

## PROJECTION LINES AND THE IPSILATERAL RETINO-GENICULATE PATHWAY IN THE HOODED RAT

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**Abstract**—The organization of the hooded rat's dorsal lateral geniculate nucleus was studied with anatomical techniques, with particular regard to the representation of temporal retina and the binocular field. The ipsilateral and contralateral retinal terminal fields were examined in three stereotaxic planes following injections of horseradish peroxidase into the eye. Projections arising from the temporal crescent of the retina were studied with silver staining techniques for anterograde degeneration products. Following discrete retinal lesions there was clear evidence that the temporal retina projects in a topographic fashion both ipsilaterally and contralaterally. The orientation of the lines of projection in the dorsal lateral geniculate nucleus was assessed by retrograde labelling of cells after cortical implants of horseradish peroxidase. Although both the lines of projection and the ipsilateral terminal field extend rostro-caudally in the dorsal lateral geniculate nucleus, their paths are oblique rather than parallel. Their intersection appears to correspond to the representation in this nucleus of conjugate retinal points. This was confirmed by administering horseradish peroxidase by iontophoresis in either the binocular or monocular representation of the primary visual cortex, while one eye received an injection of [<sup>3</sup>H]proline. Only those cortical injections in the binocular region gave rise to labelled projection lines passing through the autoradiographically-labelled ipsilateral terminal field.

The rat's dorsal lateral geniculate nucleus displays none of the cytoarchitectural lamination which is so prominent in the primate and cat. Even after labelling the input to the nucleus from one eye, there is still no obvious laminar relationship between the terminal fields from the two eyes. Despite the absence of lamination, the current results suggest that the principle of apposing the representation of conjugate retinal points in the dorsal lateral geniculate nucleus is the same in the rat as in cat and monkey.

In many mammalian species, the dorsal lateral geniculate nucleus (dLGN) of the thalamus is laminated, with each lamina receiving afferents from one or the other eye. These retinal afferents project topographically to the dLGN, creating an orderly representation of the contralateral visual hemifield in each lamina. The retinotopic maps in the laminae are in register so that "lines of projection", each representing one position in the visual field, course through the nucleus perpendicular to the laminae, passing successively through each eye's representation.<sup>2</sup> The cells in the dLGN project onto the primary visual area of the cerebral cortex, where the corresponding points of each retina are then brought together in one representation of the contralateral visual hemifield.

In the dLGN of some rodents, notably the rat, mouse, and hamster, there is no obvious cytoarchitectural lamination or discontinuity coincident with the regions or borders of segregated afferent termination from each eye. The ipsilateral retinal projection in these species is relatively small, in terms

of both the percentage of retinal ganglion cells and the volume of dLGN to which they project.<sup>4,7,8,16</sup> There is as yet no published evidence for retinotopy in this rather sparse ipsilateral retino-geniculate projection, and one single-unit electrophysiological study failed to find evidence for any visuotopic order.<sup>20</sup> This ipsilateral retinal projection occupies a medial region along the greater part of the dLGN's rostro-caudal axis. However, the "lines of projection" for the contralateral eye also traverse the nucleus primarily rostro-caudally,<sup>8,18,20,21</sup> rather than in a direction perpendicular to the field of ipsilateral termination. This raises the possibility that in the unlaminated dLGN of rodents, the principle of apposing the representation of corresponding retinal points does not apply.

A recent electrophysiological mapping study of the dLGN revealed a visuotopy within the pigmented rat's ipsilateral retino-geniculate pathway similar to that demonstrated contralaterally, and it was suggested that the two visuotopic representations are in conjugate register.<sup>26</sup> The present study provides anatomical evidence for an ipsilateral retino-geniculate topography, and presents additional evidence of how conjugate apposition may be accomplished within the dLGN of the rat.

**Abbreviations:** dLGN, dorsal lateral geniculate nucleus; LP, lateral posterior nucleus; RN, reticular nucleus of the thalamus; SC, superior colliculus; OT, optic tract; HRP, horseradish peroxidase; TMB, tetramethyl benzidine.

## EXPERIMENTAL PROCEDURES

### *Eye injections of horseradish peroxidase*

Seven adult hooded Lister rats weighing 350–550 g received unilateral eye injections of HRP. The rats were anaesthetized with a 3.0 ml/kg intraperitoneal injection of a mixture of chloral hydrate and sodium pentobarbital (2.1 g of chloral hydrate and 0.5 g of sodium pentobarbitone in 50 ml of distilled water), and placed in a stereotaxic headholder. A 5.0  $\mu$ l injection of a 25% HRP solution (Boehringer/Mannheim; made up in 0.9% saline containing 2% dimethyl sulphoxide) was delivered manually from a 10  $\mu$ l Hamilton syringe. After a 24 h survival, rats were killed with chloroform and perfused through the heart with 0.9% saline followed by 1.25% glutaraldehyde + 2.5% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2 at 20°C. Brains were removed immediately, placed in a 20% solution of sucrose dissolved in phosphate buffer, and left overnight at 4°C. Sections were cut in triplets of 100, 40 and 60  $\mu$ m. In the 100  $\mu$ m sections the anterogradely transported HRP was demonstrated using tetramethylbenzidine as the chromogen.<sup>19</sup> The adjacent 40  $\mu$ m sections were stained with Cresyl Violet acetate, and the 60  $\mu$ m sections were discarded.

### *Retinal lesions and selective silver impregnation of degenerating axons*

Nine adult hooded Lister rats, weighing 400–500 g, received lesions in the temporal crescent of the left retina (i.e. the ventral and temporal peripheral retina, which gives rise to the ipsilateral projection). The rats were anaesthetized as above, and placed in a stereotaxic headholder (level skull). Lesions were produced in two rats by inserting a sterile 25 gauge syringe needle approximately 1 mm through the sclera near the limbus, at either the most ventral or most caudal part of the eye (i.e. either low or high along the presumed temporal crescent). The remaining rats received retinal lesions from a laser ophthalmoscope (Vernon Ingram Laser Ophthalmoscope, Keeler Optical Products, Ltd). The eyelids were sutured open, a drop of 0.25% hyoscine hydrobromide was placed upon the cornea, and after dilation of the pupil a contact lens of zero dioptric power was placed on the cornea. A far temporal retinal location away from blood vessels was viewed and brought into focus through the laser ophthalmoscope. Six to fifteen laser pulses (1 ms each, 980 joules) were given in the same region, administration ceasing after a significant mark was apparent ophthalmoscopically. With the rat's head level in the stereotaxic instrument, the lesions were aimed at far lower retina, far temporal retina, or midway between these two positions (i.e. at three different locations along the presumed temporal crescent).

Four days later, seven rats were re-anaesthetized and placed in the stereotaxic headholder. A mark was made by cautery on the most dorsal part of the limbus of the left eye. Rats were then killed with chloroform and perfused through the heart with 0.9% saline followed by 10% formal-saline. Brains were removed immediately and placed in sucrose formalin for three weeks at 20°C. Left eyes were removed and with the aid of an operating microscope, the anterior chamber and lens was removed, a cut was placed from the cautery mark to the optic disk, and each retina was dissected out and flat-mounted with the cut oriented upwards. Retinae were stained with Cresyl Violet acetate. Brains were sectioned in the coronal plane at 25  $\mu$ m on a freezing microtome, and a 1 in 5 series from the posterior edge of the optic chiasma to the caudal superior colliculus was collected and processed for anterograde degeneration according to the protocol of Wiitanen.<sup>30</sup> A second series was collected and stained with Cresyl Violet acetate. Adjacent sections through the dLGN containing degeneration were drawn at a magnification of  $\times 105$  with the aid of a drawing tube attached to the microscope.

Two of the rats received HRP injections in the dLGN ipsilateral to the retinal lesion 3 days later. The rats were anaesthetized as above, and placed in the stereotaxic headholder. A small hole was drilled in the skull and enlarged with rongeurs. A single 1.0  $\mu$ l injection of 20% HRP in 2% dimethyl sulphoxide was placed stereotactically (coordinates at 4.0 mm caudal, 3.0 mm lateral, and 5.25 mm ventral to bregma), using a 1.0  $\mu$ l Hamilton syringe. After 24 h, the rats were killed with chloroform, and perfused with 0.9% saline. The left eyes were removed immediately and placed in chilled 0.1% paraformaldehyde in phosphate buffer. Retinae were dissected free and flattened on a clean slide in 2% glutaraldehyde in phosphate buffer for 30 min, followed by a rinse in phosphate buffer for 30 min. Retrogradely transported HRP was demonstrated using *p*-phenylenediamine and catechol as the chromogen,<sup>11</sup> but using the modified procedure of Perry and Linden.<sup>25</sup> After incubation, the retinae were rinsed in phosphate buffer and mounted on gelatinized slides. The retinae, the region of labelled cells (temporal crescent), and the lesion were drawn at a magnification of  $\times 40$  with the aid of a drawing tube attached to the microscope. Although such injections will label axons of passage in the optic tract, Cowey and Perry<sup>4</sup> and B. E. Reese and A. Cowey (unpublished) have demonstrated that tectal-preoptic injections of HRP which exclude the dLGN consistently label a crescent-shaped region in temporal retina no larger than that labelled when the injection site is centered on dLGN. Hence, ganglion cells labelled by transecting such fibres will not overestimate that region of retina from which the ipsilateral retino-geniculate projection arises.

### *Cortical implants of horseradish peroxidase*

Twelve adult hooded Lister rats weighing 350–500 g received unilateral or bilateral placements of HRP in the primary visual area. Rats were anaesthetized as above, and placed in the stereotaxic headholder. A small hole was drilled in the skull and then enlarged with rongeurs. The primary visual area was estimated from the map of Fukuda and Sugatini.<sup>9</sup> Horseradish peroxidase was administered either iontophoretically (see below) or more commonly as a concentrate in polyacrylamide gel surgically implanted beneath the pia under visual control. After a 15–24 h survival, rats were killed and perfused, and the brains were removed and stored, all as described after eye injections of HRP. Frozen sections were cut at 100  $\mu$ m, and every section collected and reacted. Retrogradely transported HRP was demonstrated using *p*-phenylenediamine and catechol as the chromogen.<sup>11</sup> Prior to incubation, however, the sections were soaked for 10 min in a 250 ml solution of 0.03 M sodium cacodylate buffer (pH 5.1) containing 66 mg of ammonium nickel sulfate, 100 mg of cobaltous chloride, 83 mg of catechol and 42 mg of *p*-phenylenediamine.

### *Eye injections of radioactive amino acids combined with cortical injections of horseradish peroxidase*

Six adult hooded Lister rats weighing 400–500 g were used. Rats were anaesthetized as above, and placed in a stereotaxic headholder. Each rat received a left eye injection of 3.0  $\mu$ l of <sup>3</sup>H-proline (specific activity: 48 Ci/mmol; Radiochemicals, Amersham) which had been concentrated by freeze drying and re-dissolved in sterile saline to yield 8  $\mu$ Ci/ $\mu$ l.

Four or five days later each rat was again anaesthetized and placed in the stereotaxic headholder. The right eyelids were sutured open, a drop of 0.25% hyoscine hydrobromide was placed on the cornea, and after the pupil had dilated, a contact lens of zero dioptric power was placed upon the cornea. (The left eye was not prepared, as ophthalmoscopic examination revealed the lens to have been damaged from the eye injection in some cases.) A hole overlying each primary visual area was drilled and enlarged with rongeurs. Iontophoretic injections were made in the primary visual

## Rat retino-geniculate organization

area through a micropipette with a tip width of approximately 10 µm, containing a 25% solution of HRP dissolved in 0.9% saline, with 2% dimethyl sulphoxide. The injections were positioned stereotactically with the aid of the maps provided in Fukuda and Sugatami<sup>9</sup> and Adams and Forrester.<sup>1</sup> In each hemisphere the pipette was lowered into brain tissue only once, and by electrophysiologically recording from the pipette, the tip was microdriven down to a location providing clear visually-evoked responses to the beam of a hand-held ophthalmoscope. Where evoked responses could not be obtained, the electrode was microdriven down to a depth of 750 µm. Current of 1 µA was passed for 2 min (pipette positive) after which the pipette was left in place for 5 min before being slowly removed with the microdrive. After a 24 h survival, rats were killed and perfused, the brains removed and stored, as described after eye injections of HRP. Fifty µm frozen sections were cut, with alternate sections through the dLGN collected for HRP histochemistry and autoradiography. Caudal to the dLGN, three adjacent 1 in 5 series were collected, one for HRP histochemistry, one for autoradiography, and one for Cresyl Violet acetate staining only. Retrograde transport of the HRP was demonstrated using *p*-phenylenediamine and catechol as the chromogen, as described above. The sections were lightly counterstained with Cresyl Violet acetate before being dehydrated and coverslipped. Sections for autoradiography were mounted, defatted in xylene, rehydrated, and then air dried. The slides were coated with Ilford K5 emulsion using the bubble technique of Jenkins<sup>13</sup> and stored in light-tight boxes at 4°C for 6 weeks. They were then developed for 2.5 min at 20°C in Ilford ID19, and stained with Cresyl Violet acetate before being dehydrated and coverslipped.

Every section through the dLGN in the HRP series was drawn at a magnification of ×120 with the aid of a drawing tube attached to the microscope. Every HRP-positive cell in the lateral thalamic region was plotted. Then the borders of the ipsilateral field (determined by autoradiographic label in the left dLGN and its absence in the right dLGN) were drawn after aligning the geniculate boundaries in the autoradiographs with the boundaries drawn from the adjacent HRP series. Generally, the autoradiographs fitted well to the drawings from the HRP series. In instances where the shrinkage was much greater in the sections treated histochemically, the magnification of the drawing tube was adjusted until the geniculate boundaries coincided. All geniculate drawings were completed before reconstruction of the injection sites, which were drawn at a magnification of ×65. The boundaries of the primary visual area were determined with the aid of the criteria of Krieg.<sup>14</sup>

## RESULTS

*Anterograde transport of horseradish peroxidase from the eye*

Although the field of ipsilateral termination in the dLGN of various species of rodent has received much attention, its variation as a function of rostro-caudal extent is apparent in only a few published reports.<sup>8,10,16,18</sup> The field of ipsilateral termination occupies a medial region in the dLGN along its greater rostro-caudal length when viewed in coronal section (Fig. 1). The field is represented throughout the rostral three quarters of the nucleus, with only sparse and scattered terminals detectable in the caudal quarter (Fig. 1H). In the most rostral region of the nucleus, the field occupies the complete dorsal surface, with some scattered terminals present ventrally

(Fig. 1A). Just caudal to this, but still close to the rostral margin, the field becomes restricted to the medial half of the dorsal surface (Fig. 1B). From this rostral location to its most caudal extent, the field adopts its characteristic medial position but shifts ventrally along the medial border in progressive caudal sections (Figs 1B–G). This downward shift of the field of ipsilateral termination more caudally and in relation to the overlying optic tract is clearer in parasagittal sections (Fig. 2).

In the dLGN contralateral to an eye injection of HRP, there is a region devoid of HRP-filled terminals, displaying labelled axons of passage only (Figs 1, 2, and 3). This region displays the same dorsoventral shift through the rostro-caudal extent of the nucleus as does the labelled field of ipsilateral termination in the opposite dLGN, indicating that the regions of termination for each eye's afferents are segregated from one another. It is unclear whether each eye's afferents are completely segregated, since in the ipsilateral dLGN, the labelled terminals often extend to the medial border of the nucleus, while in the contralateral dLGN there is frequently label medial to the vacated ipsilateral field. Thick sections may obscure the appearance of a complete segregation.

Far rostral coronal sections of the contralateral dLGN also display scattered terminals ventral to the main labelled region (Figs 1A, B, C). Adjacent Nissl sections indicate these rostro-ventral ipsilateral and contralateral terminals to be associated with cellular leaflets separated from the overlying main dLGN body by cell-sparse regions. The latter contain radiating fibre bundles from the internal capsule. The terminals beneath these cell-sparse regions appear as extensions from the main bodies of the ipsilateral and contralateral afferent fields caudal to this region, as can be seen in parasagittal and horizontal sections (Figs 2B and 3D). Adjacent Nissl sections in the parasagittal and horizontal planes display a distinct ventral and medial dLGN boundary caudally (the external medullary lamina), but show a less distinct rostral border where these rostro-ventral terminals reach forward. At their furthest rostral location, these terminals lie medial to the dorsal edge of the thalamic reticular nucleus (RN) and lateral to the lateral thalamic nucleus, in a cytoarchitecturally ill-defined region containing fibres from the internal capsule.

In horizontal sections, the ipsilateral field appears to lie along a parasagittal dimension, except in the most dorsal sections, where it extends laterally as it reaches the rostral pole (Fig. 3A). However, examination of the parasagittal sections indicates that there is a very gradual lateral displacement of the deepest, caudal portion of the ipsilateral field which is not apparent in horizontal section (Fig. 2).

*Anterograde degeneration after lesions in the temporal crescent*

Retinal lesions produced with the laser ophthal-

Table 1. Locus of degeneration in the ipsilateral dorsal lateral geniculate nucleus after a temporal retinal lesion

Rat	Length of ipsilateral dLGN ( $\mu\text{m}$ )	Extent of ipsilateral degeneration ( $\mu\text{m}$ )*	Location of maximal degeneration ( $\mu\text{m}$ )*	Location of maximal degeneration expressed as percentage*
H-23	2125	750-1000	875	41
H-16	2250	1000-1250	1125	50
H-17	2125	1125-1625	1250	59
H-15	2250	1250-1625	1375	61
H-18	2250	1125-1750	1500	67
H-19	2250	1625-1750	1625	72
H-24	2250	1500-1875	1625	72

\*In relation to the rostral pole of the dLGN.

moscope gave degeneration of variable extent within the dLGN and superior colliculus. Some of this variability can be attributed to slight differences in the plane of focus of the laser beam, giving rise to variable damage in the ganglion cell and fibre layers. In all rats, ganglion cells peripheral to the lesion site appeared intact and healthy. However, a pilot series with longer survival times showed extensive degeneration of retinal ganglion cells peripheral to the lesion. Because of this uncertainty, only the radial direction, and not the eccentric extent of the damage, is regarded as the independent variable. A characteristic lesion produced by the laser ophthalmoscope is shown in Fig. 4(B).

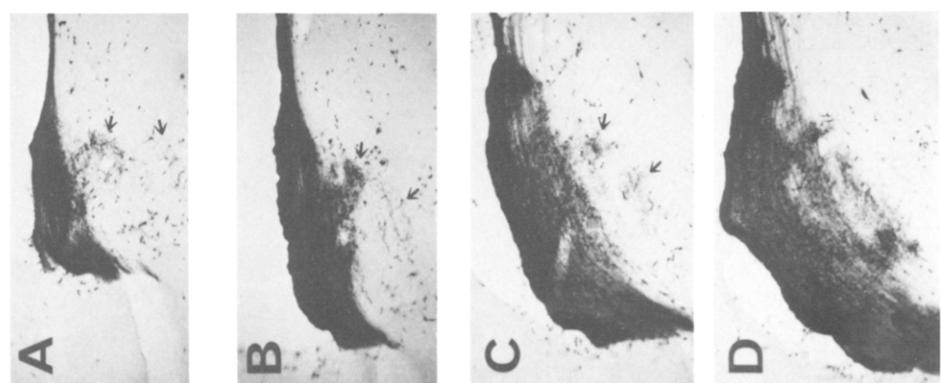
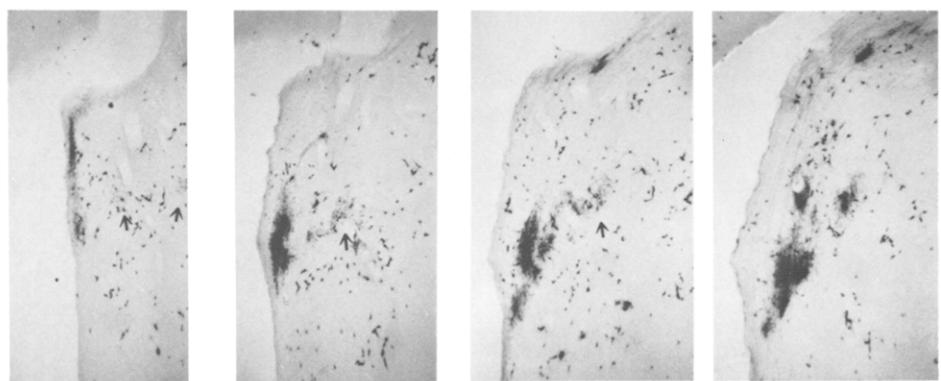
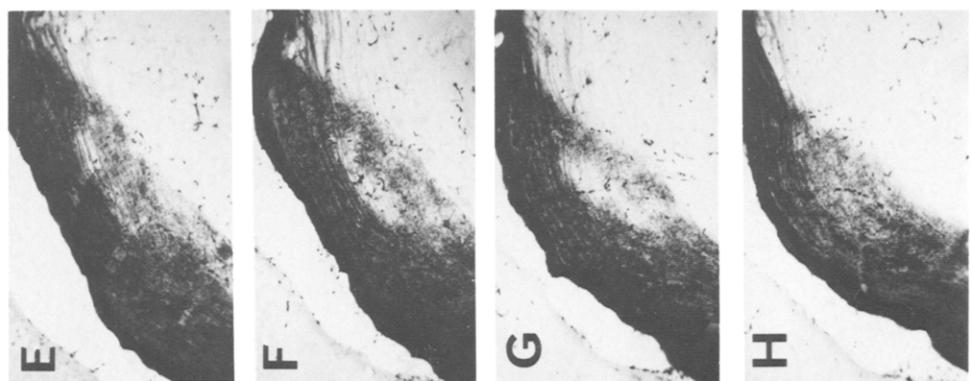
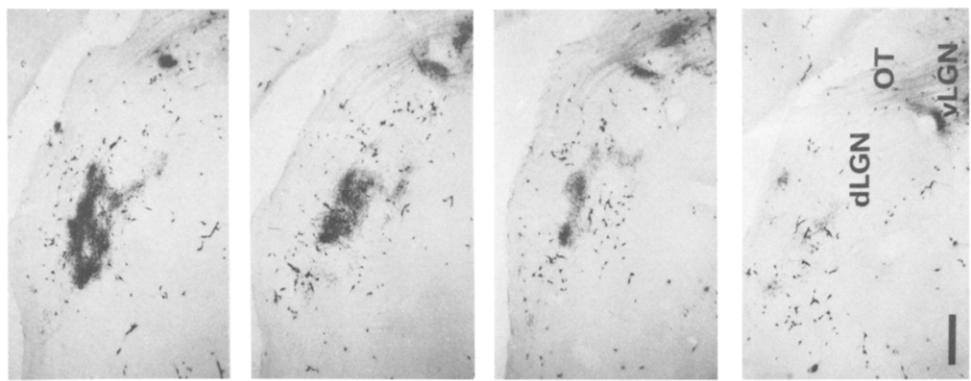
Degeneration in the ipsilateral dLGN occurred in those regions of the nucleus previously demonstrated to receive ipsilateral retinal afferents. It was densest and most widespread in one or a few adjacent sections, tapering off rostrally and caudally. The rostro-caudal location of maximal degeneration depended upon the position of the lesion in the temporal crescent: lesions intended for, and histologically verified to have occurred at, dorsal locations along the presumed crescent gave rise to degeneration caudally, whereas lesions placed further down along the crescent produced degeneration more rostrally (Figs 4C, D, E). Table 1 documents the length of the ipsilateral dLGN, the rostro-caudal extent and location of the ipsilateral degeneration, and the coronal section containing maximal degeneration, with the latter also expressed as a percentage of the length of the dLGN from the rostral pole.

Although there is a correlation between the location of the lesion site on the retina and the rostro-caudal location of degeneration in the dLGN, the shrinkage around the retinal edges and cuts (placed in the process of flat-mounting) obscures this re-

lationship. Rather, a comparison with the location of the crossed degeneration occurring in the superior colliculus (SC; which is itself retinotopically and visuotopically organized<sup>18,29</sup>) provides a clearer demonstration of the ipsilateral retino-geniculate topography. Lesions producing degeneration in the ipsilateral dLGN at rostral locations also produced degeneration in the contralateral SC at its rostro-medial border. As the degeneration occurred at progressively caudal locations in the ipsilateral dLGN, so did the degeneration in the SC shift across its rostral margin to the rostro-lateral border. Figure 4(F) depicts the location of degeneration within the contralateral SC for each animal in Table 1, plotted upon a standard surface reconstruction.<sup>29</sup>

Degeneration was also present in the contralateral dLGN of five rats, always occupying less territory and generally appearing less dense than its ipsilateral counterpart. This contralateral degeneration always occurred caudal to the location of the ipsilateral degeneration, and its focus along the rostro-caudal axis varied as a function of lesion placement (Table 2). In rats H-15, 16, 17, and 18, it occurred along the dorso-medial edge of the nucleus 'sandwiched' against the latero-posterior (LP) nucleus, while in rat H-24, it occurred further ventrally along the ventro-medial edge of the dLGN, again as a narrow band. In this latter case, the lesion may have encroached upon the central edge of the temporal crescent, since this edge increases in eccentricity further up and down the crescent. However, in the four other rats, the lesion was almost certainly within the centre of the temporal crescent. In two additional rats we attempted to make a lesion in the same retinal location, but instead of processing the brains for degeneration, HRP was injected into the ipsilateral thalamus. When the ipsilateral retinae were reacted

Fig. 1. Coronal sections of the dorsal lateral geniculate nucleus displaying the contralateral (left) and ipsilateral (right) retinal terminal fields. The sections are 100  $\mu\text{m}$ , at 200  $\mu\text{m}$  intervals, from rostral to caudal. Medial is to the center of each pair. The ipsilateral terminal field shifts ventrally as it progresses caudally. Note also the scattered clusters of terminals (arrows in A, B, C) ventral to the main retinal terminal field in rostral dLGN. Rat E-11. Tetramethyl benzidine reaction. Calibration bar = 200  $\mu\text{m}$ . (The sparse ipsilateral terminals in A, B and C and in Fig. 2B and 3D are unambiguous microscopically, but are very difficult to reproduce photographically at this magnification).



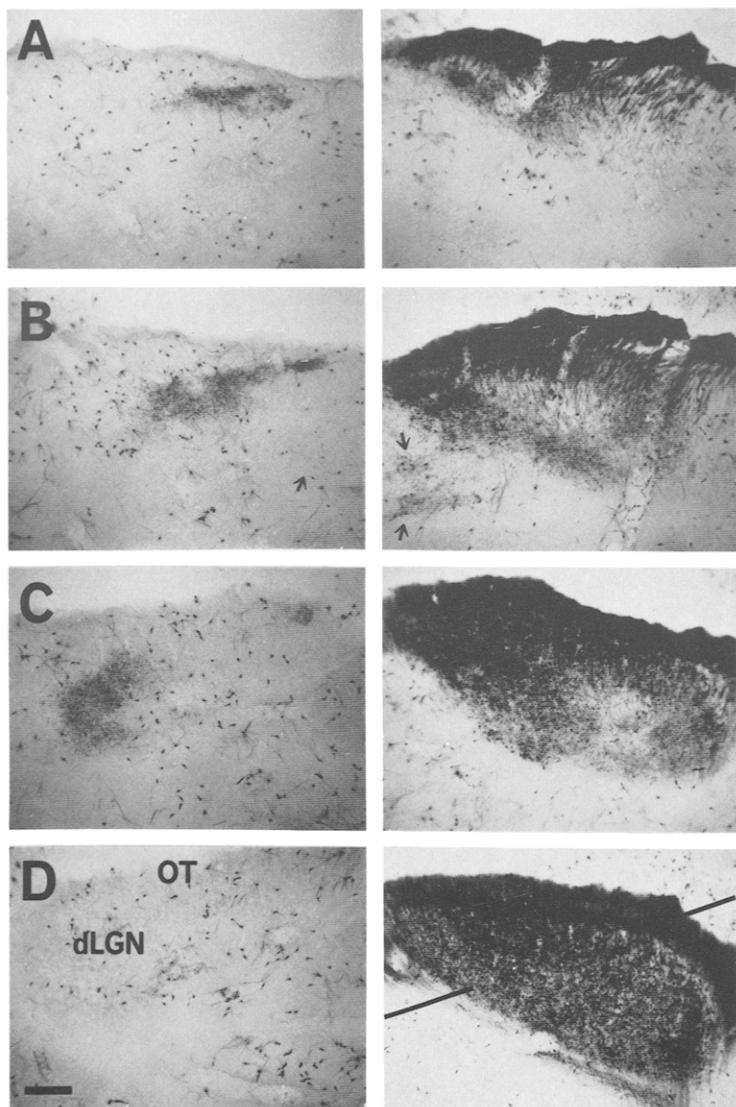


Fig. 2. Parasagittal sections of the dorsal lateral geniculate nucleus displaying the ipsilateral (left) and contralateral (right) retinal terminal fields. Sections are 100  $\mu\text{m}$ , at 200  $\mu\text{m}$  intervals from medial to lateral. Rostral is to the center. The ipsilateral terminal field shifts ventrally, and slightly laterally, from rostral to caudal, except at the rostral-most tip, which also shifts laterally. Note the terminals beneath the main terminal field in rostral dLGN (arrows in B). Rat E-37. Tetramethyl benzidine reaction. Calibration bar = 200  $\mu\text{m}$ . (Line in D indicates approximate plane of section in Fig. 6).

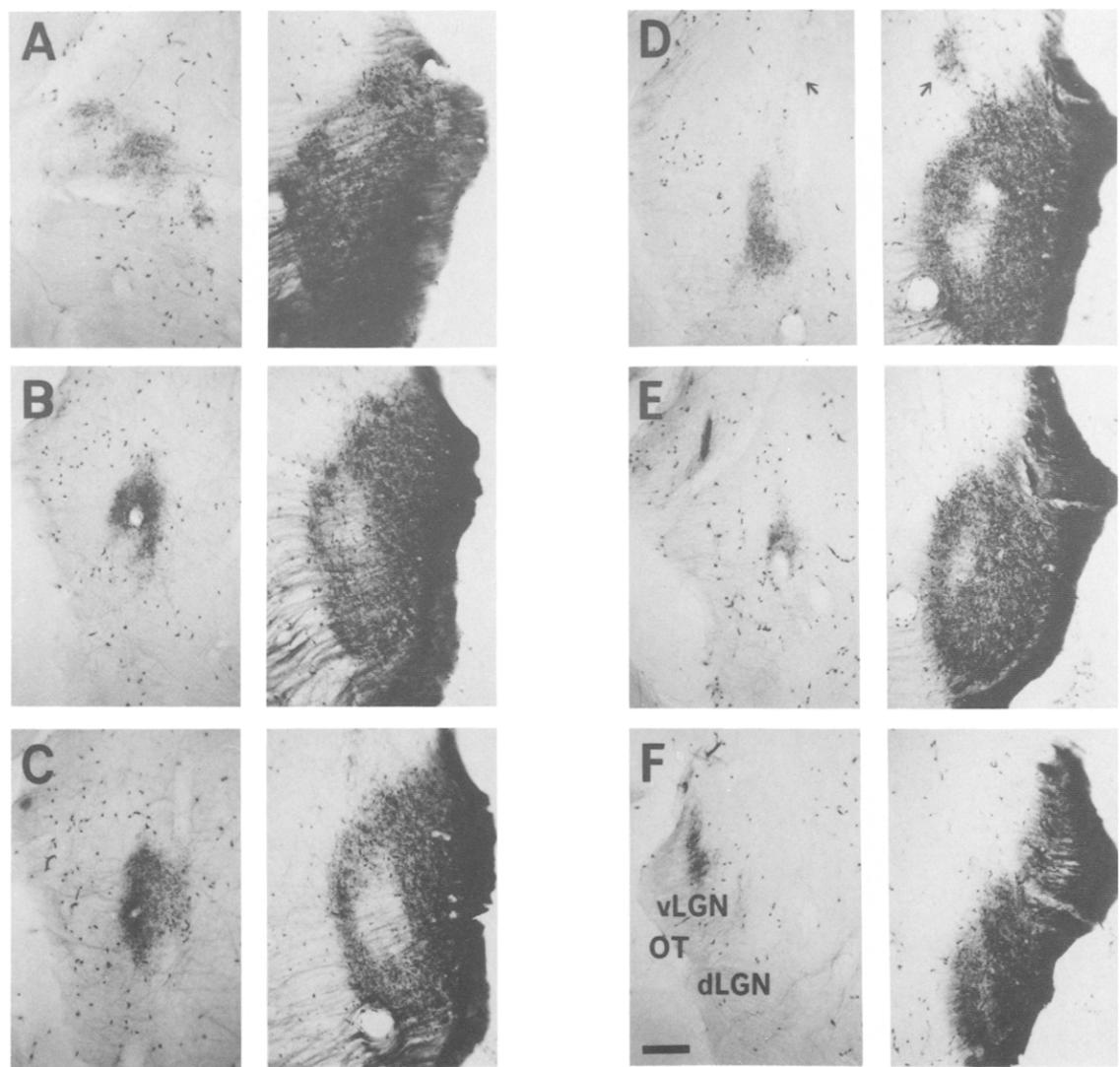


Fig. 3. Horizontal sections of the dorsal lateral geniculate nucleus displaying the ipsilateral (left) and contralateral (right) retinal terminal fields. Sections are 100  $\mu\text{m}$ , at 200  $\mu\text{m}$  intervals from dorsal to ventral. Medial is to the center of each pair, and rostral is up. The ipsilateral terminal field is oriented along a parasagittal axis, except at its rostral-most location (A). The ipsilateral terminal field in vLGN is present in the deepest sections (E and F). Note the terminals reaching forward from the main terminal fields in rostro-ventral dLGN (arrows in D). Rat E-33. Tetramethyl benzidine reaction. Calibration bar = 200  $\mu\text{m}$ .

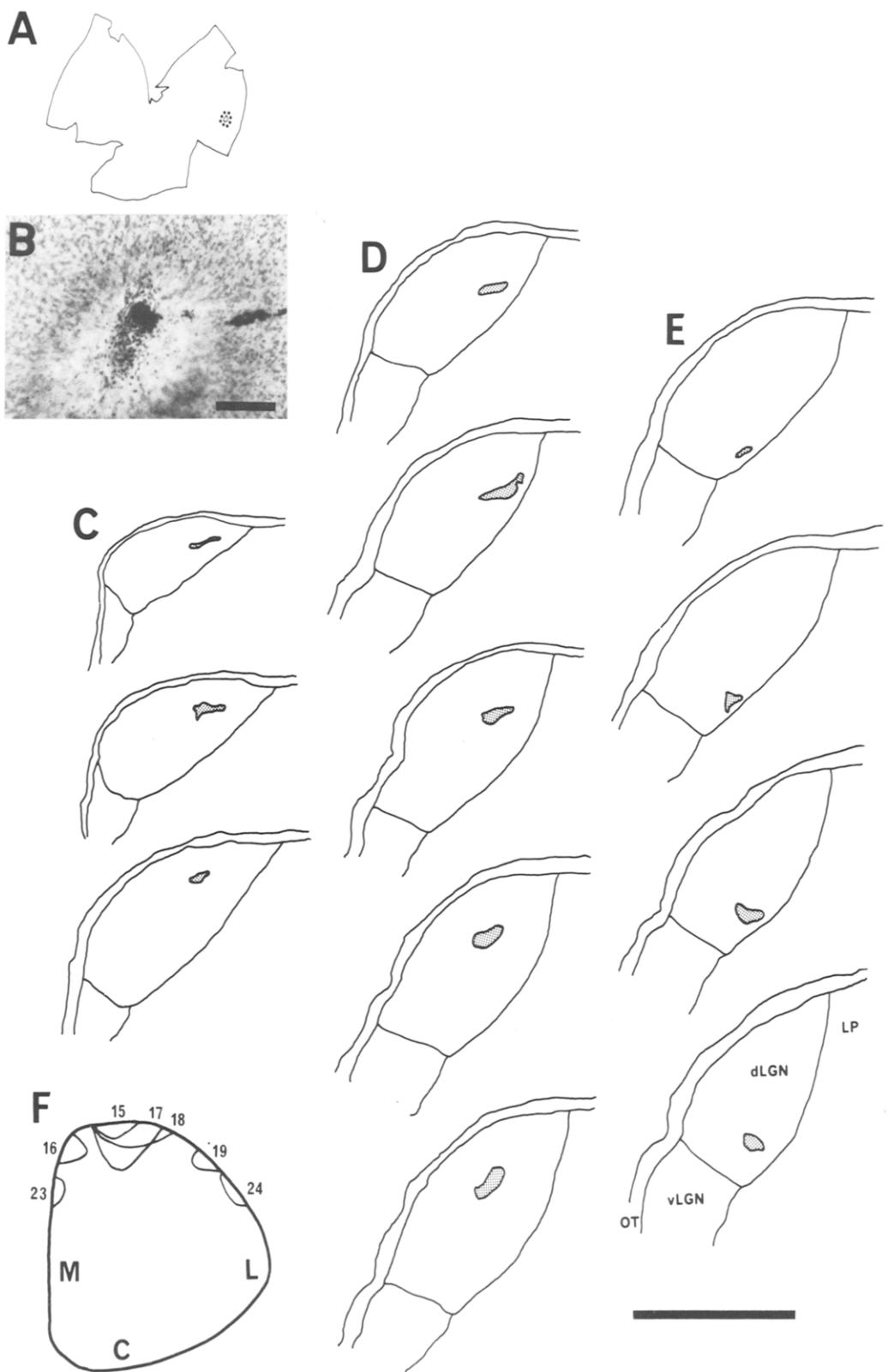


Fig. 4.

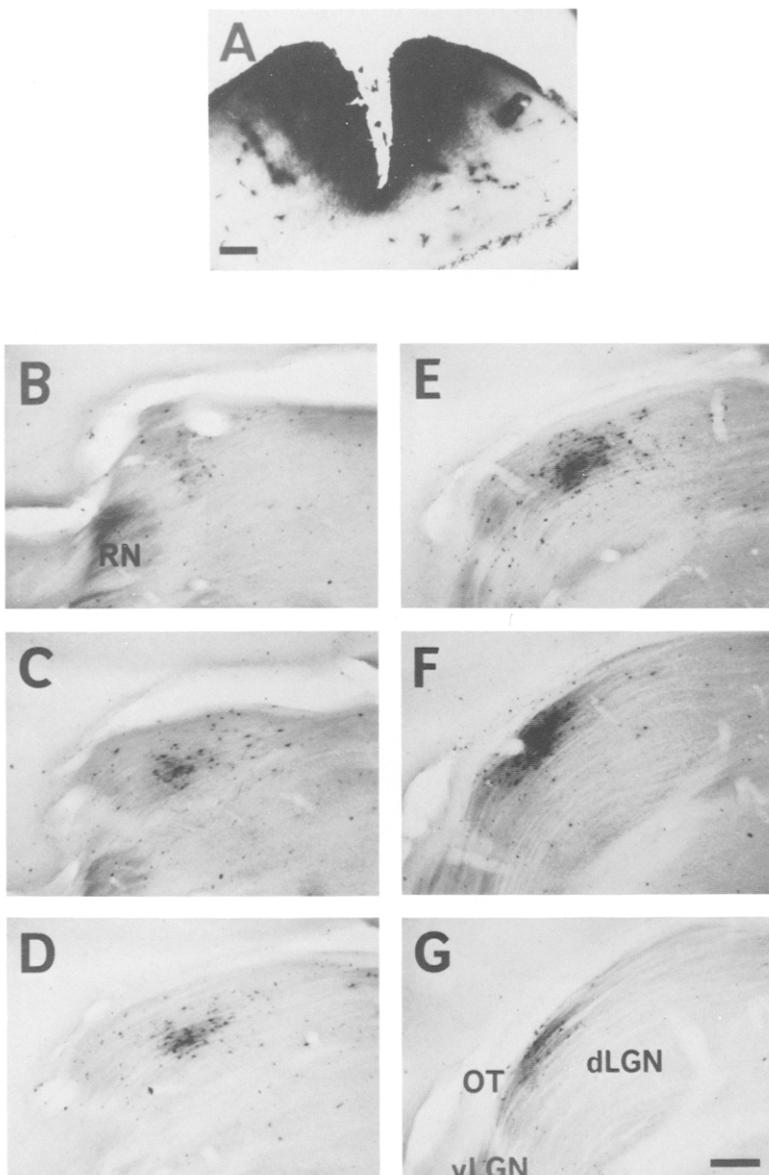


Fig. 5. A: coronal section through the left cortex displaying site of horseradish peroxidase implant in polyacrylamide gel. B-G: coronal sections through the dLGN ipsilateral to the cortical implant of HRP displaying a column of retrogradely labelled cells. The sections are 100  $\mu\text{m}$ , at 200  $\mu\text{m}$  intervals, from rostral to caudal. Medial is to the right. The labelled column of cells shifts dorsally in the nucleus in more caudal sections. Compare with the field of ipsilateral termination shown in Fig. 1. Note also the cortico-fugal projection to the reticular nucleus (RN) of the thalamus, and the reciprocal cortico-geniculate terminals oriented along the column of labelled cells. Rat E-3. *p*-Phenylenediamine and catechol reaction. Calibration bar for A = 250  $\mu\text{m}$ ; for B-G = 200  $\mu\text{m}$ .

Fig. 4. A: Histologically verified location of a retinal lesion produced with a laser ophthalmoscope upon the presumed temporal crescent of the left eye. Rat H-15. B: photomicrograph of retinal lesion produced with a laser ophthalmoscope. Rat H-15. Cresyl Violet acetate stain. Calibration bar = 50  $\mu\text{m}$ . C, D, E: drawings of coronal sections from the dLGN indicating the locus and extent of ipsilateral terminal degeneration. Medial is to the right, with rostral sections at the top. Sections at 125  $\mu\text{m}$  intervals. C: degeneration in the rostral dLGN after a retinal lesion intended for lower temporal crescent. Rat H-23. D: degeneration in the central portion of the dLGN after a retinal lesion intended for the middle temporal crescent. Rat H-17. E: degeneration in the caudal dLGN after a retinal lesion intended for upper temporal crescent. Rat H-24. See Table 1 for the specific rostro-caudal location of degeneration. Calibration bar = 1 mm. F: surface reconstruction of the location of terminal degeneration in the superficial layers of the contralateral SC. The degeneration occurs along the rostral border of the SC at various medio-lateral locations, depending upon the locus of the retinal lesion along the temporal crescent. M: medial; C: caudal; L: lateral.

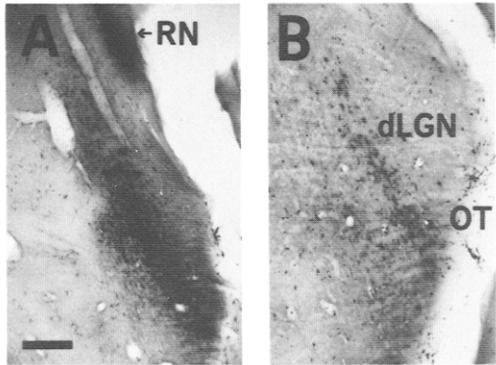


Fig. 6. Oblique (off horizontal) 100  $\mu\text{m}$  sections from the right dorsal lateral geniculate nucleus of two rats after a large (A) or small (B) ipsilateral cortical implant of horseradish peroxidase in polyacrylamide gel. (Approximate plane of sectioning is indicated in Fig. 2D.) Rostral is to the top, with medial to the left. Columns of labelled cells shift laterally as they progress caudally. Compare with the field of ipsilateral termination shown in Fig. 3. Note the cortico-fugal projection to RN, in A. A: rat A-44. B: rat A-45. *p*-Phenylenediamine and catechol reaction. Calibration bar = 200  $\mu\text{m}$ .

Fig. 7. Drawings of adjacent coronal sections from the right dorsal lateral geniculate nucleus displaying the region of ipsilateral termination (here indicated by its absence in the autoradiographs after contralateral eye injections) in relation to the lines of projection (as indicated by the retrogradely labelled cells after an ipsilateral cortical injection). Medial is to the left, with rostral sections at the top. Sections at 100  $\mu\text{m}$  intervals. The injection site of HRP in the primary visual area is shown in coronal section at the bottom of the Figure, and on a standard surface reconstruction in Fig. 8. A: rat E-25. B: rat E-22. C: rat E-21. Calibration bars for both dLGN and cortex = 1 mm.

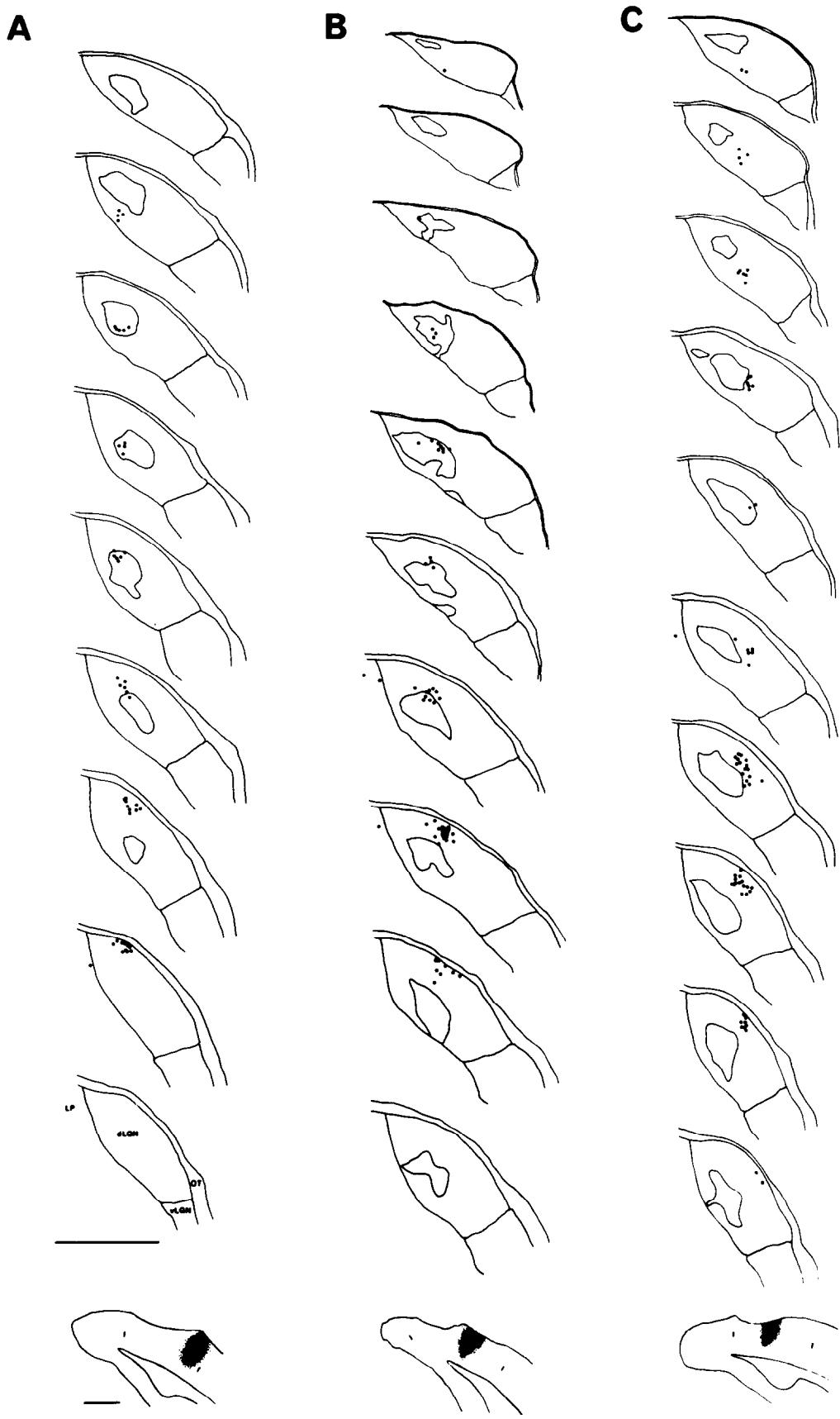


Fig. 7.

Table 2. Locus of degeneration in the contralateral dorsal lateral geniculate nucleus after a temporal retinal lesion

Rat	Length of contralateral dLGN (μm)	Extent of contralateral degeneration (μm)*	Location of maximal degeneration (μm)*	Location of maximal degeneration, expressed as percentage*
H-23	2125			
H-16	2125	1125-1500	1250	59
H-17	2000	1250-1625	1375	69
H-15	2125	1500-1625	1500	70
H-18	2250	1375-1875	1625	73
H-19	2125			
H-24	2250	2000-2125	2000	89

\*In relation to the rostral pole of the dLGN.

for HRP, the retinal lesion was found in the centre of the temporal crescent, near the crescent's widest extent, in both rats.

#### *Retrograde transport of horseradish peroxidase from the primary visual area*

Implants of HRP or its iontophoretic administration into the primary visual area of the cortex retrogradely-labelled cells along a column which traversed the dLGN obliquely, in a predominantly rostro-caudal direction. The number and scatter of labelled cells was generally smaller after iontophoretic application of HRP. Usually, a few labelled cells were found in adjacent lateral and latero-posterior nuclei of the thalamus; they were so rare when compared with labelled cells in the dLGN that every injection and implant was thought to be within the boundaries of the primary visual area<sup>3,23,27</sup> (but see also Cusick and Lund<sup>5</sup>), although these sites were not verified histologically in this series of brains.

A series of coronal sections through a column of retrogradely-labelled cells is presented in Fig. 5. Labelled columns typically shift from a ventral position rostrally (Figs 5B and C) to a more dorsal position caudally where the cells occupy the very edge of the nucleus (Fig. 5G). Rostrally, the tip of a column is not easily localized, because the number of labelled cells decreases per coronal section and the ventral border at the rostral end is not well defined, even in Nissl material. When the brain is sectioned obliquely (off horizontal) approximating the plane of the column, a better impression of its rostro-caudal extent and orientation can be obtained. A major medio-lateral shift of the column is apparent as it traverses the nucleus from rostral to caudal (Fig. 6). Note also the cortical terminals present in the reticular nucleus (RN) of the thalamus, just rostral to the dLGN<sup>17,22,28</sup> (Figs 5B and C, 6A).

#### *Combined autoradiographic and horseradish peroxidase histochemical demonstrations of the ipsilateral terminal field and lines of projection, respectively*

Iontophoretic injections of HRP in the primary visual area retrogradely labelled cells in the dLGN in 10 out of 12 cases. The two negative instances both

showed very small cortical deposits of HRP, one of which was confined to the supragranular layers. All six eye injections successfully displayed the fields of ipsilateral and contralateral termination after autoradiography, although the density of silver grains varied markedly between animals.

The position of retrogradely labelled columns of cells in the dLGN varied with the site of injection within the histologically verified primary visual area. Injections made in the caudal region of the primary visual area labelled columns which traversed the nucleus through its more rostral extent, while rostral injections labelled columns positioned more caudally. Injections near the medial edge of the primary visual area labelled columns running through the more lateral region of the dLGN. As described in the previous section, all of these labelled columns traverse the nucleus obliquely, so that, for example, a labelled column of cells in the lateral dLGN is actually ventro-lateral rostrally and dorso-lateral caudally.

Injections at progressively more lateral locations in the primary visual area labelled columns running closer and closer to the dorso-medial edge of the dLGN, and which intersected the field of ipsilateral termination as determined from the adjacent autoradiographs (Figs 7A and B). More medial injections labelled columns which traversed the nucleus lateral to the ipsilateral field (Fig. 7C). These three cortical injection sites are shown reconstructed in coronal section at the bottom of Fig. 7 and are displayed on a standard surface reconstruction<sup>6</sup> in Fig. 8.

Visual evoked responses recorded through the micropipette could be elicited in the right primary visual area through the ipsilateral eye in four out of six cases, in each of which the retrogradely-labelled column in the ipsilateral dLGN intersected the field of ipsilateral termination. In the two negative instances, one animal showed poor general responsiveness in both cortices; both injections in this animal labelled columns intersecting the ipsilateral fields. In the other instance, an evoked response could be readily demonstrated only through the contralateral eye (the left, unprepared eye). The right dLGN contained a column of labelled cells passing just lateral to the field of ipsilateral termination (Fig. 7C).

## DISCUSSION

### *Relation to previous studies*

Eye injections of HRP or radioactively labelled proline both reveal the ventral descent of the ipsilateral terminal field rostro-caudally through the dLGN. This pattern can be observed in the data of other investigators for the rat, hamster and mouse.<sup>8,10,16,18</sup> The present results support the view that the bulk of the ipsilateral terminal field is segregated from the contralateral terminal field,<sup>8,18</sup> but suggest there may be some degree of overlap along the ventro-medial border.<sup>12,24</sup>

The parasagittal and horizontal sections show that the separate ventral clusters of ipsilateral terminals seen in rostral coronal sections fuse with the main body of terminals in more caudal sections. The denser rostro-ventral terminals from the contralateral eye do the same. Adjacent Nissl sections in the parasagittal and horizontal planes indicate that these rostro-ventral terminals actually lie outside the cytoarchitecturally distinct dLGN. A contralateral projection to this region beneath the dLGN exists in the hamster,<sup>8</sup> arising from nasal retina only (Jhaiveri, 1973, as cited in ref. 8). This rostro-ventral retinorecipient region, which has yet to be accurately mapped physiologically, might be a functionally distinct sub-division of the dLGN, or simply the result of mechanical forces from the developing internal capsule, which has interrupted the nucleus and dislodged small clusters of cells on the ventral surface.

The pattern of anterograde degeneration in the ipsilateral dLGN following a localized lesion in the temporal crescent indicates a retinotopic organization similar to the visuotopic arrangement described by Reese and Jeffery:<sup>26</sup> the rostral part of the ipsilateral dLGN receives a projection from the lower temporal crescent and maps the far eccentric upper nasal visual field of the ipsilateral eye, while more caudally the ipsilateral dLGN receives a projection from upper temporal crescent and maps the far eccentric lower nasal visual field of the ipsilateral eye. Montero *et al.*<sup>20</sup> may have failed to find any visuotopic order in the ipsilateral dLGN because (a) albino rats and mice have smaller ipsilateral retino-geniculate projections<sup>7,15</sup> which terminate abnormally,<sup>16,18</sup> and/or (b) the greater scatter of single cell receptive fields in the rat may have obscured the topography.

The anterograde degeneration in the contralateral dLGN confirms the demonstration by Lund *et al.*<sup>18</sup> of a compressed projection from the contralateral temporal retina in pigmented rats, and shows that this crossed projection arises from the region of temporal retina which also gives rise to the ipsilateral projection (i.e. the temporal crescent). Two of the rats failed to show any degeneration contralaterally, which may be a consequence of making relatively small peripheral lesions in a retinal region of compressed magnification. The present results also show

that this crossed projection displays a retinotopicity similar to that observed ipsilaterally, and in accord with the visuotopic arrangement for the rest of the contralateral retina.<sup>20,26</sup> As proposed on the basis of the original findings by Lund *et al.*<sup>18</sup> the whole contralateral retina appears to project topographically to the dLGN. Not only, then, does the temporal crescent project to both sides of the brain;<sup>4</sup> it projects in a topographically appropriate fashion to both dLGNs, suggesting an analogy with the naso-temporal overlap of the primate and cat, albeit a wider one.

The cortical placements of HRP labelled columns of cells which traverse the dLGN in a manner similar to the physiologically defined lines of projection for the contralateral eye,<sup>20,26</sup> and similar to the patterns of anterograde and retrograde degeneration after contralateral retinal and ipsilateral cortical lesions, respectively.<sup>18,21</sup> These labelled columns of cells also traverse the nucleus in a manner similar to the physiologically defined projection lines for the ipsilateral eye<sup>26</sup> and to the pattern of anterograde degeneration described here after ipsilateral retinal lesions.

### *The ipsilateral field in relation to the lines of projection*

The lines of projection and the ipsilateral terminal field both course through the dLGN along its rostro-caudal extent. However, their course relative to one another is oblique rather than parallel. Moving from rostral to caudal, the field of ipsilateral termination quickly shifts medially and drops ventrally along the medial border of the dLGN, whereas the lines of projection shift dorsally and laterally. Given this arrangement, there should be opportunity for the two to intersect. However, not all the lines of projection do intersect the ipsilateral terminal field. Only those which were retrogradely labelled by cortical injections in the lateral half of the primary visual area did

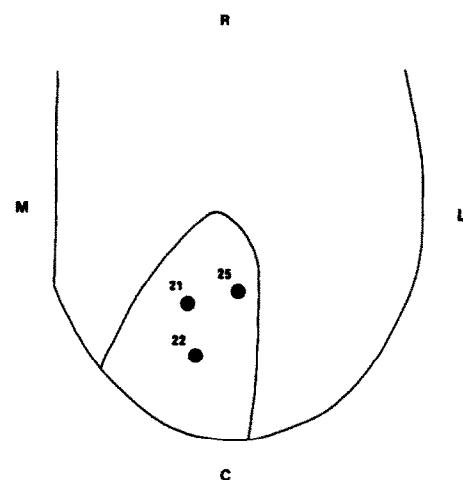


Fig. 8. Surface reconstruction of the primary visual area displaying the location of iontophoretic injection sites of horseradish peroxidase depicted in Fig. 7. R: rostral; M: medial; C: caudal; L: lateral.

so. This region of the primary visual area represents the binocular visual field<sup>1</sup> where cortical cells may be stimulated via either eye. The present results demonstrate that points in the topographic representation of the binocular visual field within the primary visual area receive input from dLGN relay cells which are aligned along columns, with separate portions of each column receiving innervation from the contralateral and ipsilateral eyes. Thus, even though the dLGN of the rat does not display the prominent lamination associated with the alignment of the two eyes' representations of the visual field, the corresponding

points of each retina are apposed in conjugate register along lines of projection.

*Acknowledgements*—It is a pleasure to thank C. Pyrah and E. Darley for histological advice and assistance, J. Broad and A. Butterworth for photographic assistance, and the University Department of Physiology for loan of the laser ophthalmoscope.

This research was supported by a grant from the M.R.C. (G971/397/B). B.E.R. gratefully acknowledges the financial support provided by an O.R.S. grant from the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom, a bridging grant from the Herbert von Karajan Neuroscience Trust, and a Sidney Perry Junior Research Fellowship from St. Peter's College, Oxford.

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(Accepted 9 July 1983)