J. exp. Biol. 153, 85–104 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

DEVELOPMENT OF THE MAMMALIAN RETINOGENICULATE PATHWAY: TARGET FINDING, TRANSIENT SYNAPSES AND BINOCULAR SEGREGATION

BY K.-F. SO

Department of Anatomy, University of Hong Kong, Li Shu Fan Building, 5 Sassoon Road, Hong Kong

G. CAMPBELL AND A. R. LIEBERMAN

Department of Anatomy and Developmental Biology, University College London, Gower Street, London, WC1E 6BT

Summary

This review is concerned with the development of the mammalian retinogeniculate projection from the perspective of our studies on the hamster and to a lesser extent on the cat. In these, and other mammalian species, axons from the two eyes initially spread throughout the dorsal lateral geniculate nucleus (dLGN) and thus completely overlap. Later they segregate, the axons from each eye coming to occupy discrete, non-overlapping territories within the dLGN. The process of segregation to establish the adult pattern coincides with the death of retinal ganglion cells projecting to inappropriate areas of the dLGN and with the loss, by degeneration or retraction, of the axons and/or axonal branches initially located within inappropriate territory of the dLGN. These events occur in the early postnatal period in hamsters, before the eyes have opened, and in cats and monkeys they occur entirely during embryonic life: thus, they do not depend on the onset of normal visual function. If one eye is removed before segregation has begun, the terminal fields of the crossed and uncrossed axons from the remaining eye do not segregate, suggesting that segregation in normal development may depend on some form of interaction between retinal ganglion cells from the two eyes. Attractive and/or repulsive influences exerted by the dLGN on retinogeniculate axons may also be involved in the formation of eye-specific territories.

Experimental ultrastructural studies in hamster and cat show that the overlap phase is associated with the formation, by inappropriately located axons, of transient synapses similar to those made by retinogeniculate axons in appropriate parts of the dLGN. In the cat, the transient synapses are made by the axon trunk and by side branches of retinogeniculate axons with terminal arbors in appropriate parts of the nucleus; the transient synapses disappear as the side branches are shed or retracted during the segregation period.

Because of good evidence that electrical activity of the retinogeniculate axons may be involved in binocular segregation of inputs, we suggest that the formation

Key words: visual system, development, transient synapses, binocular segregation, retina, dorsal lateral geniculate nucleus, hamster, cat.

and elimination of transient synapses play a significant role in the development of the orderly retinogeniculate projections.

Introduction

One of the emerging general principles governing the events that lead to the establishment of orderly connections in the developing nervous system is that of initial overlap of axonal projections followed by segregation. Thus, in many parts of the peripheral nervous system (Purves and Lichtman, 1980) and of the central nervous system (Mariani, 1983), axons growing into their target fields initially spread more widely than their mature counterparts, often overlapping with other afferents in a diffuse pattern which is later refined as the axons become restricted to more discrete, often non-overlapping areas of termination.

Binocular segregation of retinogeniculate inputs: a general feature of visual system development

The phenomenon of axonal segregation is perhaps most striking and amenable to analysis in the mammalian visual system, particularly in relation to the development of retinal inputs to the dorsal lateral geniculate nucleus (dLGN) of the thalamus. In all adult mammals, inputs from the two eyes are sharply segregated within the dLGN, terminating within different non-overlapping regions of the nucleus: this segregation of retinal inputs is a constant and major feature of the organization of the dLGN (Kaas *et al.* 1972; Guillery, 1979). In primates and carnivores, in which the dLGN is cytoarchitecturally laminated, the ipsilateral (uncrossed) and contralateral (crossed) retinal ganglion cell axons terminate in different sets of laminae which project to different areas and different sublaminae of the primary visual cortex (e.g. Rodieck, 1979). During the period of prenatal development, however, as first shown by Rakic (1977) for the monkey and subsequently by Shatz (1983) for the cat, axons from the two eyes are initially intermingled and distributed throughout the dLGN, only later undergoing a sorting out process in which they become associated with one or the other of the sets of cellular laminae.

In those mammals in which the dLGN is not overtly laminated, and in which the binocular visual field is small and the retinal projections predominantly crossed (e.g. hamsters, rats, other nocturnal rodents and rabbits), appropriate experimental methods nevertheless reveal a hidden pattern of lamination (Kaas *et al.* 1972; Reese, 1988). In particular, the relatively small numbers of uncrossed retinal axons terminate exclusively in a covert lamina (or segment) from which crossed retinal input is excluded. In these species too, establishment of the adult pattern of retinogeniculate connections involves an initial overlap of axons from the two eyes followed by a phase of segregation (So *et al.* 1978, 1984). The hamster is a particularly suitable species for the study of these events. It is extremely immature at birth (gestation is 15.5–16 days) and overlap and segregation phases both occur

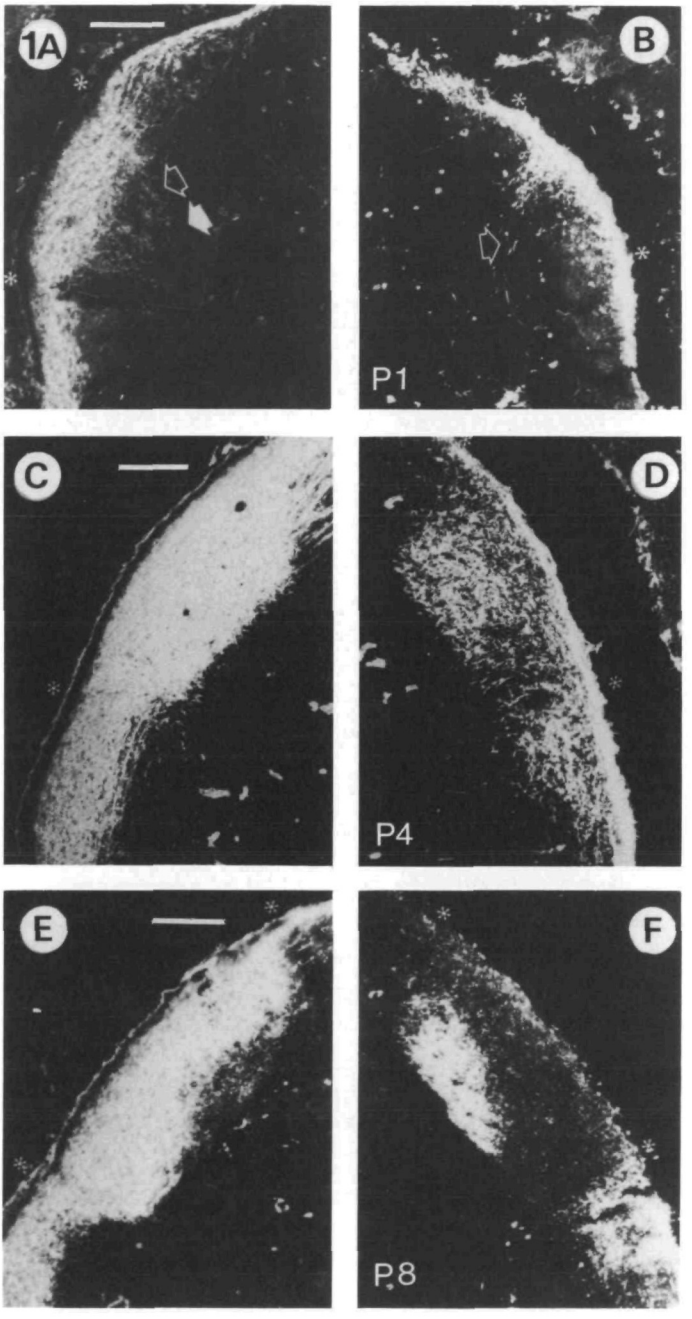
postnatally, permitting easier experimental manipulation than is possible in species, such as monkey and cat, in which these events occur prenatally. Here we review studies of the development of the retinogeniculate projections in the golden hamster by light microscopy (LM), using experimental degeneration, autoradiography and anterograde transport of horseradish peroxidase (HRP), and by electron microscopy (EM) and EM combined with HRP labelling. In addition, we will review more recent studies of the developing retinogeniculate projection in the cat. We also consider some of the mechanisms that may be involved in the process of segregation and in other key events in the development of the retinogeniculate pathway.

The developmental sequence in the hamster

Axons from the contralateral eye reach the hamster dLGN before birth. By postnatal day 1 (P1; the day of birth is designated P0) contralateral axons fill the entire dLGN and axons from the ipsilateral eye have begun to arrive. By P2 the ipsilateral input is also distributed throughout the dLGN, albeit more densely in the dorsal half of the nucleus. The contralateral and ipsilateral axons remain intermingled until P6, when there is a marked reduction in the density of ipsilateral axons in the ventral part of the nucleus. On P7 there is a reduction in the density of crossed axons in the dorsomedial part of the dLGN, leading to the establishment of the segregated adult pattern many days before the eyes have opened (eye opening usually occurs on P15). Although these events, which are illustrated in Fig. 1, occur postnatally and over a relatively short period in hamsters (So *et al.* 1978, 1984; Frost *et al.* 1979), they are similar to those that occur during the development of retinogeniculate connections in monkeys (Rakic, 1977) and cats (Shatz, 1983). Essentially the same sequence of events occurs in many other mammalian species (rat, Jeffery, 1984, Manford *et al.* 1984; mouse, Godement *et al.* 1984; squirrel, Cusick and Kaas, 1982; opossum, Cavalcante and Rocha-Miranda, 1978; brush-tailed possum, Sanderson *et al.* 1982; quokka, Coleman and Beazley, 1989; ferret, Linden *et al.* 1981) and is almost certainly a universal feature of the development of the mammalian retinogeniculate pathway. Furthermore, although different in detail, the same pattern of initial overlap of axons from the two eyes, followed by segregation into eye-specific territories, is seen in the development of retinal projections to the superior colliculus in rodents (Land and Lund, 1979; Frost *et al.* 1979; Woo *et al.* 1985; Insausti *et al.* 1985) and in other mammals (Rakic, 1977; Cavalcante and Rocha-Miranda, 1978; Cusick and Kaas, 1982; Sanderson *et al.* 1982; Chalupa and Williams, 1984; Godement *et al.* 1984; Coleman and Beazley, 1989).

Areas of ignorance

Stereotyped though the mammalian pattern of retinogeniculate development may be, our understanding of the significance of early overlap and subsequent



segregation, and our knowledge of the detailed events in the retina and brain with which they are associated, remains rudimentary. For example, it is not yet clear how retinogeniculate axons are first induced to enter the dLGN; whether or not their initial growth and arborization within the dLGN is guided by site-specific tropic or trophic cues, or by other mechanisms; whether competitive interactions

Fig. 1. Dark-field photographs of coronal sections illustrating the retinal projections to the contralateral (left-hand column) and ipsilateral (right-hand column) dLGN. Each pair of photographs was taken from one brain at the mid-geniculate level. Asterisks define the dorsal and ventral borders of the dLGN. The time of unilateral eye injection with horseradish peroxidase (HRP) is illustrated for each pair of photographs. The magnification is the same for each pair of photographs. Scale bars, 100 μm. Development of the retinogeniculate projections of normal hamsters. (A,B) Contralateral and ipsilateral dLGN, respectively, of a P1 hamster. Photomontage was needed for A and B to bring the entire dLGN into focus. The open arrows point to the retinal fibres located in the β sector. The solid arrow points to retinal fibres located within the ventrobasal nucleus. (C,D) Contralateral and ipsilateral dLGN, respectively, of a P4 hamster. The entire ipsilateral dLGN is filled with retinal fibres but the fibre density is much higher in the dorsal than in the ventral half of the nucleus. (E,F) Contralateral and ipsilateral dLGN, respectively, of a P8 hamster. The distribution of the retinogeniculate projection on P8 closely resembles the adult pattern.

between retinal ganglion cells in the two eyes, or some other mechanism, induces the retinogeniculate axons to segregate, i.e. to disappear from certain parts of the nucleus; and whether this disappearance involves death of the parent retinal ganglion cells, loss by retraction or degeneration of part of the axonal arbors of ganglion cells which persist, or a combination of the two.

Early events in the establishment of the retinogeniculate pathway

In adult rodents, the retinal axons that innervate the dLGN are predominantly or exclusively collateral branches of a subpopulation of retinocollicular axons (e.g. Linden and Perry, 1983; Martin, 1986). In the hamster, the retinocollicular projection is established by axons which grow from the retina on embryonic day 11 (E11) to reach the caudal pole of the superior colliculus by E13.5. These axons pass over the surface of the developing dLGN on E13, two or three days before birth, without emitting branches to this or other thalamic nuclei. Only much later, at around the time of birth, does the formation of the retinogeniculate projection commence (So *et al.* 1984; Schneider *et al.* 1985) as retinofugal axons in the optic tract at the level of the dLGN begin to emit collateral branches that penetrate, ramify and establish synaptic contacts within the nucleus (Schneider *et al.* 1985; Bhide *et al.* 1988*a*; P. G. Bhide and D. O. Frost, in preparation).

The question of what it is that induces the retinocollicular axons coursing over the surface of the dLGN to begin emitting collateral branches at this time is of great interest. It is possible that collateralisation reflects the operation of a genetically controlled developmental programme, but it is much more likely that it involves either: (i) a direct inductive interaction between a local cellular or extracellular constituent of the optic tract and retinocollicular axons; or (ii) a branch-inducing and attracting chemical signal diffusing to the tract axons from the underlying cells of the dLGN. We have recently made observations that favour the first of these possibilities. We have found that between E15 and P4, axons in the optic tract overlying dLGN, identified as retinofugal by anterograde HRP

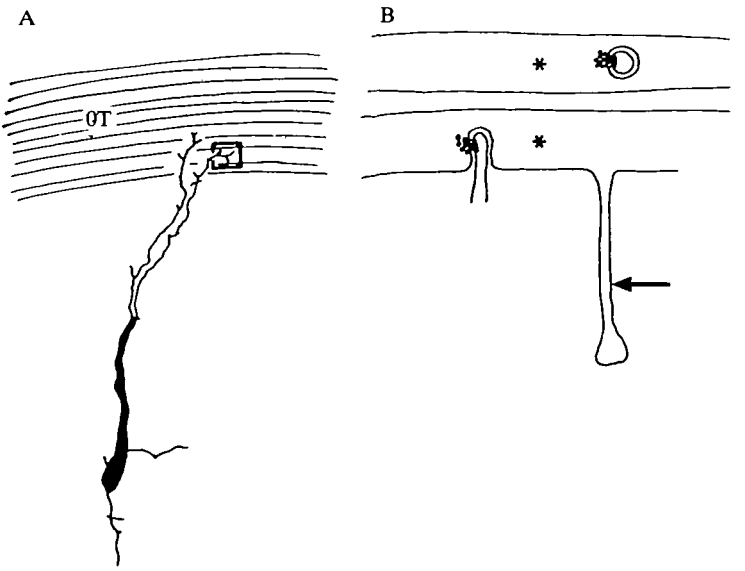


Fig. 2. Schematic drawings illustrating the invagination of retinofugal axons in the optic tract of neonatal hamsters by dendrites of geniculate neurones (based on and partially adapted from Bhide *et al.* 1988*a*,*b*). (A) A neurone with the morphological characteristics of an immature geniculocortical projection cell sends two dendrites into the optic tract (OT). (B) The tips or appendages of such dendrites invaginate the optic tract axons (*) and are postsynaptic at well-differentiated, small, synaptic contacts. A branch (arrow) emerges from the axon within a few micrometres of the invagination and extends towards the geniculate neuropile.

transport, are commonly invaginated by, and establish small, focal, precocious synaptic contact with, the tips of dendrites or dendritic appendages of geniculate neurones (Fig. 2; Bhide *et al.* 1988*a*,*b*). Invaginations are commonly located close to the site of emission of a branch directed towards the dLGN (Fig. 2B, arrow; Bhide *et al.* 1988*a*). The temporal coincidence between dendritic invagination of retinocollicular axons (most common at E15–P0, declining at P2, rare at P4, absent thereafter) and branch emission, and the indications that branches may form adjacent to the invaginations, suggests that these specialized contacts may play a role in the induction of branching.

The retinogeniculate projection is one of several projection systems in the brain, for example from the subiculum to the mammillary bodies (Stanfield *et al.* 1987) and from the neocortex to the pons (O'Leary and Terashima, 1988), which develop as collateral offshoots of parent axons that first grow to a more distant target. In the case of the corticopontine system, the projection arises as collaterals from axons projecting initially to the spinal cord and, whereas the connection with the spinal cord is retained in the case of axons arising in motor cortex, it is lost in the case of those arising in visual cortex (O'Leary and Terashima, 1988). Very recently Heffner *et al.* (1990) have presented persuasive evidence that corticospinal axons

are induced to emit collaterals to innervate the basilar pons by a diffusible chemical signal. By co-culturing embryonic cerebral cortex with various other brain areas, including basilar pons, they were able to show that the basilar pons exerts a target-specific influence, selectively on the axons of layer V pyramidal cells, causing these axons to emit branches to the pons. It might be anticipated that a similar chemotropic effect will be found to be responsible for inducing branch emission and target innervation in other fibre systems that develop in an analagous manner, but there is no direct evidence to implicate such a mechanism in the development of the retinogeniculate projection, and the role of the dendritic invagination phenomenon that we have described and its relationship, if any, to a possible chemotropic mechanism remain to be investigated.

Experimental interference with normal segregation

The mechanisms underlying the process of segregation are of special interest and amenable to a variety of experimental anatomical investigations, for example in relation to the question of whether segregation involves competition (for synaptic space or for a limited supply of trophic factor for example; Guillery, 1987) between retinal ganglion cells from the two eyes, or perhaps between retinal ganglion cells and other neurones projecting to the dLGN from some other part of the brain. The possibility that segregation depends on interactions between retinogeniculate afferents and afferents projecting to the dLGN from elsewhere in the brain is seriously undermined by the following experiments, involving two of the major non-retinal inputs to the dLGN. When the input to the dLGN from the occipital (visual) cortex is removed on postnatal day 5, or when that from the superior colliculus is eliminated on the day of birth, neither the timing nor the extent of binocular segregation is affected (Jen and So, 1983).

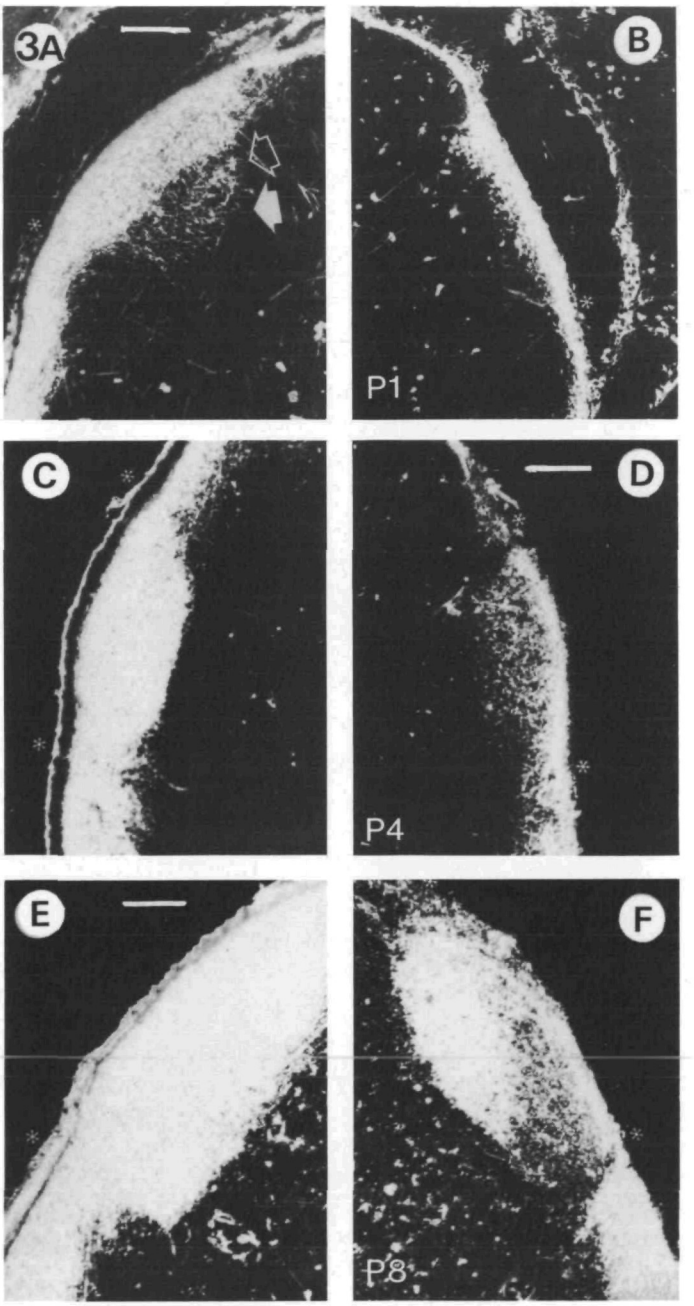
In contrast, there is persuasive evidence that interactions involving the two sets of retinal ganglion cells may play a part in binocular segregation. Thus, when one eye is removed at birth from rat (Lund *et al.* 1973) and hamster pups (So *et al.* 1978) or before segregation has taken place in foetal monkeys (Rakic, 1981) or cats (Chalupa and Williams, 1984), the retinogeniculate projections from the remaining eye, examined after the developmental stage at which segregation has normally taken place, were not found to display a laminar pattern but were instead spread throughout the entire dLGN. Although these findings are consistent with the hypothesis that interactions between retinal ganglion cells from the two eyes are responsible for segregation, they do not rule out other possibilities (see Shatz and Sretavan, 1986; Guillery, 1987). Nor do they exclude the possibility that a transient segregation, directed by some different mechanism, did occur but was followed by a subsequent sprouting of the axons from the remaining eye to occupy dLGN territory lacking retinal input. The observation that segregation also fails to occur when one eye is removed at a very early developmental stage, before axons have reached the optic chiasm (E23 cat foetuses), strengthens the evidence that binocular interactions are involved in segregation (Sretavan and Shatz, 1986*b*). A

study by So *et al.* (1984) is also relevant. Hamsters were unilaterally enucleated at birth and the pattern of projections to the dLGN from the remaining eye was examined at closely spaced intervals. In these animals the uncrossed axons from the remaining eye occupy the lateral portion of the dLGN on P1 and P2, fill the entire nucleus by P4 and at P6 and later are more extensive than in control animals, with greatest density in the dorsal portion of the nucleus but present also in the ventral portion. The pattern of crossed projections from the remaining eye was identical to that of normal animals between birth and P6. However, on P7 and subsequently, the diminution in density followed by disappearance of fibres from the dorsomedial portion of the nucleus seen in normal animals did not occur (Fig. 3). These findings, which show that segregation does not occur at any stage after elimination of the input from one eye, considerably strengthen the hypothesis that binocular interactions play a role in segregation.

It is, however, clear, that even if interactions between retinal ganglion cells in the two eyes do play a role in the segregation of axons to eye-specific domains within the dLGN, such interactions cannot, by themselves, explain the formation of eye-specific domains within the nucleus. Thus, even though uncrossed axons from the remaining eye in neonatally enucleated hamsters were spread throughout the dLGN, they were more highly concentrated in the region in which the ipsilateral sector normally forms (So *et al.* 1984). Similar observations have been reported for neonatally enucleated and congenitally unilaterally anophthalamic mice (Godement *et al.* 1980). Such observations suggest that uncrossed axons recognise this region of dLGN as an appropriate target and, more broadly, that retinogeniculate axons recognise appropriate target territory within the dLGN independently of any binocular interactions. Alternatively, the uncrossed axons may be subject to an inhibitory or repulsive influence from the inappropriate area of the dLGN. Such a conclusion is in line with much evidence of target influence on the sorting out of axons from work on the development of the lower vertebrate retinotectal projection. For example, Bonhoeffer and colleagues have shown that axons from retinal ganglion cells in the temporal region of the chick retina are repulsed by, and their growth cones induced to collapse by, cell membranes derived from the caudal tectum (Walter *et al.* 1987; Cox *et al.* 1990), suggesting that mechanisms exist *in vivo* to keep temporal retinal axons out of the part of the tectum they are not destined to innervate. (It is worth noting, however, that such evidence relates to the influence of the target on the development of appropriate

Fig. 3. See legend to Fig. 1 for a detailed description of this preparation. Development of the retinogeniculate projections in hamsters with unilateral eye enucleation on P0, and injection of the remaining eye with HRP on various postnatal days. (A,B) Contralateral and ipsilateral dLGN, respectively, of a P1 hamster. The open arrow points to retinal fibres located in the β sector. The solid arrow points to retinal fibres located within the ventrobasal nucleus. (C,D) Contralateral and ipsilateral dLGN, respectively, of a P3 hamster. (E,F) Contralateral and ipsilateral dLGN, respectively, of a P8 hamster. The entire contralateral and ipsilateral dLGN are filled with retinal fibres, although the fibres in the ventral half of the ipsilateral nucleus are much sparser than those in the dorsal half.

topography in the retinal projection rather than on its *laterality*.) However, there are other possible explanations for the apparent preferential association of uncrossed retinogeniculate axons with the dorsomedial dLGN in neonatally enucleated/unilaterally anophthalamic rodents. Thus, the segregated pattern of termination of retinogeniculate axons in the dLGN of the rat is crudely anticipated by fibre organisation in the optic tract, such that uncrossed axons are located in the



deepest part of the tract (Reese, 1987). Maintenance of this order as axons distribute to dLGN neuropile would produce a preferential distribution of uncrossed axons at the inner border of the nucleus (see also Guillery *et al.* 1982; Mastronarde, 1984, for similar considerations in the cat).

Transient synapses of retinogeniculate axons before and during segregation

Many other questions concerning the establishment of the mature pattern of retinogeniculate connections can be answered only by ultrastructural study. For example, when, where and with what cellular elements are the first synaptic and other specialised contacts established by crossed and uncrossed afferents? Do the relatively late arriving uncrossed retinal axons or axons of other afferent systems make direct contacts (specialised or otherwise) with the crossed axons? Do retinal axons that subsequently withdraw or degenerate differ from those that persist and, in particular, do axons occupying territory within the dLGN from which they will eventually disappear make synaptic or other specialised contacts in the 'inappropriate' territory? Our approach to these questions is illustrated in Fig. 4. Hamster pups received unilateral intravitreal injections of HRP at various postnatal ages to label retinal axons and terminals in dLGN. Two different areas of the dLGN were then systematically examined by EM: on the side ipsilateral to the injected eye, an area of ventrolateral dLGN that contains only crossed retinogeniculate axons in

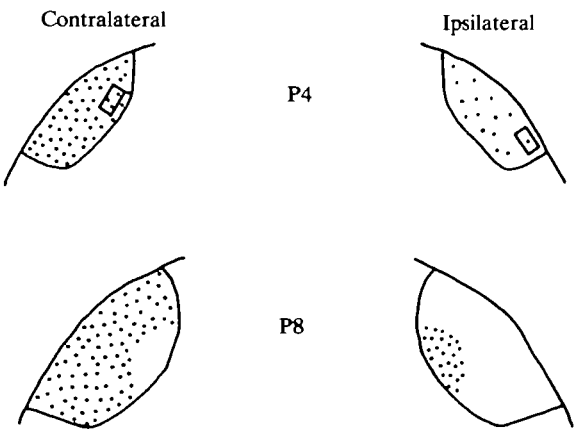
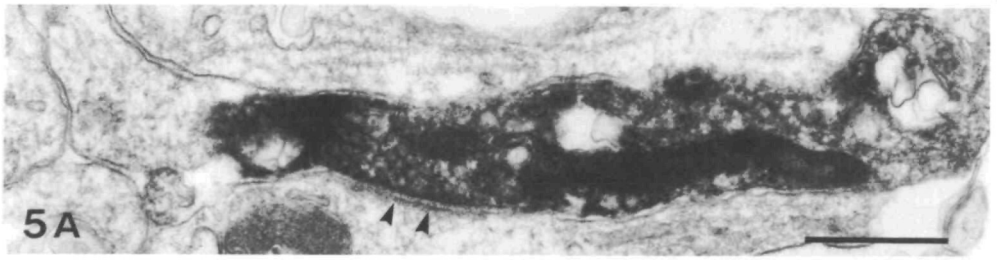


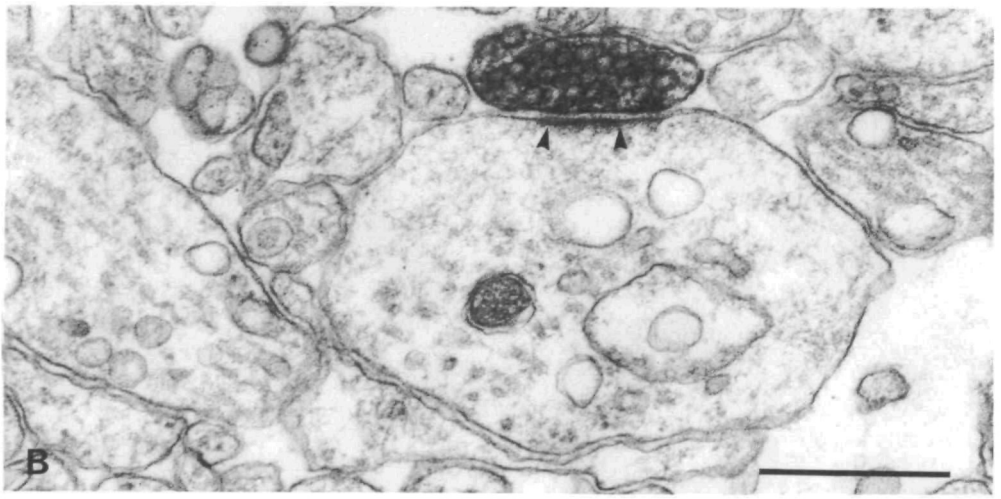
Fig. 4. The sampling method employed for the electron microscope investigation of transient retinogeniculate synapses in the early postnatal hamster (Campbell *et al.* 1984). Stippling shows the distribution and density of HRP-labelled axons in coronal sections of the ipsilateral and contralateral dLGN in P4 and P8 hamsters following unilateral intravitreal injection of the tracer. Note the overlap between crossed and uncrossed axons at P4 and that segregation has occurred by P8 (compare with Fig. 1). The boxed areas show the regions sampled by electron microscopy in each animal in a systematic search for inappropriately located synaptic terminals of crossed axons (left) and uncrossed axons (right).

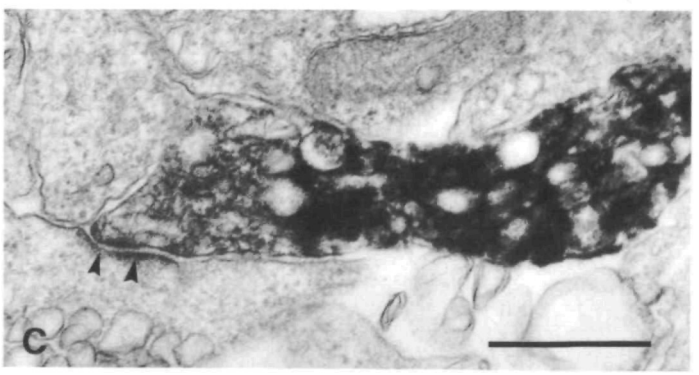
mature animals; and, on the contralateral side, a dorsomedial area at approximately the mid-rostrocaudal level of the nucleus, corresponding to the region occupied by ipsilateral axons in the mature animal. All HRP-labelled retinal axons and terminals in these areas were recorded and their relationships analysed (Campbell *et al.* 1984).

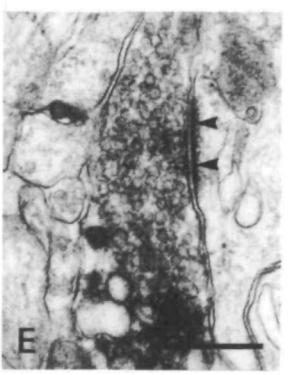
The findings are illustrated in Fig. 5. Labelled crossed and a few uncrossed axons were present at P0 and became progressively more common over the following days; appropriately located labelled uncrossed axons and terminals in the centromedial part of the nucleus (future ipsilateral sector) were relatively less common than labelled crossed axons in the ventrolateral part of the nucleus (part of the future contralateral sector), particularly between P0 and P3. Synaptic contacts established by such labelled axons, in all parts of the dLGN, were characterised by predominantly electron-lucent spherical presynaptic vesicles and a prominent postsynaptic density. At P4, labelled uncrossed axons made synaptic contact in the future contralateral sector (which is devoid of uncrossed input after P8–P10) (Fig. 5C,D) and a few crossed axons made synaptic contacts in the future ipsilateral sector (devoid of crossed input after P8–P10) (Fig. 5E,F). Such terminals and their synaptic contacts were similar to appropriately located ones in the same material (Fig. 5A,B). Inappropriately located terminals of uncrossed axons were not found in the future contralateral sector at P6, or in adults (So *et al.* 1985). No specialised contacts were observed between inappropriately located axons or terminals and either other axon terminals or glial cell processes. The absence of specialized contacts and the infrequency of direct contiguities between labelled axons and other axons during the period of segregation make it unlikely that segregation involves direct axo-axonal interactions. More important, these studies show that during the development of the hamster retinogeniculate projection, inappropriately located axons establish transient synaptic contacts with geniculate cells, and that these contacts are lost as the segregated adult pattern of projections is established.

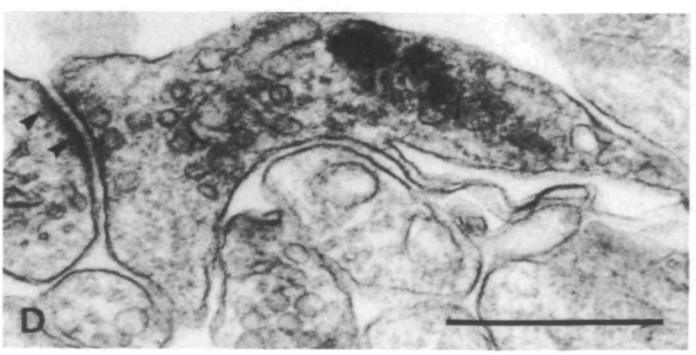
More recently, the findings in the hamster have been confirmed and extended in the cat (Campbell and Shatz, 1986; G. Campbell and C. J. Shatz, in preparation). In the cat, developing retinogeniculate axons traversing the dLGN emit short side branches into both future appropriate and inappropriate territories; the branches in inappropriate territory are lost as segregation takes place between E47 and birth at E65 (Sretavan and Shatz, 1986*a*). We used long series of thin sections to reconstruct individual crossed axons (labelled with HRP from the optic tract in an *in vitro* slice preparation) as they traversed appropriate, future contralateral territory and inappropriate, future ipsilateral territory. The inappropriate zone was identified by injecting [H^3]proline into the ipsilateral eye 24 h before making the slice preparation and processing for autoradiography 1 μm sections cut adjacent to the sections used for EM. We found (Fig. 6) that both the side branches and the main axon trunk made numerous synaptic contacts of variable degrees of maturity within both appropriate (Fig. 6A–C) and inappropriate (Fig. 6D–F) territory. The length and thickness of the postsynaptic densities were


5A


B


C


E


D

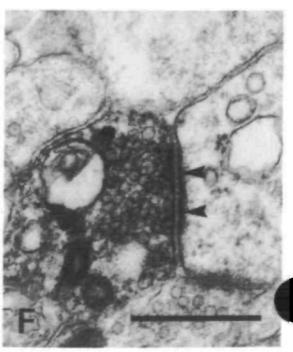

F

Fig. 5. Electron micrographs of HRP-labelled retinogeniculate axons and synaptic terminals in the dLGN of early postnatal hamsters. (A,B) Appropriately located terminals in the future ipsilateral sector at P4 (A) and in the future contralateral sector at P6 (B) establishing synaptic contact (arrowheads) with dendritic elements. The terminals are densely packed with spherical synaptic vesicles and the postsynaptic densities with which they are associated are prominent (Gray type I contacts). (C,D) Inappropriately located terminals of uncrossed axons in the future contralateral sector at P4, both in synaptic contact (arrowheads) with dendritic elements. (E,F) Inappropriately located terminals of crossed axons in the future ipsilateral sector at P4 (E) and P6 (F). In both cases the terminals contain numerous synaptic vesicles and establish Gray type I synaptic contacts (arrows). Scale bars, 0.5 μm.

similar in both territories at E53, but there were significantly larger numbers of presynaptic vesicles at synaptic contacts in appropriate territory than at those in inappropriate territory (compare Fig. 6D–F with Fig. 6A–C). This work, like the earlier work on the hamster, shows that large numbers of synaptic contacts are made by cat retinogeniculate axons in inappropriate territory prior to segregation. In the case of the cat, however, there is also evidence that these synapses are functional. Electrical stimulation of the optic nerve in slice preparations of the dLGN from foetuses at this stage of development shows that dLGN neurones receive convergent excitatory inputs from both optic nerves, whereas by birth (after the loss of side branches from inappropriate territory and, therefore, also the loss of many synapses) many neurones receive excitatory input from one optic nerve only (Shatz and Kirkwood, 1984).

There can be little doubt that these synapses *are* transient and that they are lost by the time the retinogeniculate system is mature, because: (i) all the side branches in inappropriate territory disappear (Sretavan and Shatz, 1986*a*); (ii) mature retinogeniculate axons in cat are generally, and probably always, myelinated up to the preterminal region (Famiglietti and Peters, 1972; Bowling and Michael, 1984); and (iii) geniculate neurones may be driven binocularly during the overlap period but only monocularly thereafter (Kato *et al.* 1971; Shatz and Kirkwood, 1984).

Elimination of transient axons and synapses

We do not know how the inappropriately located axons (side branches in the cat) and the synapses they make are eliminated. Part of the uncertainty stems from ignorance as to the precise nature of these axons. In the case of the cat, it is clear that the eliminated side branches and synapses are transient components of retinogeniculate axons which make terminal arbors in other, appropriate, regions of the dLGN, and persist after the branches and synapses are eliminated. The situation in rodents is much less clear, and Fig. 7 depicts four different possibilities for the nature of inappropriately located uncrossed axons and synapses in the hamster dLGN prior to segregation. (Although only inappropriately located uncrossed axons are depicted in Fig. 7 and discussed below, similar explanations would be applicable to inappropriately located axons and synapses of crossed

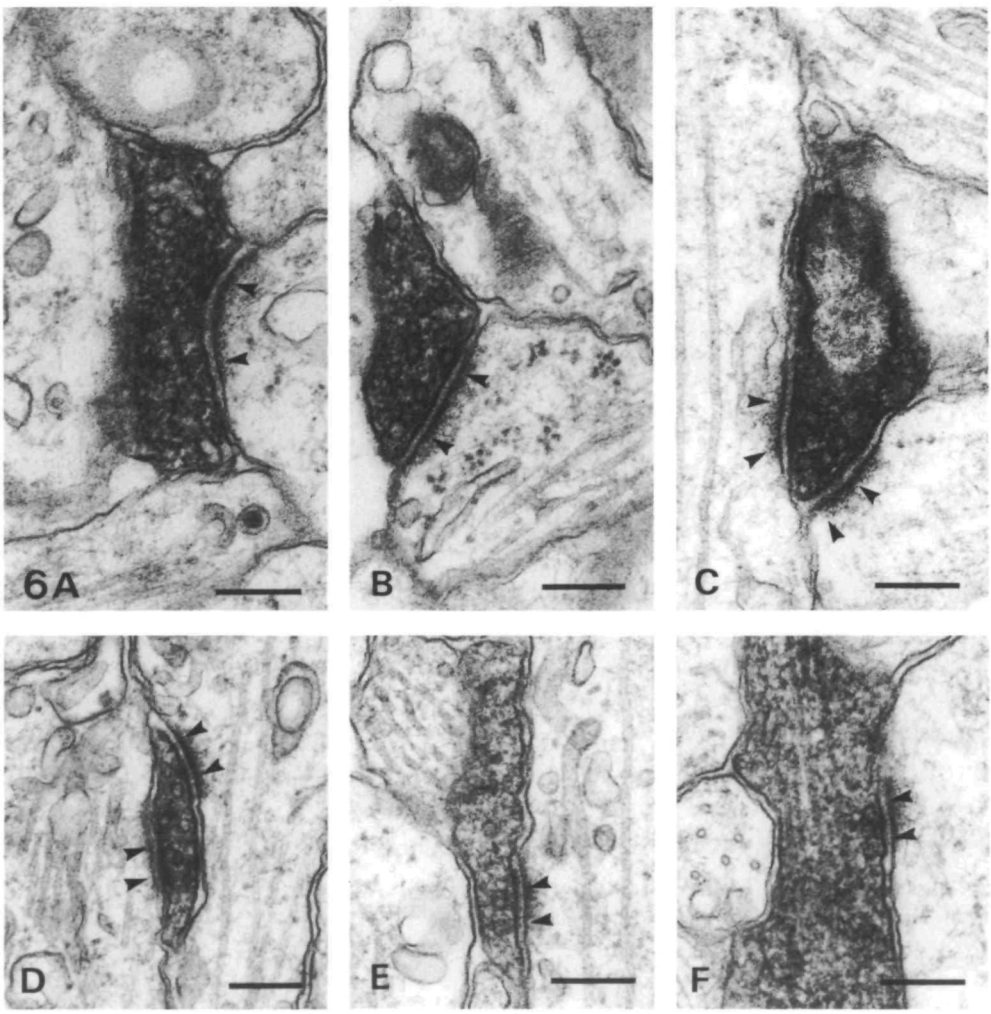


Fig. 6. Electron micrographs of retinogeniculate terminals from a serially sectioned and reconstructed HRP-filled retinogeniculate axon of a cat foetus at E53 (from Campbell and Shatz, 1986; G. Campbell and C. J. Shatz, in preparation). (A–C) Appropriately located terminals, arising from side branches of a crossed retinogeniculate axon, within the future A lamina (which receives only crossed input in the adult). The terminals establish synaptic contacts (arrowheads) with various dendritic elements. (D–F) Inappropriately located terminals arising from side branches (D and E) and from the main axon trunk (F) of a crossed retinogeniculate axon within the future A1 lamina (which receives only uncrossed input in the adult). The terminals all make immature synaptic contacts (arrowheads) with dendritic elements. Note that the numbers of synaptic vesicles adjacent to the synaptic specialisations of the transient synapses in D–F are smaller than those in A–C. Scale bars, 0.2 μm.

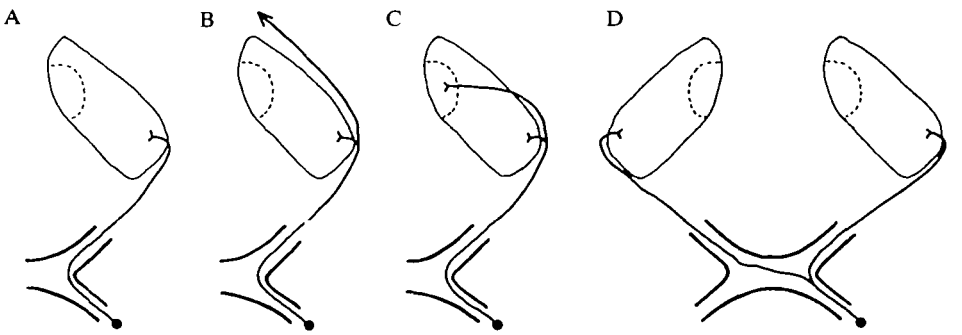


Fig. 7. Diagrams illustrating the possible nature and origins of inappropriately located uncrossed axons and terminals in the future contralateral territory of the dLGN of the hamster before segregation has taken place. (A) The axons are those of retinal ganglion cells which die. (B) The axons are collaterals of uncrossed axons projecting to the superior colliculus (and/or pretectum). (C) The axons are collaterals (and/or possibly *en passant* portions of the shaft) of uncrossed axons with their main termination site in the future ipsilateral sector of the dLGN. (D) The axons are collaterals of retinal ganglion cell axons which branch at the optic chiasm and also project to the contralateral dLGN.

axons.) The first possibility, depicted in Fig. 7A, is that these axons belong to a population of neurones that die, thus eliminating the inappropriately located axons and synapses. Since the period of segregation coincides with a period of extensive retinal ganglion cell death in the hamster (Sengelaub and Finlay, 1982; Insausti *et al.* 1984) and in the rat (Jeffery, 1984), it is a reasonable supposition that the axons degenerate as a result of cell death (although it is also possible that death of the parent cell bodies is a consequence of degeneration or atrophy of the inappropriately located axons). The idea that loss of inappropriately located axons is due to cell death is based not only on temporal coincidence between cell death and segregation (see also Rakic, 1986) but also on a general hypothesis that seeks to explain the elimination of initially excessive and extensive connections during development in terms of the death of cells that fail to compete successfully with other cells for a limited amount of trophic factor. Furthermore, there is good evidence that cell death does play a part in the elimination of topographic 'errors' by retinal ganglion cell axons and thus helps in the process of establishing correct visual topography within the brain (O'Leary *et al.* 1986).

There remain, however, serious doubts as to whether cell death is the correct and/or only explanation for the elimination of inappropriately located retinogeniculate axons. The two phenomena are not closely temporally correlated in all species (see e.g. Shatz and Sretavan, 1986). Furthermore, there is strong evidence that many neurones refine their axonal arbors during development by selectively shedding only certain branches (e.g. Stanfield, 1984); in such cases the eliminated axons must either retract and be absorbed back into the parent axon, or die back to their point of origin from the parent axon. In Fig. 7B–D are depicted several

possible explanations for the nature of transient inappropriately located ipsilateral retinogeniculate axons in the hamster, based on this supposition. First, they may be branches of axons projecting to the ipsilateral superior colliculus and/or to other retinorecipient nuclei (Fig. 7B). Such an explanation would be in keeping with what is known of the mode of development of the rodent retinogeniculate system (see above). Another possibility is that the inappropriately located axons are branches of axons (and/or possibly *en passant* axonal shafts) projecting to the appropriate part of the ipsilateral dLGN (Fig. 7C). This possibility is given considerable strength by the findings in the cat (see above). Finally, they could perhaps be branches of contralaterally projecting axons (Fig. 7D), although this last possibility has been weakened by evidence that ganglion cells with axons branching to both sides of the brain are very rare in both rat and hamster (Jeffery and Perry, 1982; Hsiao *et al.* 1984; Insausti *et al.* 1984).

Although we do not know the mechanisms that direct the loss of these axons, our results suggest that failure to make synaptic connections cannot, by itself, be responsible for parental cell death or selective retraction or shedding of collaterals. Of course, the synapses established in inappropriate territory may be somehow less efficient than those in appropriate territory (e.g. as suggested by the smaller number of vesicles in the cat – see above) or they may fail to establish the minimum number of synaptic contacts necessary for their survival, or for one reason or another fail to derive an adequate supply of trophic factor(s) from the contacted cells. At present there are no data to support or refute these possibilities. However, we do know that, in the absence of 'competing' fibres (brought about by removing one eye at birth), the transient synapses formed by the ipsilateral fibres of the remaining eye in the regions of the dLGN that do not receive uncrossed retinal inputs in normal adult animals, can be stabilised to form well-differentiated synaptic contacts with geniculate projection neurones and interneurones (Campbell *et al.* 1985; Robson *et al.* 1978).

Role of activity

Recent evidence suggests that neural activity might be involved in directing segregation (see reviews by Shatz and Stretavan, 1986; Shatz, 1990); it is certainly involved in eliminating supernumerary axons during development of the mature pattern of motor innervation of striated muscle (Purves and Lichtman, 1980). Although activity may be important, patterned visual stimulation is probably not important because the adult distribution of the retinogeniculate fibres in the hamster is established prior to the time of eye opening at around day 15. Diffuse light stimulation through the eyelid is also unlikely to be of significance, for no abnormality in the development of the retinogeniculate terminal field could be detected by light microscopy in hamsters reared in the dark from birth (So *et al.* 1982) or in the morphology or location of retinogeniculate X and Y axonal arbors in dark-reared cats (Garraghty *et al.* 1987). However, there is some evidence to

suggest that spontaneous activity in retinal ganglion cell axons may be significant in the control of segregation and possibly also of synapse elimination. Thus, injection of tetrodotoxin (TTX; a blocker of the voltage-sensitive sodium channel) into one or both eyes of rodents during the overlap and segregation phases appears to have some effect on the segregation of retinocollicular axons (Fawcett and O'Leary, 1985; Thompson and Holt, 1989). In addition, infusion of TTX intracranially above the optic chiasm of foetal cats prevents the segregation of retinogeniculate inputs (Shatz and Stryker, 1988). Further evidence for the involvement of neural activity in the segregation of terminal arbors comes from work on the formation of ocular dominance columns in the cat visual cortex. These patches do not develop when neural activity is blocked by intraocular or intracortical administration of TTX, or when a particular pattern of activity is delivered synchronously to both optic nerves, but do develop when these activity patterns are asynchronous (see Shatz, 1990). Recent evidence also suggests that correlated electrical activity between ganglion cells of a single eye may be important in segregation of retinogeniculate axons. Using a multi-electrode recording technique, Meister *et al.* (1990) have demonstrated that adjacent ganglion cells in isolated retinae from developing cats and ferrets display a highly synchronised firing pattern throughout the period of segregation, but not thereafter. If correlated activity is important for segregation, NMDA receptors may be involved, as is suggested by recent work on the development of the retinotectal projection in frogs (Constantine-Paton *et al.* 1990). They have shown that establishment of normal retinotectal topography (which involves the formation and subsequent loss/translocation of transient synapses; Gaze *et al.* 1979), is disrupted by applying to the tectum the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino 5-phosphonovaleric acid (APV), which may impair the coordinated migration of terminals arising from neighbouring retinal ganglion cells.

Thus, evidence that activity plays a role in binocular overlap and segregation is persuasive and suggests that retinal ganglion cells should form synaptic contacts before and during the segregation period and, in particular, that such synapses should initially be present in areas ultimately occupied by input only from the other eye. Our ultrastructural results support this requirement and we therefore suggest that the formation of transient synapses and their elimination is an essential feature, not just for the development of the retinogeniculate pathways but probably also for the development of other orderly connections in the central nervous system.

Work by the authors reported in this paper was made possible by financial support from several sources. We thank in particular the following organisations: KFS, The Croucher Foundation, Wing Lung Bank, Pauline Chan Medical Research Fund and University of Hong Kong; ARL, The Wellcome Trust and Danish Medical Research Council; GC, The British Council, National Science Foundation and March of Dimes.

References

Bhide, P. G., Lieberman, A. R. and Campbell, G. (1988*a*). Dendritic invagination of developing optic tract axons in the hamster. *Expl Brain Res.* **73**, 519–522.

Bhide, P. G., Lieberman, A. R. and Frost, D. O. (1988*b*). Evidence that dendrites of hamster geniculate neurons establish contact with retinal axons in the optic tract at the time and at the site of emission of retinogeniculate branches. *J. Anat., Lond.* **158**, 210–211 (Abstract).

Bowling, D. B. and Michael, C. R. (1984). Terminal patterns of single physiologically characterized optic tract fibers in the cat's lateral geniculate nucleus. *J. Neurosci.* **4**, 198–216.

Calvalcante, L. A. and Rocha-Miranda, C. G. (1978). Postnatal development of the retinogeniculate, retinopretectal and retinotectal projections in the opposum. *Brain Res.* **146**, 231–248.

Campbell, G. and Shatz, C. J. (1986). Synapses formed by individual retinogeniculate axons in inappropriate LGN territory during formation of eye-specific layers. *Soc. Neurosci. Abstr.* **12**, 589.

Campbell, G., So, K.-F. and Lieberman, A. R. (1984). Normal postnatal development of retinogeniculate axons and terminals and identification of inappropriately located transient synapses: electron microscope studies of horseradish peroxidase-labelled retinal axons in the hamster. *Neuroscience* **13**, 743–759.

Campbell, G., So, K.-F. and Lieberman, A. R. (1985). Identification of synapses formed by the aberrant, uncrossed retinogeniculate projection in the hamster after neonatal monocular enucleation. *Devl Brain Res.* **21**, 137–140.

Chalupa, L. M. and Williams, R. W. (1984). Prenatal development and reorganisation in the visual system of the cat. In *Development of Visual Pathways in Mammals* (ed. J. Stone, B. Dreher and D. Rapaport), pp. 89–102. New York: A. R. Liss Inc.

Coleman, L.-A. and Beazley, L. D. (1989). Expanded retinofugal projections to the dorsal lateral geniculate nucleus and superior colliculus after unilateral enucleation in the wallaby *Setonix brachyurus*, quokka. *Devl Brain Res.* **48**, 273–291.

Constantine-Paton, M., Cline, H. T. and Debski, E. (1990). Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. *A. Rev. Neurosci.* **13**, 129–154

Cox, E. C., Müller, B. and Bonhoeffer, F. (1990). Axonal guidance in the chick visual system: posterior tectal membranes induce collapse of growth cones from temporal retina. *Neuron* **4**, 31–37.

Cusick, C. G. and Kaas, J. H. (1982). Retinal projections in the adult and newborn grey squirrels. *Devl Brain Res.* **4**, 275–284.

Familglietti, E. V., Jr and Peters, A. (1972). The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus of the cat. *J. comp. Neurol.* **144**, 285–334.

Fawcett, J. W. and O'Leary, D. D. M. (1985). The role of electrical activity in the formation of topographic maps in the nervous system. *Trends Neurosci.* **8**, 201–206.

Frost, D. O., So, K.-F. and Schneider, G. E. (1979). Postnatal development of retinal projections in Syrian hamsters: a study using autoradiographic and anterograde degeneration technique. *Neuroscience* **4**, 1649–1677.

Garraghty, P. E., Frost, D. O. and Sur, M. (1987). The morphology of retinogeniculate X- and Y-cell axonal arbors in dark-reared cats. *Expl Brain Res.* **66**, 115–127.

Gaze, R. M., Keating, M. J., Ostberg, A. and Chung, S-H. (1979). The relationship between retinal and tectal growth in larval *Xenopus*: implications for the development of the retinotectal projection. *J. Embryol. exp. Morph.* **53**, 103–143.

Godement, P., Saillour, P. and Imbert, M. (1980). The ipsilateral optic pathway to the dorsal lateral geniculate nucleus and superior colliculus in mice with prenatal or postnatal loss of one eye. *J. comp. Neurol.* **190**, 611–626.

Godement, P., Salaün, J. and Imbert, M. (1984). Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse. *J. comp. Neurol.* **230**, 552–575.

Guillery, R. W. (1979). A speculative essay on geniculate lamination and its development. *Prog. Brain Res.* **51**, 403–418.

Guillery, R. W. (1987). Competition in the development of the visual pathways. In *The*

Making of the Nervous System (ed. J. G. Parnavelas, C. D. Stern and R. V. Stirling), pp. 356–379. Oxford: Oxford University Press.

GUILLERY, R. W., POLLEY, E. H. AND TORREALBA, F. (1982). The arrangement of axons according to fiber diameter in the optic tract of the cat. *J. Neurosci.* **2**, 714–721.

HEFFNER, C. D., LUMSDEN, A. G. S. AND O'LEARY, D. D. M. (1990). Target control of collateral extension and directional axon growth in the mammalian brain. *Science* **247**, 217–220.

HSIAO, K., SACHS, G. M. AND SCHNEIDER, G. E. (1984). A minute fraction of Syrian golden hamster retinal ganglion cells project bilaterally. *J. Neurosci.* **4**, 359–367.

INSAUSTI, R., BLAKEMORE, C. AND COWAN, W. M. (1984). Ganglion cell death during development of ipsilateral retino-collicular projection in golden hamster. *Nature* **308**, 362–365.

INSAUSTI, R., BLAKEMORE, C. AND COWAN, W. M. (1985). Postnatal development of the ipsilateral retinocollicular projection and the effects of unilateral enucleation in the golden hamster. *J. comp. Neurol.* **234**, 393–409.

JEFFERY, G. (1984). Retinal ganglion cell death and terminal field retraction in the developing rodent visual system. *Devl Brain Res.* **13**, 81–96.

JEFFERY, G. AND PERRY, V. H. (1982). Evidence for ganglion cell death during development of the ipsilateral retinal projection in the rat. *Devl Brain Res.* **2**, 176–180.

JEN, L. S. AND SO, K.-F. (1983). Effect of early removal of superior colliculus or visual cortex upon the segregation of retinogeniculate projections in hamsters. *Neurosci. Lett. (Suppl.)* **12**, S7 (Abstract).

KAAS, J. H., GUILLERY, R. W. AND ALLMAN, J. M. (1972). Some principles of organization in the dorsal lateral geniculate nucleus. *Brain Behav. Evol.* **6**, 253–299.

KATO, H., YAMAMOTO, M. AND NAKAHAMA, H. (1971). Intracellular recordings from the lateral geniculate neurones of cats. *Jap. J. Physiol.* **21**, 307–323.

LAND, P. W. AND LUND, R. D. (1979). Development of the rat's uncrossed retinotectal pathway and its relation to plasticity studies. *Science* **205**, 698–700.

LINDEN, D. C., GUILLERY, R. W. AND CUCCHIARO, J. (1981). The dorsal lateral geniculate nucleus of the normal ferret and its postnatal development. *J. comp. Neurol.* **203**, 189–211.

LINDEN, R. AND PERRY, V. H. (1983). Massive retinotectal projection in rats. *Brain Res.* **272**, 145–149.

LUND, R. D., CUNNINGHAM, T. J. AND LUND, J. S. (1973). Modified optic projections after unilateral eye removal in young rats. *Brain Behav. Evol.* **8**, 51–72.

MANFORD, M., CAMPBELL, G. AND LIEBERMAN, A. R. (1984). Postnatal development of ipsilateral retino-geniculate projections in normal albino rats and the effects of removal of one eye at birth. *Anat. Embryol.* **170**, 71–78.

MARIANI, J. (1983). Elimination of synapses during the development of the central nervous system. *Prog. Brain Res.* **58**, 383–392.

MARTIN, P. (1986). The projection of different retinal ganglion cell classes to the dorsal lateral geniculate nucleus in the hooded rat. *Expl Brain Res.* **62**, 77–88.

MASTRONARDE, D. N. (1984). Organization of the cat's optic tract as assessed by single-axon recordings. *J. comp. Neurol.* **227**, 14–22.

MEISTER, M., WONG, R. D. L., BAYLOR, D. A. AND SHATZ, C. J. (1990). Synchronous bursting activity in ganglion cells of the developing mammalian retina. *Invest. Ophthalmol. vis. Sci. (Suppl.)* **31**, 115 (Abstract).

O'LEARY, D. D. M., FAWCETT, J. W. AND COWAN, W. M. (1986). Topographic targeting errors of axons in the retinocollicular projection, and their elimination by selective ganglion cell death. *J. Neurosci.* **6**, 3692–3705.

O'LEARY, D. D. M. AND TERASHIMA, T. (1988). Cortical axons branch to multiple subcortical targets by interstitial axon budding: implications for target recognition and 'waiting periods'. *Neuron* **1**, 901–910.

PURVES, D. AND LICHTMAN, J. W. (1980). Elimination of synapses in the developing nervous system. *Science* **210**, 153–157.

RAKIC, P. (1977). Prenatal development of the visual system in rhesus monkey. *Phil. Trans. R. Soc. Ser.* B **278**, 245–260.

RAKIC, P. (1981). Development of visual centres in the primate brain depends on binocular competition before birth. *Science* **214**, 928–931.

Rakic, P. (1986). Mechanism of ocular dominance segregation in the lateral geniculate nucleus: competitive elimination hypothesis. *Trends Neurosci.* **9**, 11–15.

Reese, B. E. (1987). The position of the crossed and uncrossed optic axons, and the non-optic axons, in the optic tract of the rat. *Neuroscience* **22**, 1025–1039.

Reese, B. E. (1988). 'Hidden lamination' in the dorsal lateral geniculate nucleus: the functional organization of this thalamic region in the rat. *Brain Res. Rev.* **13**, 119–137.

Robson, J. A., Mason, C. A. and Guillery, R. W. (1978). Terminal arbors of axons that have formed abnormal connections. *Science* **201**, 635–637.

Rodieck, R. W. (1979). Visual pathways. *A. Rev. Neurosci.* **2**, 193–225.

Sanderson, K. J., Dixon, P. G. and Pearson, L. J. (1982). Postnatal development of retinal projections in the brushtailed possum, *Trichosurus vulpecula. Devl Brain Res.* **5**, 161–180.

Schneider, G. E., Jhaveri, S., Edwards, M. A. and So, K.-F. (1985). Regeneration, re-routing and redistribution of axons after early lesions: changes with age and functional impact. In *Recent Advances in Restorative Neurology: Upper Motor Neuron Functions and Dysfunctions* (ed. J. Eccles and M. R. Dimitrijevic), pp. 291–310. Basel: Karger.

Sengelaub, D. R. and Finlay, B. L. (1982). Cell death in the mammalian visual system during normal development. I. Retinal ganglion cells. *J. comp. Neurol.* **204**, 311–317.

Shatz, C. J. (1983). The prenatal development of the cat's retinogeniculate pathway. *J. Neurosci.* **3**, 482–499.

Shatz, C. J. (1990). Competitive interactions between retinal ganglion cells during prenatal development. *J. Neurobiol.* **21**, 197–211.

Shatz, C. J. and Kirkwood, P. (1984). Prenatal development of functional connections in the cat's retinogeniculate pathway. *J. Neurosci.* **4**, 1378–1397.

Shatz, C. J. and Sretavan, D. W. (1986). Interactions between retinal ganglion cells during the development of the mammalian visual system. *A. Rev. Neurosci.* **9**, 171–207.

Shatz, C. J. and Stryker, M. P. (1988). Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. *Science* **242**, 87–89.

So, K.-F., Campbell, G. and Lieberman, A. R. (1985). Synaptic organization of the dorsal lateral geniculate nucleus in the adult hamster: an electron microscope study using degeneration and horseradish peroxidase tracing techniques. *Anat. Embryol.* **171**, 223–234.

So, K.-F., Schneider, G. E. and Frost, D. O. (1978). Postnatal development of retinal projections to the lateral geniculate body in Syrian hamsters. *Brain Res.* **142**, 343–352.

So, K.-F., Woo, H. H. and Jen, L. S. (1982). Effect on segregation of retinogeniculate projections in early unilaterally enucleated or dark reared hamsters. *Soc. Neurosci. Abstr.* **8**, 816.

So, K.-F., Woo, H. H. and Jen, L. S. (1984). The normal and abnormal postnatal development of retinogeniculate projections in golden hamsters: an anterograde horseradish peroxidase tracing study. *Devl Brain Res.* **12**, 191–205.

Sretavan, D. W. and Shatz, C. J. (1986*a*). Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers within the cat's lateral geniculate nucleus. *J. Neurosci.* **6**, 234–251.

Sretavan, D. W. and Shatz, C. J. (1986*b*). Prenatal development of cat retinogeniculate axon arbors in the absence of binocular interactions. *J. Neurosci.* **6**, 990–1003.

Stanfield, B. B. (1984). Postnatal reorganization of cortical projections: the role of collateral elimination. *Trends Neurosci.* **7**, 37–41.

Stanfield, B. B., Nahin, B. R. and O'Leary, D. D. M. (1987). A transient postmammillary component of the rat fornix during development: implication for interspecific differences in mature axonal projections. *J. Neurosci.* **7**, 3350–3361.

Thompson, I. and Holt, C. (1989). Effects of intraocular tetrodotoxin on the development of the retinocollicular pathway in the Syrian hamster. *J. comp. Neurol.* **282**, 371–388.

Walter, J., Henke-Fahle, S. and Bonhoeffer, F. (1987). Avoidance of posterior tectal membranes by temporal retinal axons. *Development* **101**, 900–913.

Woo, H. H., Jen, L. S. and So, K.-F. (1985). The postnatal development of retinocollicular projections in normal hamsters and in hamsters following neonatal monocular enucleation: a horseradish peroxidase tracing study. *Devl Brain Res.* **20**, 1–13.