TRANSNEURONAL CELL DEGENERATION IN THE LATERAL GENICULATE NUCLEUS OF THE MACAQUE MONKEY

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INTRODUCTION

Recent quantitative studies of transneuronal degeneration in the dorsal nucleus of the lateral geniculate body (Cook, Walker & Barr, 1951) and in the superior cervical ganglion (Hamlyn, 1954) have served to establish that in the adult cat and rabbit the nerve cells here undergo a slow and comparatively mild process of degeneration following interruption of their afferent connexions, and that within the limits of the survival periods used no obvious cell loss occurs. In Primates, on the other hand, transneuronal degeneration in the lateral geniculate nucleus appears to be a more fulminating process, as it has been found to occur as early as 7 days after eye enucleation in the monkey (Glees & Le Gros Clark, 1941). Furthermore, in a human case at least 36 years after an eye removal Goldby (1957) has shown that the degree of cell shrinkage in the parvocellular laminae is considerably more marked than in the cat and rabbit after a survival period of 313 days, and that in these laminae about 50% of the cells had completely disappeared.

In view of the early onset of transneuronal degeneration in the monkey, experiments have been carried out to study the course of the degenerative process in this species. As this problem can only be investigated by quantitative methods, a study comparable to that of Cook *et al.* (1951) has been made in a series of monkeys during the first 4 months after unilateral eye enucleation. A point of particular interest in the lateral geniculate nucleus of the Primates is its organization into large- and small-celled laminae. The functional significance of this arrangement is not at all clear, but it appears that these two cell populations react differently both to deafferentation and to axonal section. In transneuronal changes affecting these cell groups, there is evidence that after long survival periods the large cells are less severely affected, and we have therefore sought to determine whether these differences are apparent from the beginning and continue through all stages of the degenerative process.

MATERIAL AND METHODS

Twelve mature monkeys (*Macaca mulatta*) were used in this study, though their precise ages were unknown. In each case one eye was enucleated, and in all but one animal (MG 1) an area of the visual cortex 1–2 cm.^{2} in extent was removed from one or both sides, either at the same time or at a second operation. The purpose of the cortical removals was to permit a later study of the interaction, if any, between retrograde and transneuronal degeneration in the same neurons. The animals were allowed to survive for periods varying from 4 days to 4 months (see Table 1). In

addition, one normal monkey was used as a control. The brains were fixed in 70% alcohol with 2% acetic acid, and from each a block of the thalamus containing both lateral geniculate bodies was embedded in paraffin wax and sectioned coronally at 25 μ. Every 5th section was mounted and stained with thionin.

The nature and extent of the transneuronal atrophy were assessed qualitatively in each of the twelve experiments, and quantitative observations were made in the eight experiments indicated in Table 1 and in the control brain. As it was considered desirable to investigate the changes in all six laminae, all observations were made at the junction of the posterior and middle thirds of the geniculate body, where the six laminae are circumscribed and distinct. In view of the fact that each cortical lesion had resulted in a wedge of retrograde degeneration in the central zone of the ipsilateral nucleus, quantitative observations were confined to the lateral part of each

Table 1

Animal	Survival period (days)	Eye enucleated	Side of visual cortex lesion
*MI7	4	Right	Right
MI9	6	Left	Right
*MA4	7½	Right	Left
*MI8	8	Right	Left
MI10	10	Left	Right
MI12	12	Right	Right
MA3	14	Right	Bilateral
*MA2	16	Right	Bilateral
*MA5	33	Right	Bilateral
*MG1	62	Left	None
*MI13	91	Left	Right
*MA1	129	Right	Bilateral
*MG4	Normal control	—	—

* Indicates animals in which measurements of individual neurons were made.

lamina, well away from the zone of retrograde degeneration. All comparisons of normal with atrophied cells or laminae were made at corresponding sites and at the same antero-posterior level of the two geniculate nuclei in the same brain.

For the measurement of cell shrinkage, camera lucida outlines of cell, nucleus and nucleolus for 100 neurons from each normal and atrophied lamina were drawn on mm. graph paper, the linear magnification being adjusted to exactly 1000 times. By counting the number of square mm. contained within each outline a measurement (in μ^2) was obtained of the nuclear, nucleolar and total cell areas. Sample fields were taken at random from near the middle of the lateral 'limb' of each lamina; in each field all neurons with a distinct nucleolus were measured, the presence of a nucleolus being the only criterion for their selection. The cell and nuclear outlines were always drawn in the plane of focus of the nucleolus.

In three experiments an attempt was made to detect loss of neurons by calculating their number in corresponding volumes of normal and atrophied laminae. In order to avoid the central zone of retrograde degeneration, the estimates of volume were confined to the middle three-quarters of the lateral 'limb' of each lamina. The areas of these segments were measured from projection drawings on mm. graph paper, at a magnification of fifty times, in six successive sections (125 μ apart) of the junctional region between the posterior and middle thirds of the geniculate nucleus, and the

volumes were calculated from these data. Sample counts of cell density were made throughout these regions, using an oil immersion objective and a field of known diameter. Only neurons with a distinct nucleolus were counted, and the size of the sample was of the order of 2–5 % of the cell population in the volume measured.

The results of the qualitative observations will be described first, followed by the quantitative observations which, for the sake of clarity, will be presented either graphically or in tabular form. In the assessment of cell shrinkage, mean sizes of cell, nucleus and nucleolus for normal and atrophied neurons have been compared by the *t*-test, and percentage changes, with their 95 % confidence limits, have been calculated.

RESULTS

The observations are given under four headings: (1) the appearance of the normal neurons in thionin-stained paraffin sections; (2) quantitative observations made upon neurons in the normal laminae; (3) and (4) the changes qualitatively and quantitatively detected during transneuronal atrophy. The laminae are numbered 1–6 in the ventro-dorsal direction, from the hilum (Le Gros Clark, 1941), and for the sake of brevity, the terms 'crossed' and 'uncrossed' are used to describe those laminae which receive projections from the contralateral and the ipsilateral eye, respectively.

(1) *The normal lateral geniculate neurons*

The neurons in all laminae are multipolar and irregular in profile, but are commonly ovoid in general shape (Pl. 2, figs. 3, 5). There are slight differences in appearance between neurons of large-celled and small-celled laminae, but not between those of corresponding crossed and uncrossed laminae.

In the large-celled laminae (1 and 2) the neurons have relatively abundant cytoplasm which is evenly filled with dark-staining small Nissl granules less than 1 μ in diameter, extending into the bases of the larger dendrites. Occasional neurons have one or more clear areas in the cytoplasm, some of which may represent vacuoles, and others fixation artefacts. The nucleus is oval or rounded, often eccentrically placed. The nucleoplasm is much paler than the cytoplasm and contains a little faintly staining chromatin material. There is typically one nucleolus which stains darkly and is often eccentrically placed. Two nucleoli are rarely seen in the large neurons, but are not infrequent in the smallest neurons of the large-celled laminae.

The neurons of the small-celled laminae (3–6) have, relatively to their nuclei, somewhat less abundant cytoplasm. The cytoplasm is evenly and rather closely filled with small, dark-staining Nissl granules, or a reticulum of Nissl substance, with few large aggregates. As in the large-celled laminae, there are occasional clear spaces, most or all of which may be artefacts. The nucleus tends to be approximately central, but may be eccentric in the cells with more abundant cytoplasm. The nucleoplasm is usually paler than the cytoplasm, but contains many irregularly distributed chromatin granules, which vary greatly in their size and staining intensity. The nucleolus is often eccentrically placed and stains darkly; it is usually single, but in a small percentage of neurons there may be two nucleoli of typical appearance (the incidence of these 'double nucleoli' is commonly less than 3 %, but it has occasionally been found to be as high as 10 % in one or more of the small-celled laminae).

(2) *Quantitative observations in normal laminae*

The mean areas of normal cells, nuclei and nucleoli, with standard errors of the means, are given in Tables 2–9. Table 2 shows these values for the two geniculates of a normal animal. Tables 3–9* refer to both normal and atrophied laminae in seven experimental animals after unilateral eye enucleation, and are arranged in order of increasing survival periods, from 4 days to 4 months.

These results indicate that there is no atrophy in the unaffected laminae following enucleation of one eye. For any one lamina there is a considerable scatter of values from experiment to experiment; but it may be seen that while the mean values for atrophied laminae tend to decrease with increase of survival period, the mean values for unaffected laminae remain of the same order throughout, showing no consistent alteration with time.

The means of the values of cell area for each normal lamina in the eight animals were:

Lamina	Cell area (μ^2)	Lamina	Cell area (μ^2)
1	238·5	4	140·0
2	222·7	5	139·7
3	140·2	6	132·4

Limits of error; biological and technical variations. The scatter of mean observations for normal cells from experiment to experiment was such that, for most laminae, the largest mean cell area was approximately half as great again as the smallest mean cell area; in the case of lamina 2, however, the largest value was almost twice as great as the smallest. These differences between animals may reflect real differences, i.e. they may be biological, or they may have arisen during histological preparation; however, they would not directly influence the observations on transneuronal atrophy, since normal neurons for control measurements were taken from the same brain in each experiment. Variations due to observer errors were smaller, and were limited as far as possible in the following ways: (*a*) All the camera lucida outlines were traced by the same observer, using a standardized technique. (*b*) Although areas were measured by four observers, each set of atrophied neurons was measured by the one who measured the corresponding normal neurons. (*c*) Errors in tracing outlines were assessed at various times by making duplicate outlines of neurons; the resulting differences between pairs of measurements were proportionately greater the smaller the structure concerned, but were not more than about 5 % for the smaller cell areas (of less than 100 μ^2), or about 10 % for the smaller nuclear areas (below 40 μ^2). (*d*) The variation between observers in measuring a given group of cell outlines was assessed in a sample check of 50–100 cells each, and was found not to exceed 2–3 %. The measurement of nucleoli was somewhat crude, for although their outlines could be traced fairly accurately their areas with the method used could only conveniently be assessed to the nearest μ^2; but since 100 measurements were made in each group, and since the nucleolar area was variable, with the extremes of its range lying at about $1\mu^2$ and $7\mu^2$ and with demonstrable differences between large-celled and small-celled laminae, it was felt that the mean value for each group formed a useful approximation.

* In these tables the second figure in each column is the standard error of the mean; this is 1/10 of the standard deviation, where the number in each sample is 100.

Distributions of cell areas. The scatter of cell areas is indicated by the standard errors of the means which are given for all groups of cells measured in Tables 2–9. Typical histograms of cell areas for 100 normal neurons from each lamina are shown as solid lines in Text-fig. 1. The distributions of areas in the large-celled laminae overlap considerably those in the small-celled laminae; indeed, in most animals

Table 2. *Normal control. Mean areas for* 100 *neurons from each lamina of the right and left lateral geniculate nuclei*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.	Right	S.E.	Left	S.E.
1	193·5	4·65	196·3	5·29	65·7	1·01	66·1	1·25	4·0	0·08	3·9	0·08
2	168·9	4·98	165·1	5·35	59·2	1·22	56·6	1·23	4·0	0·11	3·7	0·09
3	111·2	2·05	118·2	2·46	43·2	0·58	44·9	0·69	2·8	0·06	2·7	0·06
4	110·6	2·28	112·9	2·57	41·2	0·71	42·9	0·71	2·6	0·06	2·6	0·06
5	116·2	2·16	115·7	2·25	42·0	0·56	44·9	0·74	2·7	0·05	2·9	0·06
6	116·8	2·51	117·2	2·22	42·7	0·60	43·2	0·66	2·7	0·06	2·7	0·07

Table 3. *Eye removed* 4 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	245·8	6·71	223·1	5·20	82·0	1·58	78·3	1·50	3·7	0·08	3·3	0·07
2	231·2	5·20	228·4	5·28	79·1	1·35	81·0	1·20	3·8	0·08	3·5	0·08
3	138·6	2·93	129·2	2·63	55·9	0·76	57·1	0·79	2·7	0·06	2·5	0·06
4	136·9	2·98	123·2	2·63	57·0	0·99	53·5	0·76	2·7	0·06	2·4	0·06
5	142·9	2·78	124·7	2·16	53·3	0·63	54·9	0·76	2·4	0·06	2·3	0·05
6	119·8	2·30	99·7	1·74	50·7	0·75	45·0	0·76	2·4	0·05	2·2	0·06

Table 4. *Eye removed* 8 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S. E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	282·3	7·90	256·9	6·60	90·5	1·98	80·1	1·52	4·0	0·09	3·1	0·09
2	296·8	8·80	225·9	6·01	87·1	1·88	75·8	1·44	3·7	0·09	3·2	0·08
3	156·2	4·21	116·0	2·25	57·6	0·95	51·5	0·68	2·4	0·07	2·2	0·06
4	145·9	3·09	133·3	2·27	52·2	0·84	55·4	0·63	2·5	0·07	2·3	0·05
5	173·8	2·96	121·5	3·13	62·3	0·61	53·2	0·94	2·3	0·05	2·2	0·05
6	158·4	3·06	131·1	2·60	58·9	0·94	56·5	0·79	2·7	0·06	2·1	0·05

Table 5. *Eye removed* 16 *days previously*

(150 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	222·3	6·16	199·8	4·33	71·0	1·46	68·6	1·02	3·5	0·07	3·3	0·08
2	243·0	6·86	186·3	5·46	78·1	1·61	61·8	1·26	3·9	0·09	3·0	0·08
3	140·0	2·82	113·3	2·03	53·8	0·83	48·1	0·60	2·5	0·06	2·1	0·05
4	135·6	2·63	112·4	2·08	53·0	0·75	46·6	0·59	2·3	0·06	2·2	0·04
5	137·3	2·68	103·0	1·91	49·4	0·62	45·8	0·61	2·5	0·05	2·2	0·05
6	131·0	2·75	110·3	2·02	49·8	0·63	44·1	0·55	2·5	0·05	2·2	0·04

one or two cells were found in laminae 1 and 2 which were as small as any in laminae 3–6. On the other hand, it was very rare to find any cell in laminae 3–6 equal to the largest in laminae 1 and 2. Within each lamina, these histograms suggest a unimodal distribution and a single population of neurons. Polyak (1957) has described neurons of different types in single laminae in Golgi preparations, but all were found to undergo retrograde degeneration. In order further to test for the

Table 6. *Eye removed* 33 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	246·5	7·97	144·2	3·79	79·3	2·12	64·4	1·36	4·0	0·10	3·2	0·08
2	191·8	5·42	156·0	4·72	73·4	1·80	61·1	1·47	4·0	0·11	3·0	0·10
3	127·7	2·70	107·8	2·24	54·7	0·94	47·9	0·94	2·3	0·06	2·2	0·05
4	175·3	3·94	89·6	1·56	65·2	1·11	44·2	0·73	2·4	0·06	2·0	0·05
5	129·6	2·76	114·3	2·49	55·2	0·80	47·0	0·85	2·6	0·05	2·1	0·05
6	157·5	2·77	86·6	1·73	61·0	0·89	40·1	0·76	2·5	0·06	2·0	0·05

Table 7. *Eye removed* 62 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	206·7	6·12	146·7	4·51	74·2	1·61	56·1	1·42	4·4	0·12	3·0	0·09
2	186·7	7·06	140·3	3·90	66·6	1·91	52·9	1·09	3·7	0·10	3·1	0·08
3	140·8	3·19	90·2	1·63	49·5	0·78	38·1	0·52	3·0	0·07	2·2	0·06
4	133·7	2·94	83·6	1·85	47·1	0·79	33·5	0·57	2·8	0·07	2·0	0·06
5	121·5	2·95	81·5	1·53	45·6	0·87	31·4	0·53	2·6	0·06	1·9	0·05
6	114·2	2·11	74·2	1·52	44·6	0·58	31·9	0·58	2·8	0·06	2·1	0·05

Table 8. *Eye removed* 91 *days previously*

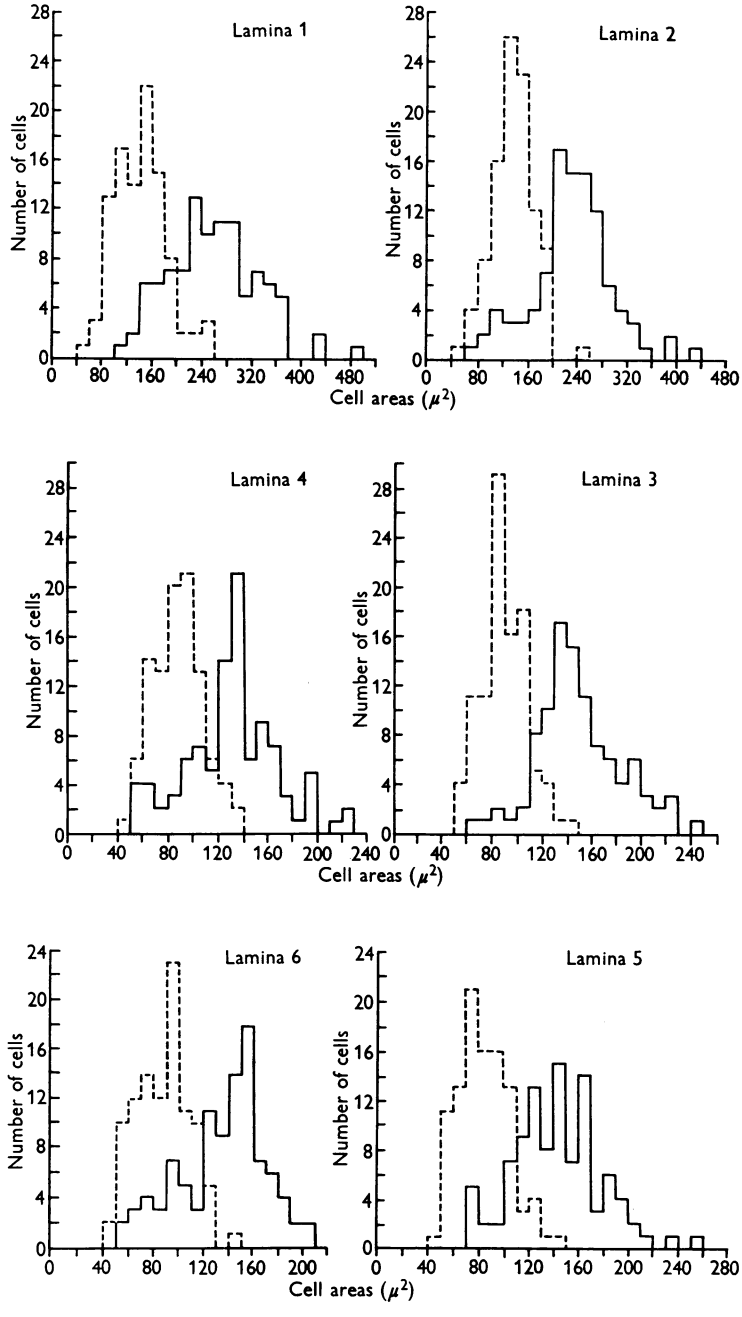
(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	251·1	7·07	98·8	2·94	73·6	1·39	39·0	1·04	4·8	0·11	2·4	0·08
2	234·4	6·15	142·9	3·15	71·0	1·46	48·5	0·95	4·3	0·10	3·1	0·08
3	153·5	3·05	88·6	1·57	53·1	1·20	37·3	0·52	2·8	0·12	2·1	0·06
4	151·2	4·10	82·2	1·76	49·5	0·88	33·2	0·50	2·9	0·10	1·9	0·04
5	154·2	3·80	91·2	1·33	51·9	1·34	35·7	0·60	2·7	0·06	2·1	0·05
6	125·8	2·83	72·9	1·44	48·1	0·79	34·8	0·55	3·1	0·06	2·2	0·04

Table. 9. *Eye removed* 129 *days previously*

(100 neurons in each sample.)

	Cell areas (μ^2)				Nuclear areas (μ^2)				Nucleolar areas (μ^2)			
Lamina	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.	Normal	S.E.	Atrophied	S.E.
1	258·5	7·22	140·7	4·07	70·6	1·23	53·2	0·96	3·1	0·08	2·2	0·06
2	230·3	6·43	136·4	3·28	76·4	1·44	49·6	0·85	3·5	0·09	2·2	0·07
3	149·9	3·39	89·6	1·83	57·1	0·82	36·6	0·51	2·4	0·05	1·6	0·06
4	129·6	3·66	87·3	1·92	44·4	0·87	37·2	0·55	2·3	0·08	1·8	0·05
5	142·3	3·43	84·1	2·00	53·2	0·74	31·9	0·53	2·4	0·06	1·8	0·06
6	135·3	3·45	87·0	2·12	46·6	0·99	36·0	0·63	2·2	0·07	1·9	0·05



Text-fig. 1. Histograms to show the distributions of cell areas for 100 normal and 100 atrophied neurons from each lateral geniculate lamina of a macaque, 129 days after enucleation of the right eye. Solid lines: normal neurons; broken lines: atrophied neurons. The group intervals are $20\mu^2$ for laminae 1 and 2, and $10\mu^2$ for laminae 3–6. The crossed laminae (1, 4 and 6) are on the left.

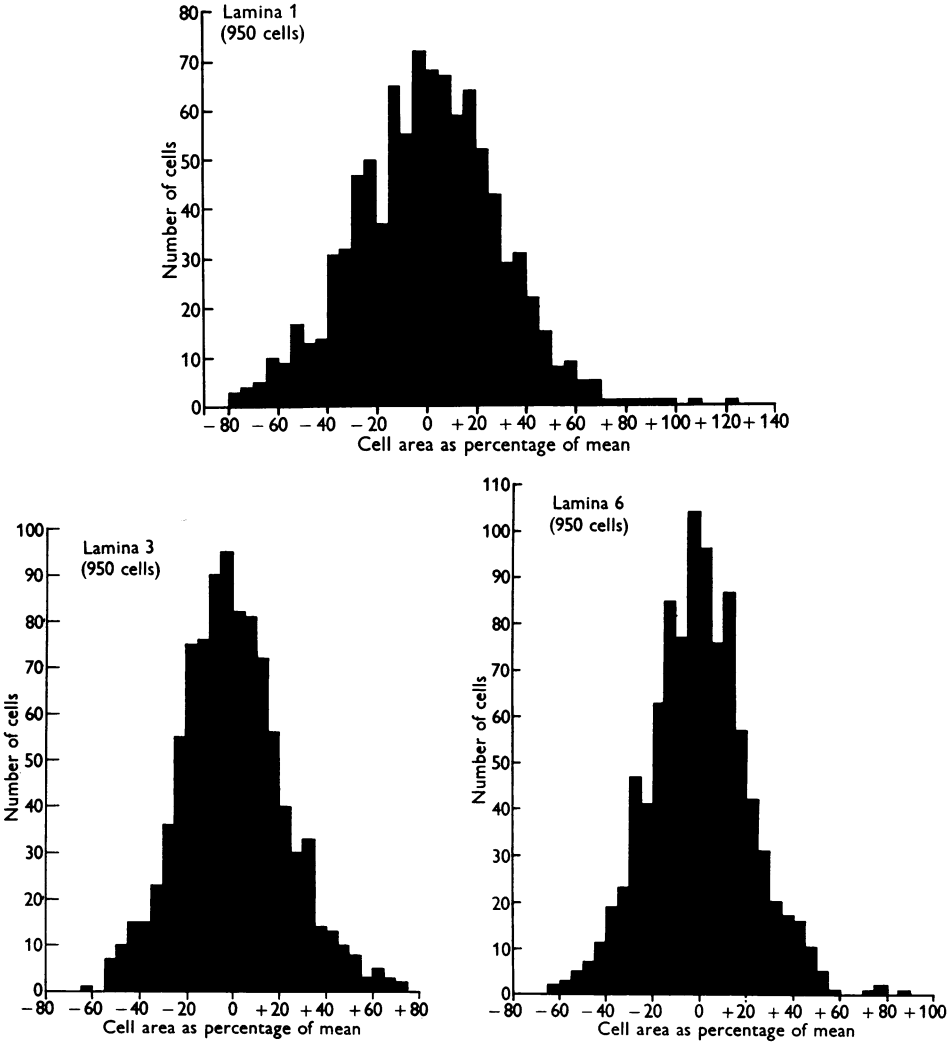
presence of distinct groups of neurons differing in size in normal laminae, various sets of comparable cell areas were pooled to increase the number of observations. Histograms were plotted for neurons of the same lamina showing similar mean cell areas in several different animals, and for the four small-celled laminae from single animals; these all showed unimodal distributions. As a more critical test, each group of cell areas for a given lamina was plotted with a grouping which represented percentage variation about its mean value, and the distributions so corrected for differences of the mean were added to give a histogram based on the measurement of 950 cell areas (the maximum number available for each lamina). This was done for laminae 1, 3 and 6, with a group interval in each case equal to 5% of the mean, and the resulting histograms are shown in Text-fig. 2. It is clear that each of these histograms is effectively unimodal.

Neurons of different types might, however, differ in the extent to which they undergo transneuronal atrophy, or in the time-course of this atrophy, even if not differing in their original size. To test this possibility, the coefficients of variation of cell areas were analysed. Comparison of these coefficients of variation for normal and atrophied neurons at seven different survival times ranging from 4 to 129 days showed no consistent change; but for each lamina, at one or more survival times, there was a decrease in the coefficient significant by the *t*-test, and only once in one lamina (lamina 5, at 8 days) was there any significant increase. A decrease in the coefficient of variation of cell area might arise if the smaller neurons shrank disproportionately less, or more slowly, than the larger neurons, and would be transient if the difference were confined to the rate of shrinkage. But since shrinkage need not be directly proportional to the area of cross-section, changes in the coefficient of variation of cell area are not conclusive evidence for differences in behaviour of neurons within the same lamina during transneuronal atrophy. The present observations as a whole, therefore, are not inconsistent with the view that the neurons of any given lamina form a single population, within the region studied.

Differences of mean cell area between laminae. Apart from the obvious distinction between large-celled and small-celled laminae, there may be lesser differences within these groups. Chacko (1949), in a preliminary study based upon one human and one macaque brain, found gradients of cell size from lamina to lamina of the normal lateral geniculate. In the human brain, within the six-laminar central vision area of the geniculate, the neurons of lamina 2 were found to be somewhat larger than those of lamina 1, and there was a slight but fairly consistent gradient of decreasing size from lamina 3 to lamina 6. Similar relationships were found in the central vision area of the macaque lateral geniculate, and in the peripheral vision area of the human geniculate—where pairs of small-celled laminae fuse—laminae (3+5) had larger neurons than those of laminae (4+6).

In the present study neurons were measured in the six-laminar region of the geniculate, that is, in the 'central vision area'. Comparison of the mean cell areas for different normal laminae has shown that their relationships are rather variable. There is, however, a definite tendency for a decrease in cell area between laminae 3 and 6, although a continuous gradient is uncommon. In addition, if pairs of small-celled laminae are taken together, the cells of the uncrossed laminae (3+5) are often slightly larger than those of the crossed laminae (4+6).

These conclusions are based upon observations in eight animals. It may be objected, however, that seven of these had unilateral eye enucleations. In these seven animals the normal crossed and uncrossed laminae were in opposite geniculates, so that any differences between them may have been due to differences in cell size between the



Text-fig. 2. Histograms to show the distributions of normal cell areas, plotted as percentage deviations from the mean, for three lateral geniculate laminae. The grouping interval is 5 % of the mean. The derivation of the histograms is described in the text.

two geniculates. In the normal control animal, however, no significant differences in area were found between cells of the same lamina on the two sides (the greatest difference was 6 % in lamina 3, which was significant only at the 1 in 20 level). It is therefore unlikely that there are regularly significant differences in size between normal cells in the two geniculates; and, indeed, it is on the assumption that there

are not such differences that any quantitative study of transneuronal atrophy is based.

One animal (Table 7) showed a continuous gradient of decreasing cell area from lamina 3 to lamina 6, similar to that found by Chacko, but unlike her finding the neurons of lamina 2 were smaller than those of lamina 1. For the large-celled laminae this was the commonest relation, and was found in six of the eight animals, including the normal control; in these six animals the cell areas for lamina 2 were, on average, 12% smaller than those for lamina 1. Among the small-celled laminae there was most commonly little difference of mean cell area, but there was a tendency for lamina 6 to show the smallest values. Thus, in four animals, including the normal control, the mean cell areas of laminae 3–5 were approximately equal to each other, in two cases exceeding, but in two cases also equalling that of lamina 6. But for the small-celled laminae a comparison of laminae 3 and 5, or 4 and 6, is possibly more interesting. The mean cell areas of laminae 3 and 5 were approximately equal in five of the eight animals, whereas the cell area for lamina 4 more often exceeded than equalled that for lamina 6 (greater in four, equal in three animals). Only once, and in the same animal (Table 4), were the neurons of lamina 5 and lamina 6 greater than those of laminae 3 and 4, respectively. Crossed and uncrossed laminae were compared; for the small-celled laminae it was found that in five animals the mean of the cell areas for laminae 3 and 5, taken together, exceeded that for laminae 4 and 6 together, but this did not apply to the large-celled laminae, for only once, and in a different animal, was the value for lamina 2 greater than that for lamina 1.

Incidence of double nucleoli. Neurons having two nucleoli ('double nucleoli') were present in small numbers in most normal laminae. In two of the thirteen animals studied, double nucleoli were seen in all laminae. They were commoner in the small-celled than in the large-celled laminae, for they were found in all the small-celled laminae of eleven animals, but were seen in both large-celled laminae only in four animals. They appeared, moreover, to be rather commoner in lamina 1 than in lamina 2. As has already been noted, double nucleoli in the large-celled laminae tended to occur in the smaller rather than in the larger neurons.

The percentage incidence of double nucleoli on the whole was low, the highest values being 8–10%, seen in one or more small-celled laminae in each of three animals. Approximate percentage incidences were obtained in eight animals for all laminae by observing the number of cells with double nucleoli in the samples drawn for measurement of area; in one of these animals larger samples of about 1000 were taken in the small-celled laminae. The means of the percentage incidences for each lamina, in the eight animals, were:

Lamina	Percentage of neurons with double nucleoli	Lamina	Percentage of neurons with double nucleoli
1	1·1	4	2·0
2	0·7	5	2·2
3	2·4	6	1·1

The mean incidence of double nucleoli in the large-celled laminae, taken together (0·9%), was approximately half as great as that for the small-celled laminae (1·9%).

'*Nuclear-cytoplasmic ratios*'. For each normal lamina, the mean nuclear area was subtracted from the mean cell area to give a figure for the 'mean area of cytoplasm'.

This figure was then divided by the mean nuclear area to yield an approximate 'nuclear-cytoplasmic ratio'. The 'nuclear-cytoplasmic ratios' for the large-celled laminae were almost invariably lower than those for the small-celled laminae, and this confirmed the impression previously noted of a relative abundance of cytoplasm in the large cells; the mean 'nuclear-cytoplasmic ratio' in eight animals for laminae 1 and 2 together was 1:2·07, and for laminae 3–6 together, 1:1·70. No other consistent relationship was noted between these ratios for pairs or groups of laminae.

(3) *Qualitative assessment of the atrophy following eye enucleation*

The changes in appearance of neurons during transneuronal atrophy are conspicuous at an early stage, because they affect whole laminae and also because the unaffected laminae are immediately adjacent for comparison. The adjacent normal laminae provided a convenient standard of reference in describing the changes of atrophy. The changes detected in the atrophied laminae, by comparison with adjacent normal laminae, could be confirmed by reference to the normal laminae at the appropriate level in the opposite geniculate. A description was recorded of the cells in each lamina, normal and atrophied, and these descriptions were later compared.

As early as 4 days after eye enucleation, which was the shortest survival period, a slight change in staining intensity of the atrophic laminae was apparent on low-power inspection. This did not appear to be equal on the two sides; whereas the crossed atrophic laminae (1, 4 and 6) were definitely paler than the adjacent normal laminae (Pl. 2, fig. 7), the uncrossed atrophic laminae (2, 3 and 5) were only just perceptibly paler than the adjacent normal crossed laminae. Measurements of cell areas at this stage showed that the cells of the crossed atrophic laminae were all significantly shrunken, those of lamina 6 showing most change (see Table 10), while of the uncrossed atrophic laminae only the cells of lamina 5 showed any significant shrinkage. At 6 days, all atrophic laminae were definitely paler than the adjacent normal laminae, but the uncrossed laminae now showed a rather more profound decrease in staining intensity than the crossed laminae, and the neurons of all three uncrossed laminae appeared shrunken (Pl. 1, fig. 1). Measurement in two laminae (3 and 4) at 7½ days, and in all laminae at 8 days, showed a considerable decrease in mean cell area in the uncrossed laminae, which exceeded the shrinkage in the crossed laminae (Pl. 2, figs. 8, 9). At the same stage, an increase in density of glial cells was noted in the uncrossed laminae: this will be termed 'apparent gliosis', since it might be caused by shrinkage of neurons and loss of neuropil, as well as by a true increase of glial cells. First appearing in the uncrossed laminae at 7½ days and seen more definitely at 8 days, this apparent gliosis was well marked at 10 days, but at 12 and 14 days it was less, and at 16 days it was no longer certainly present (although it was seen in these laminae after longer survival periods). Both qualitative and quantitative observations showed that between approximately the sixth and the tenth day after eye enucleation the cells of the uncrossed laminae underwent a marked and rapid shrinkage, while from then until 16 days there was no evidence of further shrinkage. The early period of apparent gliosis observed in these laminae thus coincided closely in time with the early rapid shrinkage of their cells.

Meanwhile the cells of the atrophic crossed laminae, which underwent an even

earlier and quite marked shrinkage during the first 4 days after eye enucleation, showed no further decrease in size between 4 and 8 days, nor, with the exception of a small decrease in lamina 4, between 8 and 16 days. Apparent gliosis in the crossed laminae began slightly later than in the uncrossed laminae, and was somewhat less well marked at 10 days; unlike that in the uncrossed laminae, however, it persisted at the same intensity at 12, 14 and 16 days. This less intense and more prolonged period of gliosis thus coincided with cell shrinkage only in lamina 4.

Between 16 and 33 days, the cells of the crossed laminae underwent the greatest degree of shrinkage common to them all, and showed little consistent change thereafter; whereas from 16 days onward the uncrossed laminae showed no major consistent change, but rather an overall slow progression of shrinkage until 3 months. Pl. 1, fig. 2, shows the appearance of the left lateral geniculate nucleus 3 months after enucleation of the left eye. At 33 days, and at 2, 3 and 4 months, there was apparent gliosis of moderate and roughly equivalent degree in all six atrophied laminae.

The appearance of atrophied geniculate neurons 4 months after unilateral eye enucleation, with normal neurons from the opposite side for comparison, is illustrated in Pl. 2, figs. 3–6.

Cytoplasmic changes. The decrease in overall staining intensity of atrophic laminae, which was among the earliest changes observed, was due to a decrease in staining intensity of the cytoplasm of the cells, and this in turn appeared to be due to changes in the Nissl granules. The Nissl granules, or reticulum of Nissl substance, became progressively paler and less conspicuous, and no longer formed dark aggregates in the large cells; there was no obvious decrease in the size of the granules, however, nor any change in their density or their distribution within the cytoplasm. But the decrease in staining intensity was great enough after 1 or more months to give an almost homogeneous, ground-glass appearance to thick cell sections, and the granules were then only well seen in thin sections of cells. The next change to become qualitatively apparent was a shrinkage of the neuron, with an attenuation of the dendrites. This shrinkage was measured in eight animals, and will be presented with the quantitative observations. No increase in the small number of apparent vacuoles was seen in atrophic cells.

Nuclear changes. There was no decrease in staining intensity of the nucleus of the atrophic neurons. The nuclear membrane became more conspicuous at an early stage, remaining so thereafter, but this appeared to be largely illusory, due to the increasing pallor of the cytoplasm, especially in the large-celled laminae where the normal cytoplasm was so dark as often to obscure the nuclear membrane completely. In many neurons of the small-celled laminae, after 1 or more months of atrophy, there appeared an irregular, slight thickening of the nuclear membrane, which made it even more conspicuous. As the neuron became smaller, the nuclear area decreased less than the cytoplasmic area; and it became less common to find an eccentrically placed nucleus, particularly in the small-celled laminae (in which this was rare after 6–8 days). In the large-celled laminae eccentric nuclei were definitely uncommon from 33 days onward.

Nucleolar changes. Apart from a decrease in area, which followed roughly that of the nucleus, the nucleolus showed one early and possibly one late change in the

atrophied neurons. First, in the small-celled laminae, from about 8 days onward the rounded clear areas which were present in the normal nucleoli of most of these animals became indistinct, and by 1 month they had virtually disappeared. This process also occurred to a lesser extent in the larger nucleoli of laminae 1 and 2. Secondly, in the animal with the longest survival period (4 months) there was a considerable increase in the incidence of double nucleoli in all the atrophied laminae, the mean percentage of neurons with double nucleoli in the normal laminae being 1·2%, and in the atrophied laminae 4·4%. This observation is based on over 8000 cells in this animal; in other animals not more than between 1000 and 2000 cells have been sampled and these were probably too few to show small changes. In one other animal, at 33 days, double nucleoli were not found in the normal small-celled laminae but were seen in three of the atrophied small-celled laminae; the incidence in these laminae, however, was only of the order of 1%.

Nucleolar satellite. In the four animals in which typical nucleolar satellites were observed, no obvious change was found in their appearance or position in the atrophied neurons, but no quantitative study was made. This is in accordance with the findings of Cook *et al.* (1951) in the cat.

(4) *Quantitative observations in atrophied laminae*

Shrinkage of neurons. The mean areas of atrophied cells, nuclei and nucleoli, with standard errors of the means, are given in Tables 3–9, which are arranged in order of increasing survival periods, from 4 days to 4 months. In Text-fig. 1 are shown the histograms of cell areas for 100 normal and 100 atrophied neurons from each lamina after 4 months' survival. Although the histograms for the atrophied neurons appear more compact than those for the normal neurons when plotted on the same scale, the coefficient of variation of the populations is in fact little altered, and only for lamina 4 is it significantly decreased. The percentage changes in area of cell, nucleus and nucleolus at each survival period are given in Tables 10–12, together with the results of the *t*-test for the significance of each comparison. Each percentage change is calculated with reference to its appropriate normal control. These Tables, and the graphs constructed from them, permit some assessment of the rate of transneuronal atrophy, though allowance must be made for the possibility of 'biological variation', since with a single exception only one animal was used for quantitative study at each survival time.

The time-course of the shrinkage, and the similarities and differences of behaviour within and between groups of laminae, are illustrated graphically in the succeeding figures, in which mean areas for atrophied neurons are plotted as percentages of the mean normal control areas. The initial 'control' observation shows the values obtained by comparing the two geniculate nuclei of a normal animal; this has been done using the laminae corresponding to the left eye as the standard of reference, since the enucleation was on the right side for all except two of the experimental animals (see Table 1). Text-fig. 3 shows cell, nuclear and nucleolar areas plotted together against survival period in days for each lamina of the crossed and uncrossed groups. The cell area, which is some index of the cytoplasmic volume, has almost always shrunken proportionately more than the nuclear area, and the nucleolar area, though crudely measured, has followed the nuclear more closely than the total cell

area. (The shrinkage of the nucleolus may have been somewhat under-estimated, for in the atrophied neurons chromatin particles were often apposed to the nucleolus, obscuring its outline.) The difference between the shrinkage of nucleus and cell tended to be greater following periods of rapid cell shrinkage, as, for example, at

Tables 10–12. *Percentage changes in area of cell body, nucleus and nucleolus in lateral geniculate neurons at different periods after unilateral eye enucleation*

Asterisks indicate level of significance by the *t*-test. * significant at 5 % level; ** significant at 1 % level; *** significant at 0·1 % level.

Table 10

Survival time (days)	Percentage change of cell area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	+1·5	+2·3	−5·9*	+2·1	+0·4	+0·3
4	−9·3**	−1·2	−6·8*	−10·0***	−12·7***	−16·8***
8	−9·0*	−23·9***	−25·7***	−8·6***	−30·1***	−17·2***
16	−10·1*	−23·3***	−19·1***	−17·1***	−25·0***	−15·8***
33	−41·5***	−18·7***	−15·6***	−48·9***	−11·8***	−45·0***
62	−29·0***	−24·9***	−36·0***	−37·5**	−32·9***	−35·0***
91	−60·7***	−39·1***	−42·3***	−45·6***	−40·9***	−42·1***
129	−45·6***	−40·8***	−40·2***	−32·6***	−40·9***	−35·7***

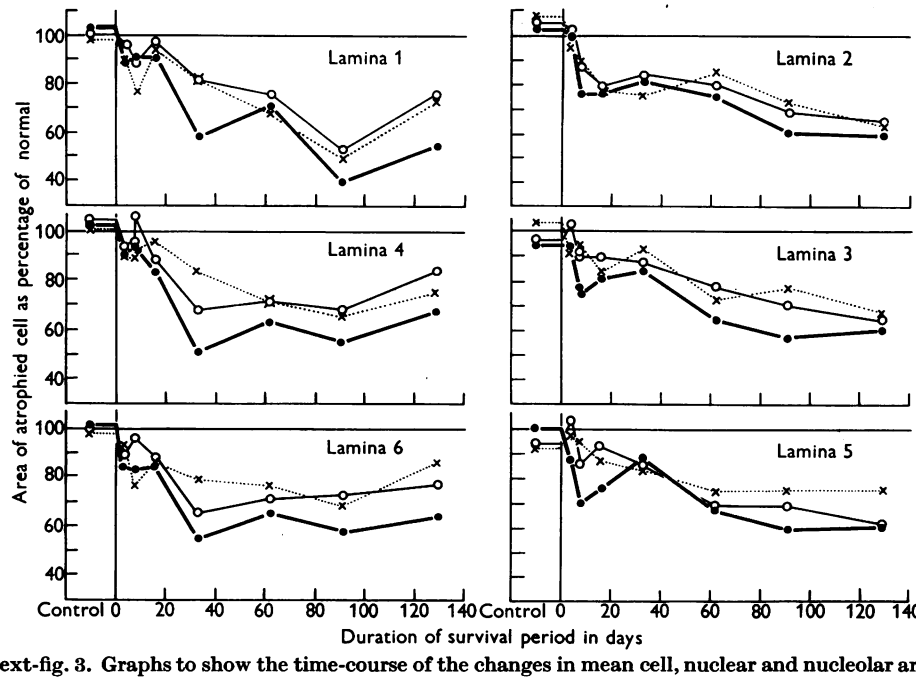
Table 11

Survival time (days)	Percentage change of nuclear area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	+0·5	+4·6	−3·7	+4·2	−6·5**	+1·0
4	−4·5	+2·4	+2·2	−6·1**	+3·0	−11·2***
8	−11·6***	−13·0***	−10·6***	+6·0**	−14·5***	−4·0
16	−3·4	−20·9***	−10·7***	−12·2***	−7·4***	−11·6***
33	−18·8***	−16·8***	−12·5***	−32·3***	−15·0***	−34·3***
62	−24·5***	−20·6***	−22·9***	−28·8***	−31·1***	−28·5***
91	−47·1***	−31·6***	−29·8***	−32·8***	−31·2***	−27·6***
129	−24·6***	−35·1***	−35·9***	−16·3***	−40·0***	−22·8***

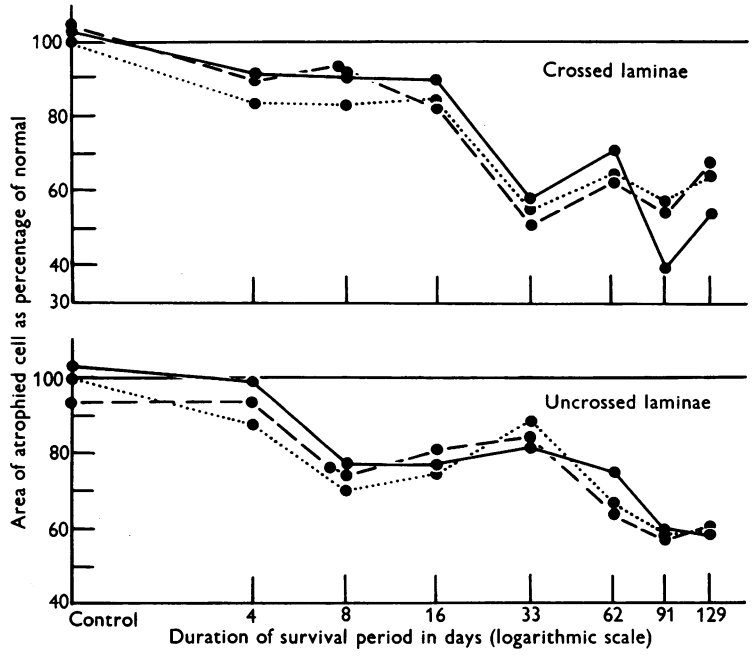
Table 12

Survival time (days)	Percentage change of nucleolar area from normal					
	Lamina 1	Lamina 2	Lamina 3	Lamina 4	Lamina 5	Lamina 6
Control	−2·1	+6·7	+3·3	+0·8	−7·3*	−1·1
4	−10·5***	−5·6	−9·3**	−10·2**	−3·4	−6·8*
8	−22·9***	−11·5***	−6·4	−8·1*	−5·2	−23·0***
16	6·0*	−22·3***	−15·9***	−4·3	−13·4***	−13·7***
33	−18·4***	−25·0***	−7·4*	−16·6***	−16·9***	−20·5***
62	−32·0***	−15·6***	−27·2***	−28·5***	−25·7***	−23·6***
91	−50·5***	−27·7***	−23·1***	−34·6***	−25·0***	−30·9***
129	−27·5***	−36·3***	−33·1***	−24·9***	−24·4***	−13·9***

33 days for the crossed laminae and 8 days for the uncrossed laminae; this suggests that the nuclear atrophy is slower than that of the cytoplasm though, in the uncrossed laminae at any rate, it may not ultimately be any less. It is also evident that the crossed laminae resemble each other more closely than they resemble the uncrossed laminae, and vice versa. Within each group, however, the two small-celled laminae (4 and 6, or 3 and 5) resemble each other rather more closely than they resemble the large-celled lamina (1 or 2). This difference might reflect a difference of function between large and small cells, but it might possibly have arisen as an

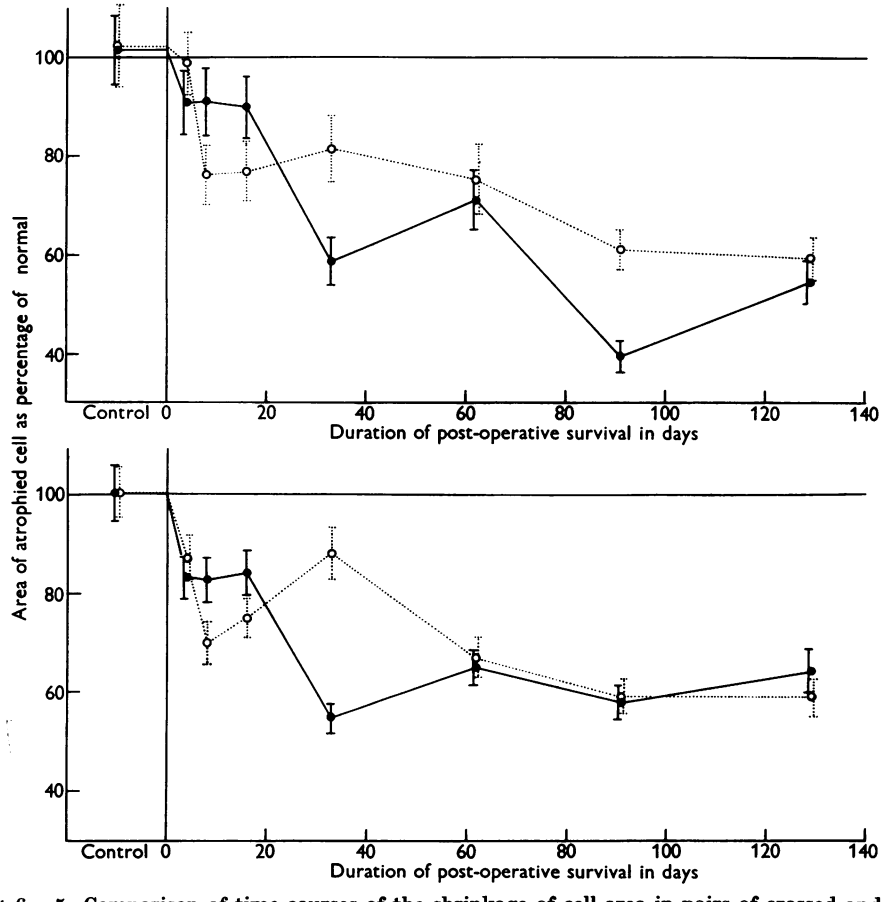


Text-fig. 3. Graphs to show the time-course of the changes in mean cell, nuclear and nucleolar areas for each lamina following eye enucleation. Each mean value for the atrophied neurons is plotted as a percentage of the mean normal value for that animal. The crossed laminae are on the left. ● = cell area, ○ = nuclear area, × = nucleolar area.



Text-fig. 4. Graphs to show the differences in the time-course of changes in cell area between the crossed and the uncrossed laminae, and the similarities within these groups. Upper graph: solid line, lamina 1; broken line, lamina 4; dotted line, lamina 6. Lower graph solid line, lamina 2; broken line, lamina 3; dotted line, lamina 5.

error inherent in sampling, owing to the greater variance of the population of neurons in the large-celled laminae which is apparent from a comparison of the histograms in Text-fig. 2. When the laminar groups as a whole are compared, crossed with uncrossed, their behaviour is seen to differ appreciably during the first month after eye enucleation, but only slightly from 2 months (62 days) onward, especially



Text-fig. 5. Comparison of time-courses of the shrinkage of cell area in pairs of crossed and uncrossed laminae; linear scales. The vertical bars represent 95% confidence intervals for the percentage changes. Upper graph: ● = lamina 1, ○ = lamina 2; lower graph: ● = lamina 6, ○ = lamina 5.

if lamina 1 is excluded. The differences within the first month are illustrated by a semi-logarithmic plot in Text-fig. 4, in which a logarithmic scale is used for the survival period, and the mean cell areas for the three laminae of each group are plotted linearly as percentages of normal. This serves to expand the early part of the time scale, which appears to be of most interest. Text-fig. 4 shows that, at 4 days, cell areas in all the crossed laminae are already decreased by 9–17%; the shrinkages are statistically significant and remain substantially unchanged until some time after 16 days. Of the uncrossed laminae, only lamina 5 has a significant decrease of cell

area at 4 days, but at 8 days all show considerable shrinkage which exceeds that of the crossed laminae, being between 20 and 30%. The difference at 8 days was confirmed for laminae 3 and 4 by measuring groups of fifty cells from an animal killed $7\frac{1}{2}$ days after eye enucleation; as shown in the graphs, there was close agreement of the two sets of observations. From 8 to 33 days the uncrossed laminae show a slight recovery in cell area, while in the crossed laminae there is a further marked shrinkage, of 30%, between 16 and 33 days. The difference between the laminar groups at 33 days is a striking one, for it is well over 20%. Unfortunately, however, its validity is a little uncertain, for in this animal the normal neurons were considerably larger in the crossed than in the uncrossed laminae. The fact that the nucleus and nucleolus have behaved differently in the two laminar groups in relation to cell area in this animal indicates a difference, but it is much less well established than that existing within the first fortnight. Text-fig. 5, which is plotted with linear scales, shows the time-course of changes in cell area for two pairs of laminae from opposite groups, for direct comparison.

Assessment of possible cell loss. No loss of neurons from the atrophied laminae, following transneuronal degeneration, could be demonstrated at 16 or at 91 days. At 129 days, which was the longest survival period, three of the atrophied laminae (1, 2 and 6) showed a slight decrease in the population of neurons within the sample volume studied, but this change was significant only in lamina 6. For all atrophied laminae together, however, the mean decrease in population was 7·8%, and this, though significant, is a very small change.

DISCUSSION

The three most significant findings of the present study are: (1) the marked rapidity of onset of the transneuronal changes in the monkey; (2) the greater severity of the neuronal atrophy in the lateral geniculate nucleus of the monkey than in the cat and rabbit; and (3) a distinct difference in behaviour between the cells of the crossed and the uncrossed laminae which is in striking contrast with the similarity in behaviour of the cells of the three laminae in each group. An incidental finding which is of interest, although it is unrelated to the problem of transneuronal degeneration, is the observation that there does not appear to be any characteristic gradient of cell size from the ventral to the dorsal small-celled laminae.

The cells of the lateral geniculate nucleus of the monkey undergo essentially the same changes as have been described in the lateral geniculate nucleus of the cat and rabbit (Cook *et al.* 1951) and of man (Goldby, 1957). In general these changes consist of a reduction in cell size affecting all components of the cell, but particularly the cytoplasm, together with a diminution in the amount of Nissl material as assessed by the intensity of staining. From the evidence available at present, both for the lateral geniculate nucleus and for other sites, it may be concluded that in adult animals of all species transneuronal atrophy appears qualitatively to be the same. There are two possible qualifications, however, for Cook *et al.* (1951) described distinct vacuolation of the degenerating cells of the lateral geniculate nucleus of the cat and rabbit after 8–10 months, and Penman & Smith (1950) found that the medium-sized cells of the spinal nucleus of the trigeminal nerve in man had undergone

swelling and chromatolysis four months after alcohol injection of the trigeminal ganglion.

In their paper on the termination of optic fibres in the lateral geniculate nucleus of the monkey, Glees & Le Gros Clark (1941) mention that transneuronal cell degeneration had occurred in one of their animals as early as 7 days after section of the optic nerve. The present study has confirmed this observation of the rapidity of onset, and has provided quantitative evidence of the severity of the cellular degeneration. Thus, as can be seen from the results summarized in Table 10, significant cell shrinkage occurred in the animal with the shortest survival period of this series (4 days), and the shrinkage progressed during the course of the next 1–2 months until the cross-sectional area of the cell was reduced to approximately 60 % of the normal. The degeneration, however, does not appear to be a uniform process; there are distinct differences in the rate at which it occurs in the cells of different laminae and in the extent to which the cytoplasm, nucleus and nucleolus of the cells are affected.

An analysis of the time-course suggests that in the monkey the degeneration occurs in three phases. There is an early rapid shrinkage of neurons in all six laminae which reaches its peak in the crossed laminae by 4 days and in the uncrossed laminae by 8 days. This early phase is followed by a period of relative stability lasting for approximately 2–3 weeks. After this the degeneration enters its third phase, which is characterized by a progressive shrinkage in mean cell area during the next 2 months. Thereafter the degenerative process appears either to have stabilized, or to have become considerably slower. Previous quantitative studies of transneuronal degeneration have shown nothing comparable to the early phase of shrinkage, and only in man has such a severe degree of atrophy been described (Goldby, 1957). In their detailed study of this type of degeneration in the lateral geniculate nucleus of the cat, Cook *et al.* (1951) found no evidence of cell shrinkage earlier than 63 days after eye enucleation, and thereafter the degeneration as estimated by cell size remained more or less constant until 313 days, which was their longest survival period. In the rabbit they found no consistent signs of degeneration until 5–6 months after enucleation of the eye.

That the cytoplasm, nucleus and nucleolus do not behave uniformly during transneuronal degeneration is also clearly seen in Text-fig. 3. From this figure it is apparent that at nearly every interval studied the degree of shrinkage of the cytoplasm exceeds that of the nucleus. Although the nucleolar measurements are somewhat less accurate, the evidence available suggests that during transneuronal degeneration the changes in the nucleolus follow those in the nucleus more closely than they follow the cytoplasmic changes. Regarding the severity of the cellular degeneration, however, there appear to be distinct differences between species in the degree of atrophy ultimately reached. In the lateral geniculate nucleus of the cat Cook *et al.* (1951) found the shrinkage to be of the order of 20–30 %; in the lateral geniculate nucleus of the rabbit they describe the shrinkage as being slightly less, although they give no figures for this species. In the macaque, and in man, the end-result appears to be more severe; the present observations have shown that after 129 days the cell shrinkage in all laminae is of the order of 30–40 %, which is only slightly less than the shrinkage found by Goldby (1957) in a human lateral geniculate nucleus at least 36 years after eye enucleation. Not only the cell area, but also the

nuclear and nucleolar areas, show more marked changes in the monkey than in the cat (20–30 %, as compared with approximately 15 %).

In the lateral geniculate nucleus of the Primates, moreover, there is after relatively long survival periods a difference in the degree of atrophy between the cells of the large-celled and small-celled laminae. This difference has been noted by Le Gros Clark (1941) and Polyak (1957) in the macaque, and in man by Hechst (1933) and Goldby (1957); Goldby measured the actual degrees of shrinkage of these cells in his human case. It is also well known that these cells behave differently after visual cortex ablation (Brouwer, 1930; Walker, 1935; Powell, 1952). The finding in the present study that during the first 4 months after eye enucleation there is no essential difference either in the time-course or in the severity of transneuronal degeneration between the large- and small-celled laminae can probably be explained by the fact that these survival periods were comparatively short. That the observed difference in behaviour between the large and the small cells is not merely a function of the size of the cell soma, but is due to some more fundamental difference in the neuron or in the organization of its connexions, is suggested by recent evidence from the primary auditory nuclei that neurons of markedly different size may undergo transneuronal degeneration at the same rate and to the same degree (Powell & Erulkar, unpublished).

Cell loss. Despite the rapidity and severity of the cellular changes in transneuronal degeneration found in the present material there is no good evidence of any appreciable cell loss during the first 4 months after enucleation of the eye. In only one animal (4 months' survival) was there any evidence of cell loss; here there was a slight loss in three laminae, but only in lamina 6 was it statistically significant. This finding may represent the initial stage of a slow but progressive cell loss.

The question whether or not cell loss occurs in transneuronal degeneration is of considerable interest. In Primates severe cell loss has been described in the lateral geniculate nucleus following eye removal. Polyak (1957) found 'complete disappearance of most of the nerve cells and a considerable increase in the neuroglia' in the affected laminae of the lateral geniculate nucleus of a macaque in which the retina had been scooped out at birth, and which was then allowed to survive for 2 years. In the human case described by Goldby (1957) the atrophic laminae of the lateral geniculate nucleus showed a cell loss of approximately 50 % many years after removal of the eye as a result of a war injury. Similarly, Penman & Smith (1950) described 'a definite reduction in the number of the neurons' in the main sensory nucleus of the trigeminal nerve of man, and in the spinal nucleus 'clumps of microglial cells are seen suggesting neuronophagia', 4 months after injection of alcohol into the trigeminal ganglion. On the other hand, in the lateral geniculate nucleus of the cat and rabbit Cook *et al.* (1951) found 'no evidence of neuronophagia' up to 313 days. Whether the increasing vacuolation which they found in the animals with the longest survival periods is an indication that the degenerative process might progress to cell loss is unknown. In the ventral cochlear nucleus and lateral superior olive of the cat, however, detailed counts throughout the nuclei showed no evidence of cell loss 319 days after destruction of the cochlear nerve (Powell & Erulkar, unpublished). In marked contrast to these results in adult cats, Torvik (1956) has described almost complete cell loss in restricted areas of the pontine and inferior olivary nuclei as

early as 5–8 days after lesions of the cerebral hemispheres and brain stem in young kittens (8–20 days old).

From these observations it seems clear that the degree of cell loss occurring in any given species is a function at least of the age of the animal at the time of operation and of the duration of the post-operative survival period. It would be of interest to test experimentally the validity of this deduction by a study of the time-course and severity of transneuronal degeneration in the lateral geniculate and cochlear nuclei of young rabbits and kittens, or in the pontine and inferior olivary nuclei of mature cats, and in the lateral geniculate nucleus of monkeys after longer survival periods. The fact that in transneuronal degeneration in adults appreciable cell loss has only been described in man might suggest that species is also a determining factor.

Differences in behaviour between crossed and uncrossed laminae. A point of considerable theoretical interest which has emerged from this study is the finding that the neurons of the crossed and uncrossed laminae show differences both in the severity and time of onset of the initial phase of transneuronal atrophy. Thus the cells of the crossed laminae have virtually reached the end of this phase by 4 days, while the neurons of the uncrossed laminae show little morphological evidence of atrophy until somewhat later. At 8 days, however, the degree of shrinkage which they have undergone is greater than that seen in the crossed laminae, and this observation has been confirmed in one animal which was allowed to survive for $7\frac{1}{2}$ days in which the severity of the cell shrinkage was essentially the same. In contrast to this difference between the cells of the crossed and uncrossed laminae, the neurons of all three laminae of either group consistently showed the same pattern of degeneration, except during the first few days when there was a greater degree of atrophy of the cells of the dorsal small-celled laminae (i.e. lamina 5 of the uncrossed and lamina 6 of the crossed group).

Qualitatively there appear to be no morphological differences between the neurons of the crossed and uncrossed laminae in the normal lateral geniculate nucleus. The only known quantitative difference is the finding that the mean cell area of the uncrossed small-celled laminae exceeds that of the crossed, but in the large-celled laminae this relationship is reversed. No differences have been described either in the number or in the form of the synaptic endings in the crossed and uncrossed laminae. Indeed, when discussing their experimental study of the termination of the optic nerve fibres in the monkey, Glees & Le Gros Clark (1941) state that 'it seems probable that no cell in any of the laminae receives more than one bouton', and from their description it appears that all synaptic terminals are of the axo-somatic type. In this connexion, however, it may be apposite to recall that the appearance of the terminal degeneration at 3 days as described by these authors differed in the crossed and uncrossed laminae. In laminae 2, 3, and 5 they found the terminal boutons to be 'in many instances thickened, somewhat enlarged, and stained more densely than in normal preparations'. In the crossed laminae 1, 4 and 6 similar changes were observed, but in addition they state that 'the interior of the ring-like boutons in these laminae is often found to be filled with a dense, opaque mass which on close scrutiny appears to be composed of a very fine neurofibrillar network. In other instances the boutons have become converted into

small, solid, black end-bulbs.' From a knowledge of the time-course of bouton degeneration it seems justifiable to infer from this description that the terminal degeneration had proceeded further in the crossed than in the uncrossed laminae.

Two other differences in the afferent fibres which may be of importance in this connexion are the greater length of the crossed fibres and the relative diameters of the crossed and uncrossed components of the optic tract. It would be of interest to know, for example, whether in Primates there is a difference in mean fibre diameter between the crossed and uncrossed optic tract fibres comparable to that found in the cat by Bishop, Jeremy & Lance (1953), and whether optic nerve fibres of different diameters have differing rates of degeneration such as van Crevel (1958) has shown in the cat. In view of these known differences in the afferent fibres of the cat, it would be of interest, also, to determine whether transneuronal degeneration differs in its time of onset in the crossed and uncrossed laminae of this species.

General observations on the process of transneuronal degeneration. Despite all the work which has been done in recent years, the factors which determine whether or not a cell will degenerate after removal of some or all of its afferent connexions are not clearly known; but the severity and time-course of the degeneration appear to depend upon at least three main variables. The first of these is the age of the animal at the time of operation. All other neuronal degenerations which have been adequately studied so far have proved to be more severe in younger animals, and the interesting work of Torvik (1956) has indicated that transneuronal atrophy is no exception to this rule. His work has shown that transneuronal changes are both more severe and more rapid of onset in young animals than in adults; indeed, it is probable that this type of degeneration would be found to occur more widely if young animals were used in such experimental studies.

The second factor of importance is the species of the animal used. Its effect upon the time-course is illustrated by the marked difference in the time of appearance of transneuronal changes in the monkey, cat and rabbit; in the monkey definite changes become apparent within a few days of deafferentation, but in the cat and rabbit the changes can only be seen after about 2 months. In consequence of such differences of time-course, it is obvious that the duration of the survival period required to demonstrate unequivocal transneuronal degeneration may vary markedly from species to species. The differences in the severity of neuronal changes between cat, rabbit, monkey and man, at the longest survival periods for which data are available, have been mentioned above; it would appear for the first two species at least that, once transneuronal degeneration is established, further survival (up to 1 year in the cat) makes little difference to the degree of cellular shrinkage. On the other hand, it is not certain whether cell loss would occur at some later stage, and it would be of interest to know if after very long survival periods some slow, but progressive change, such as the increasing vacuolation which was described by Cook *et al.* (1951), would lead to cell loss comparable to that found by Goldby (1957) in the human lateral geniculate nucleus. The cell loss in the human lateral geniculate is not necessarily dependent upon the long post-operative survival, however, in view of the observations of Penman & Smith (1950) on the trigeminal nuclei.

The differences in transneuronal degeneration of the visual system in these four species are sufficiently great to suggest that there is some fundamental difference in

the lateral geniculate neurons themselves, or in their afferent connexions. It is impossible at present to decide which of these factors is the more critical, as there are known differences in each. For example, the lateral geniculate nucleus shows distinct differences in behaviour after visual cortex ablation: in Primates all the cells undergo complete degeneration (Poljak, 1933), but in the cat a definite proportion of cells remains (Minkowski, 1913). Whether these persisting cells are principal neurons which have survived axonal section, or represent the short-axon cells described by O'Leary (1940), is not known. Differences have also been described in the afferent terminals: Glees & Le Gros Clark (1941) found only a single axosomatic bouton on each lateral geniculate cell of the monkey, but in the cat and rabbit Glees (1941, 1942) has described multiple endings of both the axosomatic and axodendritic types.

The third variable which may be considered is the nuclear formation in which transneuronal degeneration is studied. Here the evidence available suggests that in adult animals of the same species, transneuronal degeneration will probably be found to show very much the same severity and time-course in all regions in which it occurs. In an unpublished series of observations on the cochlear nuclei and lateral superior olive of the cat, the transneuronal degeneration following section of the cochlear nerve has been found to show a time-course and a degree of neuronal shrinkage virtually identical with those described for the lateral geniculate nucleus in this species. In a study of the superior cervical ganglion of the rabbit after preganglionic nerve section, Hamlyn (1954) found cell shrinkage of up to 18% after 3–4 months, which is probably of the same order as that described qualitatively by Cook *et al.* (1951) in the rabbit lateral geniculate, though occurring slightly earlier. It would obviously be of interest to obtain quantitative data on transneuronal degeneration in the superior cervical ganglion of the cat and monkey, and in the auditory nuclei of the rabbit and monkey. Structural or functional differences between groups of neurons may, however, produce some differences in transneuronal atrophy, as indicated, for example, by the differences in the time-course of the changes between crossed and uncrossed laminae of the monkey lateral geniculate nucleus, and in ultimate severity of the degeneration between the large and small neurons of the lateral geniculate nucleus in man and in the monkey.

The question of the region affected by transneuronal degeneration in any given species may not be important as far as the time-course and severity of the degeneration are concerned; the site certainly is significant, however, in determining whether or not transneuronal degeneration does occur after interruption of the afferent connexions. To take but one example, the absence of transneuronal degeneration in the ventral posterior nucleus of the thalamus of the monkey after section of the medial fillet is in striking contrast to the unequivocal degeneration found in the lateral geniculate nucleus of the same animal (Glees & Le Gros Clark, 1941). The factor critical in determining the occurrence of transneuronal degeneration after deafferentation of neurons is almost certainly the extent to which afferents have been destroyed. The absence of transneuronal degeneration in the ventral thalamic nucleus is probably explicable on this basis, and Cook *et al.* (1951) have suggested the same explanation for its absence in the anterior horn cells of the spinal cord following ablation of the motor cortex and dorsal root section. The precise propor-

tion of afferents which has to be destroyed for transneuronal degeneration to occur is unknown, and may vary from nucleus to nucleus. The only evidence bearing on this point is Torvik's (1956) statement for the young kitten that 'heavy degeneration is observed even though at least one-half of the afferents are intact (as evidenced by the bilateral changes in the olive in unilateral lesions)'.

SUMMARY

1. A quantitative study has been made of transneuronal atrophy in the lateral geniculate body of *Macaca mulatta*. One eye was enucleated from each of twelve mature monkeys, and they were allowed to survive for periods varying from 4 days to 4 months. The neurons of the atrophied laminae in each geniculate were compared with those in the normal laminae of the opposite geniculate innervated from the intact eye. One unoperated monkey was used as an additional control.

2. The appearance of the normal neurons is described. A small percentage of normal neurons had two nucleoli; this was commoner in the small-celled laminae (3–6) than in the large-celled laminae (1 and 2). Measurement of normal neurons has shown that regular gradients of cell area from lamina to lamina are uncommon, and that the neurons of any given lamina cannot be grouped into more than one population, when assessed according to the size of their cell bodies.

3. During transneuronal atrophy, the neurons shrink and the staining intensity of their Nissl substance diminishes. In the first few months, the cytoplasm shrinks proportionately more than the nucleus and nucleolus. At 4 months there was an increase in the incidence of neurons with two nucleoli.

4. The shrinkage of neurons has been found to show two phases, an early rapid phase which affects the laminae receiving crossed fibres (1, 4 and 6) earlier than those receiving uncrossed fibres (2, 3 and 5), and which is succeeded by a period of relative stability, and a later phase in which there is no consistent difference in behaviour between the groups of laminae. Within each group, the neurons of all three laminae of each crossed or uncrossed set behaved rather similarly, except during the first few days when it was the dorsal small-celled lamina (5 or 6) which was the most affected.

5. The early rapid shrinkage of neurons reached its peak in the crossed laminae by 4 days, and in the uncrossed laminae by 8 days; this shrinkage was more profound (24–30 % of control area) in the uncrossed than in the crossed laminae (9–17 % of control area). This phase coincided with an onset of apparent gliosis in the atrophic laminae.

6. The later phase of atrophy began towards the end of the first month, or during the second month, and was possibly earlier in the crossed laminae. By 4 months, further shrinkage of neurons appeared to have ceased, at least in the uncrossed laminae; the mean area of the atrophied neurons was now about 60 % of the mean area of the control neurons in the opposite lateral geniculate.

7. No indication of loss of neurons was found until 4 months after eye enucleation, when there appeared to be a slight but significant loss of neurons in lamina 6.

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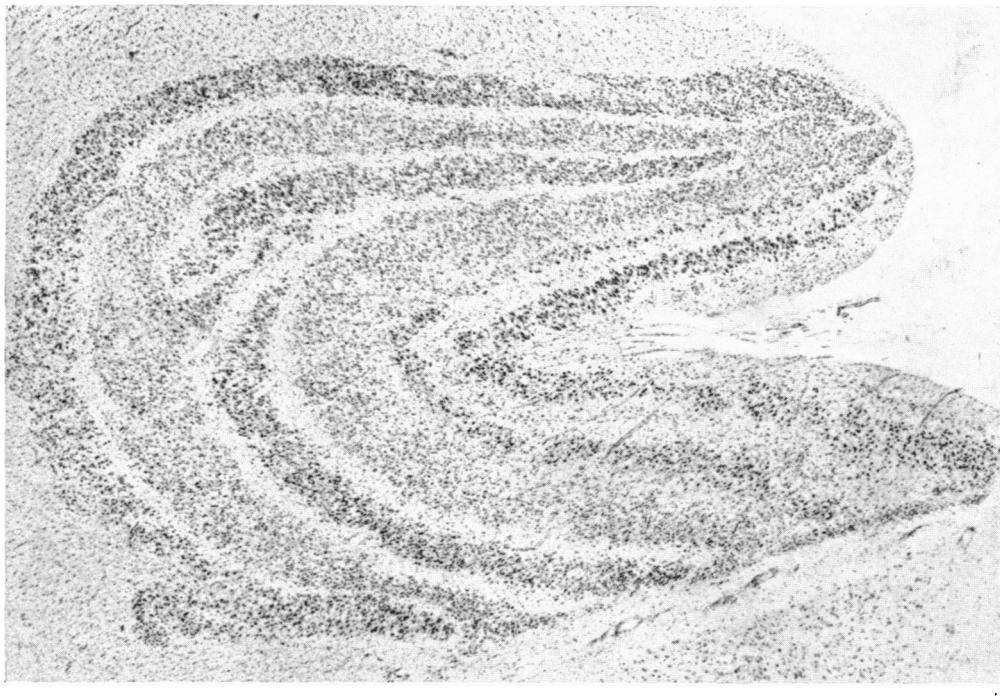
EXPLANATION OF PLATES

All sections were 25 μ in thickness and were stained with thionin.

PLATE 1

Fig. 1. Coronal section from the posterior half of the left lateral geniculate nucleus of a macaque, 6 days after enucleation of the left eye. There is distinct atrophy of neurons in the uncrossed laminae (large-celled lamina 2, small-celled laminae 3 and 5). The crossed laminae (1, 4 and 6) are normal. × 24.

Fig. 2. Coronal section from the posterior half of the left lateral geniculate nucleus of a macaque, 91 days after enucleation of the left eye. The neurons of the three uncrossed laminae are severely shrunken. × 24.



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(*Facing p.* 168)

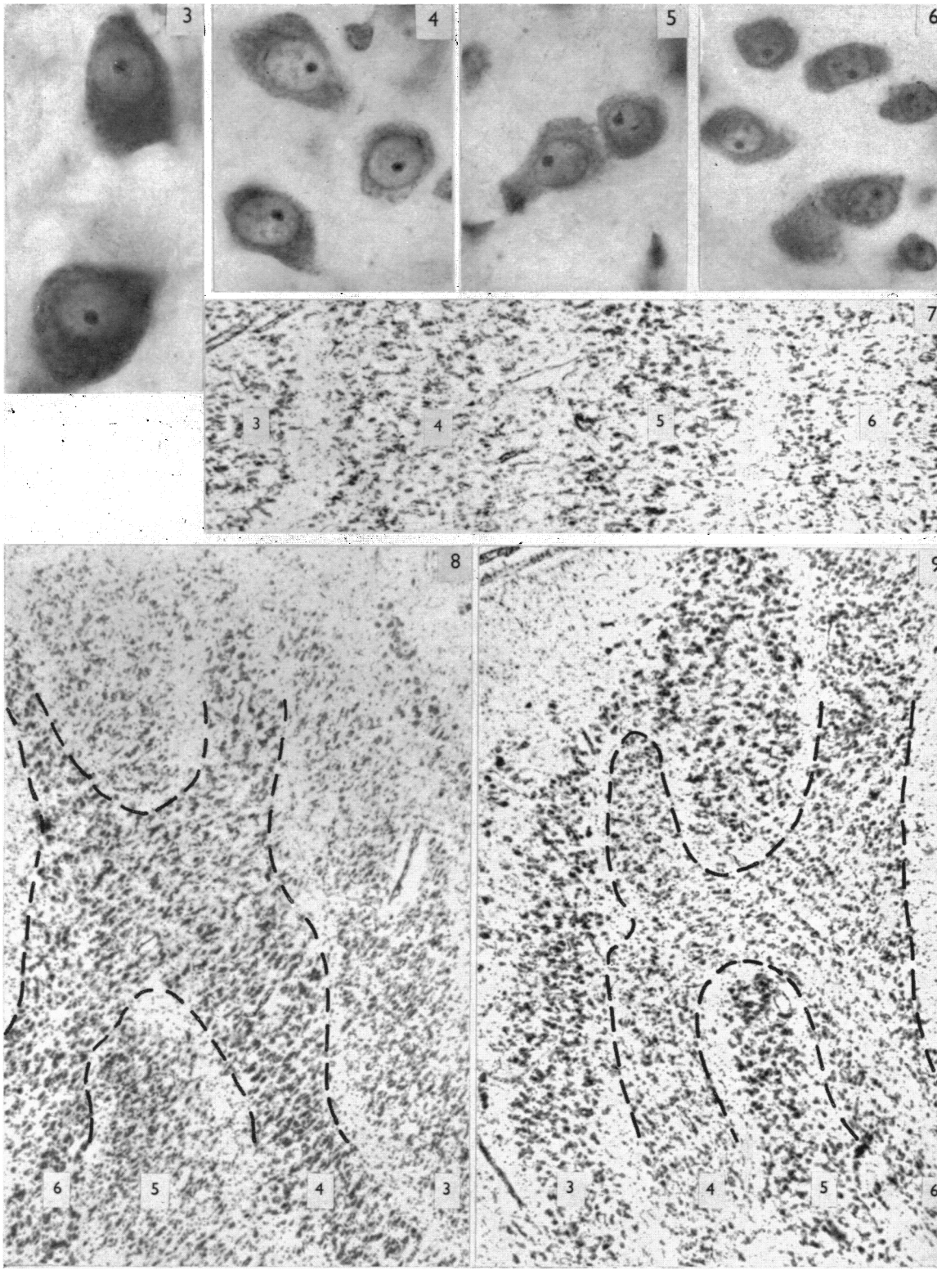
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PLATE 2

Figs. 3 and 4. Normal (Fig. 3) and atrophied (Fig. 4) neurons from lamina 1 of the lateral geniculate nuclei of a macaque, 129 days after removal of one eye. × 1000.

Figs. 5 and 6. Normal (Fig. 5) and atrophied (Fig. 6) neurons from lamina 5 of the lateral geniculate nuclei of a macaque, 129 days after removal of one eye (same animal as Figs. 3 and 4). × 1000.

Fig. 7. Part of a coronal section from the left lateral geniculate nucleus of a macaque, 4 days after enucleation of the right eye. This strip passes perpendicularly across the small-celled laminae 3–6, which are numbered, and shows the pallor and shrinkage of neurons in the crossed laminae, 4 and 6. The uncrossed laminae on this side are normal. × 72.

Figs. 8 and 9. Parts of coronal sections at corresponding levels of the two lateral geniculate nuclei of a macaque, 8 days after enucleation of the right eye. Portions of the lateral 'limbs' are shown, and the small-celled laminae are numbered. On each side, a band of cells connects laminae 4 and 6 at this level, interrupting lamina 5; this is indicated by a dotted line. These photographs are complementary, showing the shrinkage of the uncrossed laminae in the right lateral geniculate (Fig. 8) and of the crossed laminae in the left lateral geniculate (Fig. 9). × 72.