: in A, B and C = 200μm; in D = 200μm.
Figure 32. Photomicrographs showing the cortical label in the telencephalon of a 22 day PY following a placement of injection of WGA-HRP in the diencephalon.

A. The dark field view of the label in the telencephalon showing the robust thalamocortical projection entering into and distributing in the depth of the telencephalon. The anterograde label is most dense in the SP where the optic radiation (OR) lies, but is also very substantial in much of the CP, leaving only the thin cell compact zone uninvaded by thalamic afferents at this age.

B. A bright field view of a thionin stained section adjacent to A showing the cytoarchitectonics of the telencephalon at 22d. Arrow head indicates the cell compact zone of the CP. Even at this stage, it only comprises a small proportion of the whole depth of the CP.

Bar = 200µm.
Figure 33. Photomicrographs of cross sections of the telencephalon in a 28d PY.

A. The dark field view of anterograde label in the telencephalon after a extensive placement of detergent soaked HRP chips in the diencephalon, covering both LGNd and LP. Label is substantial in upper part of the IMZ, lower part of the CP and MZ. Faint label is also present in the cell compact zone. It remains unclear why the anterograde label in the MZ at this stage is even more intense than that seen at 35d and 45d.

B. The bright field view of an adjacent thionin stained section showing the cytoarchitectonics of the labelled region in A.

Bar = 100µm.
Figure 34. Camera lucida drawings of injection of WGA-HRP in the diencephalon and the resulting label in the telencephalon at 35d. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Rostral, direction of sequence of sections. Cross hatching represents areas with a high concentration of injected HRP, whereas single hatching represents areas with a lower concentration of injected HRP. Note the injected HRP is widespread in much of the dorsal half of the diencephalon and the medial wall of the telencephalon, although areas with dense HRP products are largely limited over the future LGNd and LP. In cortical label, dots represent anterogradely labelled terminals and fine lines represent anterogradely labelled axons. Labelled axons can be followed from the diencephalon in the optic radiation to where they innervate the visual cortex, reaching the caudal occipital pole. Micrographs of the label are shown in the following figure.
Figure 35. Photomicrographs of cross sections showing HRP label in the telencephalon in a 35 dayPY after a placement of WGA-HRP in the diencephalon. Camera lucida drawings for this animal are shown in figure 34.

A. The dark field view of the HRP label in the caudal part of the telencephalon. Anterogradely labelled terminals of thalamic afferents are densely and evenly distributed in the upper part of the IMZ including the SP and almost whole the depth of the CP except for the cell compact zone.

B. Bright field view of thionin stained section adjacent to A to show the cytoarchitectonics of the telencephalon at this stage. Arrow head indicates the cell compact zone of the CP.

C. The high power view of the HRP label in the telencephalon showing the presence of sparse anterogradely labelled terminals of thalamic axons in the MZ (arrow heads).

Bar: in A and B = 200µm; in C = 100µm.
Figure 36. Photomicrographs showing HRP label in the telencephalon in a 45d PY after receiving a large placement of WGA-HRP in the diencephalon covering both LGNd and LP.

A. A dark field view of the HRP label showing the distribution of anterograde label and retrograde label in the depth of the telencephalon. Most noticeable at this age is the first appearance of retrogradely labelled cells in the cell compact zone (arrow head). Also note the level of anterograde label above this zone in the MZ is largely increased compared to that at 35d (Fig. 35). Beneath the cell compact zone anterograde label is densely distributed, in particular in the low half of the CP and SP. In rest of the IMZ beneath the SP anterograde label is also present with less intensity.

B. A bright field view of an adjacent thionin stained section showing the cytoarchitectonic features of the labelled region in A.

C. A magnified photomicrograph showing the detail of the retrogradely labelled cells in the cell compact zone (large arrow head) and anterogradely labelled terminals in the MZ (small arrow head).

Bar: in A and B = 100µm; in C = 100µm.
Figure 37. Photomicrographs of cross sections of telencephalon at 45d showing the HRP label in the rostral part of the visual cortex after an intensive placement of WGA-HRP in the diencephalon covering both LGNd and LP.

A. Dark field view of the distribution of HRP label in the lateral part of the rostral visual cortex. Arrow points dorsally. The anterograde label in this part of the visual cortex is only obvious in the MZ. It is mainly accumulated in the lower part of the MZ (large arrow head). The retrogradely labelled cells are first seen at this stage in the telencephalon. They are confined into two bands (small arrow heads): one in the cell compact zone of the CP with more widespread distribution dorsally, and the other in the lowest part of the CP with a restricted extent in the ventral part of the telencephalon.

B. Bright field view of a thionin stained cross section adjacent to A. Arrow heads indicate the positions of two bands of labelled cells in A. Arrow points to the cell sparse zone in the CP. Note that cells in the lower part of the CP are absent in regions where the cell sparse zone is absent.

C. A magnified view of labelled cells in the cell compact zone and in the lowest part of the CP (arrow heads).

Bar: in A and B =200µm; in C = 100µm.
Figure 38. Camera lucida drawings of location and extension of a placement of WGA-HRP in the diencephalon and the resulting label in the telencephalon at 54d. Sections in the series are numbered from the caudal occipital pole. Arrows: Dorsal, dorsal surface of brain; Caudal, direction of sequence of sections. The cross hatching represents regions with a high concentration of injected HRP, and the single hatching represents a low concentration of injected HRP. The HRP injection in this case is very extensive and may have a region of uptake in other thalamic structures, as well as in the LGNd and LP. In the cortex, large dots represent retrogradely labelled cells. Small dots represent anterogradely labelled terminals, and fine lines represent labelled axons. The terminal label is very much confined to the mediodorsal part of the visual cortex, corresponding approximately in position to area 17 which however can not be identified at this stage on the basis of cytoarchitecture. Where the label stops abruptly may correspond to the area 17/18 border. The anterograde label becomes very densely distributed in the MZ and is still evenly concentrated in much of the CP, leaving only the cell compact zone without significant label. The retrogradely labelled cells are most obvious in the low part of the CP in the relatively ventral part of the telencephalon. In the upper part of the CP just beneath the cell compact zone, they are only detectable dorsally in the caudal part of the cortex. Photomicrographs of the label are shown in figures 39-41.
Figure 39. Photomicrographs of HRP label in the caudal part of the telencephalon following an injection of WGA-HRP in the diencephalon covering both LGNd and LP at 54d. Camera lucida drawings of this animal are shown in figure 38.

A. A low power dark field view of HRP label in the caudal part of the developing cortex showing the distribution of the anterogradely labelled terminals of thalamocortical axons at 54d. The thalamic axon termination appears restricted in the dorsolateral part of the cortex and stops rather abruptly (small arrow head). Within this region, they are robust in most parts of the CP and in the MZ (see a magnified view in Fig. 40). In the cell compact zone, they have a faint presence. Ventral to the end of the terminal label only retrograde label in the low part of the CP is present (large arrow head).

B. The thionin stained cross section adjacent to A showing the cytoarchitectonic arrangement of the labelled region. The arrow indicates the cell sparse zone in the CP. Note at this stage there is still no sign of cytoarchitectonic differences between areas above and below the end of the anterograde label seen in A.

Bar = 1mm.
Figure 40. Photomicrographs of cross sections of presumptive area 17 showing the laminar distribution of the anterogradely labelled thalamic axons in the visual cortex at 54d.

A. Dark field view of the terminal label in the visual cortex. Note the thalamic afferent termination becomes very dense in the MZ at this stage (arrow head). In the CP, thalamic axons are still evenly and densely distributed in most of the depth of the CP, although in the cell compact zone their presence is less significant. Beneath the CP, anterograde label is also seen in the upper part of the IMZ, the SP.

B. A bright field view of an adjacent thionin stained cross section showing the cytoarchitectonics of the labelled region in A.

Bar = 200µm.
Figure 41. Photomicrographs of high power views of the laminar distribution of retrogradely labelled cells in presumptive areas 17 and 18 at 54d following a placement of WGA-HRP in the diencephalon, which covered both LGNd and LP.

A. Area 17. The cell sparse cleft in this area is not as obvious as that in the more ventrolaterally situated area 18. Retrogradely labelled cells have become displaced inwards into the less densely packed part of the CP (upper arrow) from their initial positions in the cell compact zone at 45d (figure 37). Also note that cells in the low part of the CP become more substantially labelled (lower arrow). Also shown are anterogradely labelled terminals indicated by the presence of many particles of HRP products between cell bodies.

B. The high power view of retrograde label in the presumptive area 18 shown in an unstained cross section. Retrogradely labelled cells are forming two bands (arrows).

C. An adjacent cross section to B, counterstained with thionin, showing the distribution of these two bands of retrogradely labelled cells in the depth of the CP. The upper band of labelled cells is situated just beneath the cell compact zone, whereas the lower band of cells is situated in a region below the cell sparse cleft.

Bar = 100µm.
**Figure 42.** Photomicrographs of cross sections showing the laminar distribution of the HRP label in the visual cortex at 65d and 76d after the placement of WGA-HRP in the thalamus.

A. The dark field view of the laminar distribution of the HRP label in the depth of the visual cortex at 65d following an injection of WGA-HRP largely focused in the LP. The anterograde label is concentrated in the MZ (arrow head) and the low part of the CP, while in the IMZ only a faint distribution of the label can be seen. Retrogradely labelled cells are now seen underneath the cell compact zone. The lower band of cells is absent presumably because the LGNd was not included in the injection site.

B. The adjacent thionin stained cross sections to A, showing the cytoarchitectonics of the labelled region in A.

C. The dark field view of the laminar distribution of the retrogradely labelled cells in the visual cortex at 76d. They are confined into two bands: while the superficial one appears in the future layer V just beneath the forming layer IV, the lower one is beneath the cell sparse cleft in the future layer VI of the visual cortex. Anterograde label is not present in this section.

D. The cytoarchitectonics of the labelled region shown in A. Note the first appearance of the granule cell zone underneath the cell compact zone and the cell sparse cleft zone between future layers V and VI.

Bar = 100µm.
Figure 43. Camera lucida drawings of series of cross sections showing the cortical label of HRP (upper) and the site of HRP injection in the thalamus (lower) covering both the LGNd and LP at 82d. Sections in the series are numbered from the caudal occipital pole. Arrows, Dorsal, dorsal surface of the brain; Rostral, direction of sequence of sections. Dashed lines: borders between 17 and 18 and 18 and VTP.

Upper: The distribution of the cortical label of HRP. Large dots represent retrogradely labelled cells, and small dots represent anterogradely labelled terminals. Label is intensive in area 17, 18 and V-TP. Note the particularly enhanced label in the area 18, where connections with the LP are remarkably concentrated in the adult (see Chapter 2, Fig. 11). In area 17, there are signs that the initially evenly distributed thalamic afferent termination in the CP is beginning to be concentrated into bands corresponding to the forming cortical layers at this stage (see Fig. 44 for more details). The retrogradely labelled cells are situated in layers V and VI. In area 18, anterograde label is substantial in all layers but particularly dense in layers I and IV. In area V-TP, anterograde label is only seen with less intensity in a region adjacent to area 18, whereas retrogradely labelled cells have a more extended distribution dorsoventrally and rostrocaudally. They are confined into layers V and VI.

Lower: The location and extent of injected WGA-HRP in the dorsolateral thalamus. The cross hatching represents areas with a high concentration of HRP, and the single hatching represents areas with a low concentration of HRP. It appears that while both LGNd and LP are intensively filled by the injected HRP, areas surrounding them may also be involved in HRP uptake.
Figure 44. Photomicrographs showing the cortical HRP label in an 82 day PY following the thalamic injection of WGA-HRP which covered both LG Nd and LP.

A. The dark field view of the HRP label in area 17. The initially evenly distributed HRP label has become distributed with different intensity in the low part of layer III and layers IV, V and VI. The most intense label is found in layer VI, whereas in layers III and V the label is less intensive. In layer IV, label is rather faint. Label is also present moderately in the MZ.

B. The bright field view of an adjacent thionin stained cross section showing the cytoarchitectonics of the labelled region shown in A. The retrogradely labelled cells can be seen in the future layers V and VI.

C. The dark field view of the HRP label in area 18 close to the area 17/18 border region. The anterograde label is much enhanced in all layers I, III, IV and V in this region than in area 17.

D. The bright field view of an adjacent thionin stained cross section showing the cytoarchitectonics of the labelled region in C. Labelled cells, with a stronger appearance than those in area 17, can be seen in layer V.

Bar: in A, B, C and D = 100µm.
Figure 45. Photomicrographs showing the laminar distribution of the HRP label in area 17 following a placement of WGA-HRP largely confined to the LGNd in a 99 day PY.

A. The bright field view of the HRP label in area 17 taken from an unstained cross section. The anterograde label now becomes primarily concentrated into a band of very high density, overlying layer IV although its presence in layer VI, in the overlying forming layer III and, less obviously, in layer I, is also visible. Retrogradely labelled cells are largely confined into layer VI, except in rare cases one or two of them are present in layer V (arrow), reflecting the possibility that LP in this case may be also marginally involved in the uptake of HRP,

B. The bright field view of an adjacent section counterstained with thionin to show the HRP label and the cytoarchitectonics of the labelled part of area 17. The band of very dense anterograde label corresponds to layer IV, the major termination of the LGNd afferents in the adult (see Chapter Two, Fig. 8 and 9). Retrogradely labelled cells are concentrated over layer VI.

C. The dark field view of the HRP label in area 17. Apart from the robust label in layer IV and VI, substantial amounts of label extended from layer IV into the overlying layer III and underlying layer V is also obvious. In addition, label is present in the superficial part of the layer I.

Bar: in A and B = 200µm; in C = 100µm.
Figure 46. Photomicrographs illustrating the cortical HRP label at 118d following a massive injection of WGA-HRP in the visual thalamus including the LGNd and LP.

A. The dark field view of the laminar distribution of the anterograde and retrograde label in area 17. The anterograde label is largely concentrated in layers I and IV, with additional distribution in layers III, V and VI, while retrogradely labelled cells are concentrated in layers V and VI (arrow heads).

B. The bright field view of an adjacent thionin stained cross section showing the cytoarchitectonics of the labelled region in area 17.

C. The dark field view of the anterograde and retrograde label in the area 18 close to the area 17/18 border. Note the particular enhancement of the anterograde label in layer I, III/IV and of the retrograde label in layer VI. The arrow indicates the border between areas 17 and 18.

D. The bright field view of the adjacent section to C, with thionin staining to show the cytoarchitectonics of the labelled region in area 18.

Bar: in A, B, C and D = 200µm.
Chapter 5. Concluding Remarks

The visual cortex of the tammar wallaby (Macropus eugenii) is constructed according to a scheme similar to that seen in a number of representative placental mammals in that the whole thickness of the visual cortex is divided into six distinct cellular layers. It is interesting that the cellular organization of area 17 in the tammar is remarkably similar to that seen in some lower primate species, as evidenced by the presence of a cell sparse zone overlying the layer IV granule cell zone, the equivalent of the stria of Gennari which is most distinct in the primate. The similarity of the visual cortical organization between the tammar and some primate species was further suggested by the similar pattern of the laminar distribution of the geniculate afferent termination seen in both the tammar and certain primate species. A unique feature is that layer II in area 17 of the tammar is particularly prominent with its high cellular density and staining quality, which is not seen in placentals.

In the construction of the visual cortical connections the tammar also follows the general plan which applies in all mammalian species. Features that delineate the areal and laminar specificities of the placental visual cortical connections are also seen in the tammar. It is particularly intriguing that, despite the fact that this species, being one of the Australian marsupials, has been separated from the evolutionary course taken by placentals for more than 100 million years, the areal and laminar distribution of afferents and efferents in the visual cortex is so strikingly similar to that seen in the placentals.

This similarity in the neuronal organization between the marsupials and the placentals provides the relevant base for the comparison of the present findings on development with those established in the placentals.

In table III, the relative timing for the occurrence of formation of a given visual cortical connection in the tammar is compared with those in some of the representative placental mammals. This interspecies comparison suggests that the time course of the
formation of the visual pathways in the tammar is different from that seen in the placentals in that, judged by the proportion of the caecal period (the period between conception and eye opening), the relative timing of the visual pathway development in the tammar precedes that of placentals considerably in the first half of the caecal period. For example, both the retinogeniculate projection and the geniculocortical projection are formed about 20% of the caecal period earlier in the tammar than in placentals. However, in the second half of the caecal period, this lead in timing by the tammar is lessened due to the considerable delay in the formation of the descending corticothalamic projection and lost by the time that the corticocollicular projection appears. As a result, although the whole duration of visual pathway development in the tammar is considerably prolonged, by the time the corticocollicular projection arises, its relative timing in the tammar (67% of the caecal period) becomes synchronous with the other species compared (67-69% of the caecal period).

After thalamocortical fibres first enter the telencephalon, contrary to what is described in the placentals, geniculocortical axons in the tammar do not wait in the SP. Rather, they enter the CP and become densely and evenly distributed, leaving only the thin cell compact zone without significant thalamic afferent termination. While the use of a more sensitive method of tracing neuronal connections in the present study may account partly for the remarkable difference between present findings and those seen in the placentals, a true interspecies difference may be the main cause explaining the different mode of the ingrowth of the thalamic axons into the CP. One of the most obvious differences between marsupials and placentals is the cellular construction of the telencephalon during the ontogeny of the visual cortical laminar organization. The relatively sparsely packed CP in the tammar may be more permissive to the invading axons from the thalamus.

At the early stages of development, the thalamic afferents do not terminate in their appropriate target layers of the visual cortex. Instead, they are evenly distributed
in the whole thickness of the CP except for the cell compact zone. They become relegated to their target cortical layers nearly 3 months after they first arrive in the telencephalon, when their principal cortical layers are differentiated. In contrast to the widespread distribution of the thalamocortical axons in the depth of the CP, the cortical cells projecting to the thalamus have already settled into their appropriate cortical layers when they first send their axons into the thalamus, about two months prior to the establishment of the laminar specificity of the thalamic afferents in the visual cortex. Similarly, corticocollicular cells were found in layer V, their appropriate origin in the adult, when the connection was first seen.

From the present findings, emerge several lines of research interests which might be appropriate to follow. For example, a systematic analysis of the neurogenesis in the visual cortex giving the date of birth of cells comprising individual cortical layers is needed if the precise temporo-spatial relationship between thalamic afferents and their target cortical cells is to be properly addressed. Also, this study has concentrated on establishing the earliest times of formation of connections between cortical and subcortical centres and the time course of lamination of the afferents and efferents in the cerebral cortex. Once this is laid down there remains some 2 months of development before eye opening. What is happening during this time and in particular what role might impulse activity and synaptic transmission have in the formation of anatomically mature projections before the first optical images strike the retina?
Table III. Time Course of the Development of Visual Pathways

<table>
<thead>
<tr>
<th>Species</th>
<th>Rat</th>
<th>Cat</th>
<th>Macaca</th>
<th>Tammar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caecal Period</strong></td>
<td>36 days</td>
<td>72 days</td>
<td>123 days</td>
<td>162 days</td>
</tr>
<tr>
<td><strong>Start of retinal projection into the LGNd</strong></td>
<td>16 days</td>
<td>32 days</td>
<td>78 days*</td>
<td>39 days</td>
</tr>
<tr>
<td></td>
<td>44% CP</td>
<td>44% CP</td>
<td>63% CP</td>
<td>24% CP</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(6)</td>
<td>(10)</td>
<td>(13)</td>
</tr>
<tr>
<td><strong>Start of retinal projection into the SC</strong></td>
<td>17 days</td>
<td>32 days</td>
<td>78 days*</td>
<td>39 days</td>
</tr>
<tr>
<td></td>
<td>47% CP</td>
<td>44% CP</td>
<td>63% CP</td>
<td>24% CP</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(6)</td>
<td>(10)</td>
<td>(13)</td>
</tr>
<tr>
<td><strong>Start of LGNd projection into the cortex</strong></td>
<td>18 days</td>
<td>42 days</td>
<td>78 days*</td>
<td>42 days</td>
</tr>
<tr>
<td></td>
<td>50% CP</td>
<td>58% CP</td>
<td>63% CP</td>
<td>26% CP</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(7)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td><strong>Start of cortical projection into the LGNd</strong></td>
<td>23 days</td>
<td>48 days</td>
<td>84 days</td>
<td>72 days</td>
</tr>
<tr>
<td></td>
<td>64% CP</td>
<td>67% CP</td>
<td>68% CP</td>
<td>45% CP</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(8)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td><strong>Start of cortical projection into the SC</strong></td>
<td>25 days</td>
<td>48 days</td>
<td>84 days</td>
<td>108 days</td>
</tr>
<tr>
<td></td>
<td>69% CP</td>
<td>67% CP</td>
<td>68% CP</td>
<td>67% CP</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(8)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td><strong>Start of cortical projection into opposite cortex</strong></td>
<td>28 days</td>
<td>62 days</td>
<td>112 days</td>
<td>126 days</td>
</tr>
<tr>
<td></td>
<td>78% CP</td>
<td>86% CP</td>
<td>91% CP</td>
<td>78% CP</td>
</tr>
<tr>
<td></td>
<td>(4)(5)</td>
<td>(9)</td>
<td>(12)</td>
<td></td>
</tr>
</tbody>
</table>
References for Table III

(1) Bunt et al., '83;
(2) Lund and Mustari, '77;
(3) Dreher and Robinson, '88b;
(4) Thong and Dreher, '86;
(5) Lund et al., '84;
(6) Shatz, '83;
(7) Shatz and Luskin, '86;
(8) Anker, '77;
(9) Innocenti, '81;
(10) Rakic, '76;
(11) Shatz and Rakic, '81;
(12) Dehay et al., '88;
(13) Wye-Dvorak, '84.

Footnote For Table III

* The earliest time tested. These events probably occur much earlier in the macaque and is in synchrony with those in cat and rat, in the light that:

1). The relative timing for the the generation of retinal ganglion cells in the macaque is similar to that in the cat and rat (macaque: 29-50%CP; cat: 28-49%CP; rat: 29-50%CP), as is the timing of the generation of lateral geniculate cells (macaque: 29-35%CP; cat: 31-44%CP; rat: 33-44%CP).

2). The retinal axons enter the optic nerve and reach the peak number earlier in the macaque (32%CP, 53%CP) than in the cat (39%CP, 54%CP) and in the rat (42%CP, 56%CP).

See Dreher and Robinson ('88a) for review.

Note in this particular case CP = caecal period.
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