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Further observations on transneuronal degeneration in the lateral geniculate nucleus of the macaque monkey

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INTRODUCTION

An account was recently given (Matthews, Cowan & Powell, 1960) of the course of transneuronal degeneration in the macaque during the first 4 months after eye enucleation. Estimates of cell population showed that, 4 months after enucleation, there had been little or no loss of degenerating lateral geniculate neurons. Goldby (1957), however, in a human brain, found a loss of about 50 % of the affected lateral geniculate neurons, between 36 and 40 years after removal of one eye. To test for the occurrence of cell loss, therefore, another macaque has been allowed to survive for 1 year following eye enucleation and the results are presented in this paper. In addition, in some of the original series of twelve monkeys, small lesions were placed in the visual cortex at the time of enucleation of the eye, in order to study the consequent interaction of retrograde degeneration with transneuronal degeneration; this paper includes some observations on this interaction.

MATERIAL AND METHODS

The experimental observations were made upon nine mature monkeys (*Macaca mulatta*). One eye was enucleated from each monkey, and in eight of the animals an area of the visual cortex 1–2 cm.^{2} in extent was removed from the lateral aspect of one cerebral hemisphere at the same operation. In three of these monkeys a similar lesion was also made in the opposite visual cortex, at a second operation. Only the cortical lesions produced at the time of the eye enucleation have been used in the present study. The intervals allowed for survival are shown in Table 1. Each brain was fixed by immersion in 70 % alcohol with 2 % acetic acid, and a block of the thalamus containing both lateral geniculate bodies was embedded in paraffin wax and sectioned coronally at 25μ. Every 5th section (every 10th for MG2 and MG5) was mounted and stained with thionin.

The changes due to transneuronal degeneration have already been described qualitatively for each animal except MG2 (1 year survival), with quantitative data in some cases (Matthews *et al.* 1960). One control brain (MG4) was the same as that used previously. All observations on cell size were made at the junction of the posterior and middle thirds of the lateral geniculate nucleus, where the six laminae are circumscribed and distinct. Since the wedge of retrograde degeneration resulting from each cortical lesion lay in the central zone of the ipsilateral nucleus, the observations upon transneuronal degeneration were confined to the lateral part of each lamina, well away from the zone of retrograde degeneration. All comparisons of normal with degenerate cells or laminae were made at the same antero-posterior

level in the two lateral geniculate nuclei of the same brain, and whenever possible at corresponding sites in the laminae.

Cell shrinkage was measured from outlines traced with the aid of a camera lucida, as described in the previous paper (Matthews *et al.* 1960). In MG2 (survival 1 year) the total populations of normal and degenerate neurons were estimated in laminae 1 and 2, and in the pairs of laminae 3 and 5, and 4 and 6, by determining the volumes of the laminae and the densities of neurons within them. By means of a projection apparatus, the outline of each lamina was traced at a magnification of 50 times in

Table 1. *Details of the operative lesions and survival times in the experimental animals; the control animals are also listed*

Animal	Survival interval (days)	Eye enucleated	Side of visual cortex lesion studied
MG4	Control	—	—
MG5	Control	—	—
MG2	365	Right	None
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	*Right
MA2	16	Right	*Right

* In these animals a similar lesion was placed in the opposite visual cortex at a second operation.

every 10th section, 25 μ thick, throughout the antero-posterior extent of each lateral geniculate nucleus. From these tracings, the areas occupied by the laminae were measured and laminar volumes were calculated. The small-celled laminae were treated together in pairs, since they are continuous with each other (3 with 5, and 4 with 6) in the anterior part of the nucleus and are only completely separate for a short part of their extent posteriorly. In all the sections used for the determination of area, sample counts of neuronal density were made in each lamina, using an oil-immersion objective and a field of known diameter. The number of sample fields taken per lamina in each section was kept in constant proportion to the area occupied by the lamina, and the appropriate number of fields was spaced as evenly as possible. By this procedure it was hoped to avoid errors due to variations in cell density in any lamina from one part of the nucleus to another, such as were found by Le Gros Clark (1941). Only neurons with a distinct nucleolus were counted, and the size of the sample was between 1·75 and 1·8 % of the cell population in each lamina.

The laminae are numbered 1–6 in the ventro-dorsal direction, from the hilum (Le Gros Clark, 1941), and the terms 'crossed 'and 'uncrossed' are used to describe those laminae which receive projections from the contralateral and the ipsilateral eye, respectively.

RESULTS

Transneuronal degeneration after 1 *year*

In the lateral geniculate nuclei of the monkey which was allowed to survive 1 year after eye enucleation, the deafferented laminae are so severely atrophied as to be quite inconspicuous when viewed at low magnification.

The neurons of these laminae are greatly shrunken and their cytoplasm is only faintly stained. The intensity of cytoplasmic staining, however, varies from cell to cell, and lies occasionally within the range observed in normal neurons. This is not infrequent in the large-celled laminae (1 and 2). Many of the neurons in the degenerate small-celled laminae appear little larger than neuroglial cells, but are distinguishable from these by the finer texture of their nuclei, containing small but typical nucleoli, and the slight residual basophilia of their cytoplasm.

Table 2. *Mean areas for* 100 *neurons from each normal and each degenerate lamina of the lateral geniculate nuclei of a macaque,* 1 *year after unilateral eye enucleation*

Lamina	Normal	S.E.	Degenerate	S.E.	Difference (%)
			Cell areas (μ^2)		
1	236·2	8·7	108·0	4·5	54·3
2	250·2	9·8	100·5	4·0	59·9
3	197·7	5·2	67·4	2·2	65·9
4	172·5	4·6	58·1	1·8	66·4
5	156·8	4·3	54·3	1·5	65·4
6	157·4	3·7	52·5	1·6	66·6
			Nuclear areas (μ^2)		
1	70·5	1·8	42·7	1·4	39·4
2	72·4	1·8	40·9	1·3	43·5
3	60·7	1·1	28·4	0·9	53·3
4	55·7	0·9	21·0	0·8	62·3
5	49·3	1·3	21·5	0·7	56·4
6	41·5	1·3	21·4	0·7	48·4
			Nucleolar areas (μ^2)		
1	3·58	0·10	1·96	0·07	45·3
2	3·43	0·12	1·86	0·07	42·3
3	2·56	0·06	1·37	0·05	46·5
4	2·47	0·07	1·18	0·04	52·2
5	2·31	0·06	1·21	0·04	47·6
6	2·26	0·06	1·25	0·04	44·7

The mean areas of the cell body, nucleus and nucleolus for 100 neurons from each normal and each degenerate lamina are shown in Table 2, together with the difference in each case, as calculated from the means and expressed as a percentage of the mean normal value. The shrinkage of neurons, to rather less than half the normal area, exceeds that found previously, 4 months after eye enucleation (Matthews *et al.* 1960), when the mean cell area was decreased by 33–46 % of the mean normal value. As judged from the cell and nuclear areas, the shrinkage of neurons after 1 year appears to be slightly less severe in the large-celled than in the small-celled laminae,

but there is no difference in degree of shrinkage between the degenerate neurons of the crossed laminae (1, 4 and 6) and the uncrossed laminae (2, 3 and 5).

The results of the determinations of laminar volume and of cell population are shown in Table 3. All the degenerate laminae are shrunken, and show a loss of neurons. There has also been an increase in the packing density of neurons per mm.^{3}. The loss of neurons is rather evenly distributed throughout each affected lamina. The estimates in the small-celled laminae should be the more reliable, because of their greater volume. In the degenerate lamina 2 less shrinkage has been detected, and there appears to have been only a 4% decrease in cell population; but these results are possibly inaccurate, since this degenerate lamina is greatly attenuated and difficult to define, and its volume may have been over-estimated, with a consequent reduction in the apparent loss of neurons. There seems to be no reason for expecting lamina 2 to behave differently in this respect from the other laminae, and from lamina 1 in particular.

Table 3. *Total volumes, mean numbers of neurons per mm.^{3} and estimated total populations of neurons for normal and degenerate laminae of the lateral geniculate nuclei 1 year after unilateral eye enucleation*

	Volume of lamina (mm.^{3})			Mean number of neurons per mm.^{3}			Estimated population of neurons		
Lamina	Normal	Degenerate	Difference (%)	Normal	Degenerate	Difference (%)	Normal	Degenerate	Difference (%)
1	4·273	2·162	−49·4	27,975	43,000	+53·7	119,537	92,966	−22·16
4+6	13·85	9·706	−29·9	65,072	79,546	+22·2	901,247	772,073	−14·40
2	2·449	2·163	−11·7	26,664	28,944	+8·6	65,300	62,606	−4·13
3+5	10·11	6·288	−37·8	55,097	73,793	+33·9	557,031	464,010	−16·70

If the populations of neurons for normal laminae in the nuclei of opposite sides are added, then cell populations are obtained of approximately 185,000 for the two large-celled laminae, and 1,460,000 for the four small-celled laminae. These are of the same order as the figures of 209,000 and 1,590,000 obtained by Le Gros Clark (1941). The increase in neuronal packing density in an antero-posterior direction, then observed, has been confirmed for all laminae.

In the course of the counts for cell density (53,770 cells counted in 3193 fields) a record has been kept of the number of neurons encountered which have two nucleoli of completely typical appearance, since in the previous study an apparent increase in the proportion of such neurons was noted at 4 months after eye enucleation. The results of these observations are given in Table 4. There again appears to have been an increase in the proportion of these neurons. This might, however, be due to a decrease in the proportion of nuclei fragmented in the course of sectioning in the degenerate laminae, rather than to any multiplication of nucleoli, or improved chance of survival of neurons possessing two nucleoli.

The interaction of retrograde with transneuronal degeneration

Fig. 1 shows diagrammatically the distribution of the various types of degeneration in the lateral geniculate nuclei of the four monkeys in which a lesion of the ipsilateral visual cortex was made at the time of the eye enucleation. In four other

Table 4. *Percentages of neurons found to have two nucleoli in normal and degenerate lateral geniculate laminae, 1 year after unilateral eye enucleation*

Lamina	Normal	Number in sample	Degenerate	Number in sample	Ratio, normal : degenerate
1	0·33	2,098	1·51	1,655	1 : 4·6
4+6	0·56	15,874	1·41	13,597	1 : 2·5
2	0·62	1,147	2·16	1,114	1 : 3·5
3+5	0·35	10,026	1·85	8,249	1 : 5·2

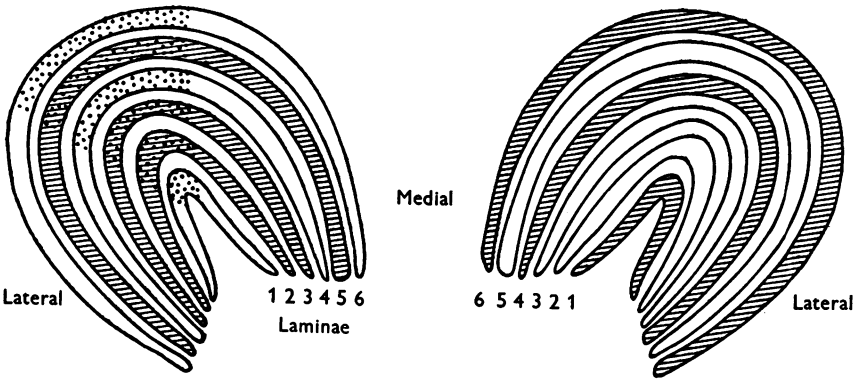

Fig. 1. Diagram to show the distribution and overlapping of transneuronal degeneration (cross-hatched) and retrograde degeneration (stippled), near the posterior end of the lateral geniculate nuclei, after enucleation of one eye and the placing of a lesion in the macular region of the visual cortex of the ipsilateral hemisphere.

monkeys the cortical lesion was contralateral. The periods permitted for survival in these animals are shown in Table 1. In studying the changes in appearance of the degenerate neurons, only the high-power and oil-immersion objectives have been used, since under low magnification the impression given by each lamina of degenerate neurons is governed principally by the degree of gliosis and general shrinkage in the lamina, rather than by the appearance of the neurons themselves. During the first 16 days retrograde degeneration produces no obvious laminar shrinkage or gliosis whereas these are pronounced in the laminae undergoing transneuronal degeneration, in association with the loss of terminal afferent fibres which occurs during this period (cf. Glees & Le Gros Clark, 1941; Glees, 1958; Matthews *et al.* 1960). In consequence, with increase of survival period the regions of retrograde degeneration appear progressively paler, under low magnification, than either the transneuronal or the 'retrograde with transneuronal' degeneration, although the

retrogradely degenerating neurons themselves do not differ greatly in appearance from the other degenerating neurons. Under high magnification, gliosis and laminar shrinkage may be ignored and the neurons alone compared.

The changes produced by transneuronal degeneration in these lateral geniculate neurons have already been described, qualitatively and in some cases quantitatively (Matthews *et al.* 1960). As judged from this same series, retrograde degeneration produces, during the first week, an increasing pallor of the cytoplasm which is very similar to that due to transneuronal degeneration, and is accompanied by a comparable, or slightly lesser, degree of shrinkage. During the second week (after 8 or 10 days) there are greater shrinkage and more intense pallor than in the neurons undergoing transneuronal degeneration. At 16 days (the longest survival period) the neurons affected by retrograde degeneration are much shrunken and have barely any Nissl granules while those showing transneuronal degeneration alone are visibly less shrunken and less pale, with more obvious Nissl granules, than those undergoing retrograde degeneration. In the neurons undergoing retrograde degeneration, no change has been observed in the distribution of Nissl substance, nor any vacuolation with nuclear displacement, and there is no obvious loss of neurons.

When retrograde degeneration is combined with transneuronal degeneration of the same duration, the changes in the neurons are similar in character to those produced by either form of degeneration alone. During the first 16 days there appears to be no loss of neurons. At each interval studied the cytoplasmic pallor and general shrinkage of the neurons which result from the combined degeneration are more severe than those due to transneuronal degeneration alone. When the neurons affected by the combined degeneration are compared with those undergoing retrograde degeneration alone, the result is less clear-cut. At 4 days, the shortest survival period, the combined degeneration seems more severe, the affected neurons appearing paler and smaller than those with retrograde degeneration. This difference is less obvious at 6 days, and at 7½ days and 8 days it is not evident or is beginning to be reversed; at 10 days and 12 days, the neurons undergoing the combined degeneration tend to be slightly less pale than those showing retrograde degeneration alone. By this stage of the second week, however, both these sets of neurons are much more shrunken, and at 14 and 16 days they again appear closely similar, in both pallor and degree of shrinkage. It is interesting that, at 12, 14 and 16 days, a few neurons have been found in the laminae involved by 'retrograde with transneuronal' degeneration, which have an appearance typical of chromatolysis, with a large clear area occupying much of the cytoplasm, the nucleus displaced toward the margin of the cell and the Nissl substance confined to the periphery. This is commoner in the large-celled than in the small-celled laminae, and has only been seen in the uncrossed laminae, which happen to be the ones undergoing the combined degeneration in these three experiments. No typical chromatolysis has been seen in the neurons affected by retrograde degeneration alone. Thus, during the first 2 weeks, concurrent transneuronal degeneration does not appreciably increase the degree of change in appearance of lateral geniculate neurons undergoing retrograde degeneration, and it may even temporarily favour the retention of Nissl substance and also the change in metabolic activity of which chromatolysis is an index.

Quantitative observations on the interaction of degenerative processes

As shown in Fig. 1 the distribution of the three types of degeneration was such that normal neurons for comparison were available in the same lateral geniculate nucleus for some laminae and in the opposite nucleus for other laminae. Since this was expected to be a source of variation, the scope of the cell measurements has been

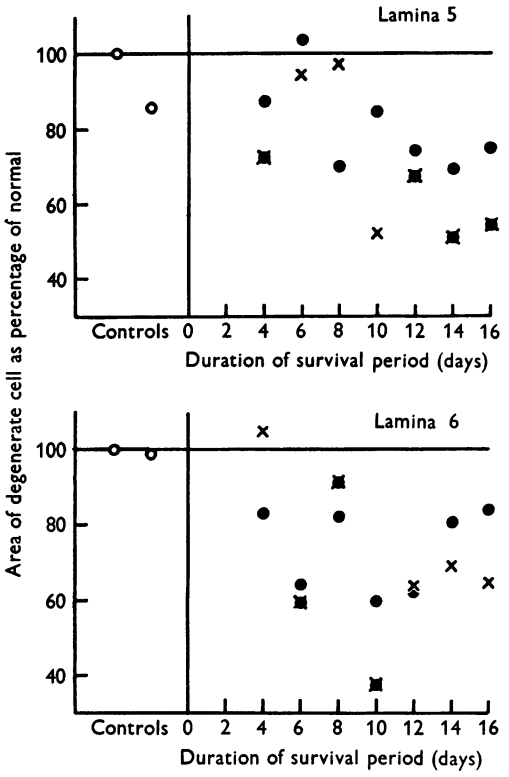

Fig. 2. Graphs to show the changes in mean cell area produced in laminae 5 and 6 by transneuronal degeneration, retrograde degeneration and 'retrograde with transneuronal' degeneration, between the 4th and the 16th post-operative days. One animal has been used for each survival period. Each mean value for the degenerate neurons is plotted as a percentage of the mean normal value for that animal. ●, Transneuronal degeneration; ×, retrograde degeneration; ⧓, 'retrograde with transneuronal' degeneration; ○, control.

restricted. Small samples of 25 or 30 neurons have therefore been measured in the small-celled laminae 5 and 6 alone, and these have been taken, as appropriate, either from the central region of the lamina or from its lateral 'limb'. Since the transneuronal degeneration is somewhat variable the relevant samples have been increased to 50 neurons (100 neurons at 12 days) but sampling has not been extended to the other four laminae. These observations are summarized in the graphs of Fig. 2, which show the percentage changes of mean cell area in the various types of degeneration. In general, these results suggest that the combination of retrograde with

transneuronal degeneration causes greater cell shrinkage than is caused by either degeneration alone, and that after the first 8 days retrograde degeneration tends to produce more shrinkage than does transneuronal degeneration. Except in the case of transneuronal degeneration, however, the samples of neurons were small, and in view of this and the other sources of variation these results should not be given too much weight. They agree fairly well with the qualitative impressions.

DISCUSSION

It was interesting to find that there had been some loss of neurons in the lateral geniculate laminae undergoing transneuronal degeneration, one year after eye enucleation, as no evidence of cell loss due to transneuronal degeneration has previously been obtained in adult experimental animals after a comparable survival period (lateral geniculate of cat and rabbit (Cook, Walker & Barr, 1951); auditory relay nuclei of cat (Powell & Erulkar, 1962)); although cell loss has been seen in young animals (lateral geniculate of newborn macaque (Polyak, 1957); pontine and inferior olivary nuclei of young kitten (Torvik, 1956)) and in adult man (lateral geniculate (Goldby, 1957); sensory nuclei of trigeminal nerve (Penman & Smith, 1950)). The age and species of animal, and the site affected, are probably all important factors in determining whether transneuronal degeneration will lead eventually to loss of neurons, just as they also appear important in deciding whether this transneuronal form of atrophy will occur at all, and in governing its rate and extent (Matthews *et al.* 1960).

The cell loss found in this macaque after 1 year was milder than that observed in man after a much longer period by Goldby (1957), who concluded that only about half the neurons had survived in the affected lateral geniculate laminae, between 36 and 40 years after removal of an eye for injury. He found no difference in the packing density of neurons in normal and in degenerate small-celled laminae, and suggested that, following the shrinkage of the laminae to about half their previous volume, loss of neurons might have occurred as a consequence of crowding, until the original packing density was restored. In the macaque one year after eye enucleation the percentage loss of neurons is slightly less than half the percentage shrinkage of the corresponding laminae, and there is a greater density of neurons in the degenerate than in the normal laminae, so that if the process suggested by Goldby is occurring it is as yet incomplete and must, as he thought, be a slow one. The percentage shrinkage of surviving neurons in the degenerate laminae in this macaque was greater than the shrinkage which Goldby found in the human case (mean change in cell area -32% in large-celled, -48% in small-celled laminae) and this may reflect a species difference. There was the same tendency toward lesser cell shrinkage in the large-celled laminae, with better retention of Nissl substance in some of these large neurons. Data on normal dimensions, cell density and cell content of the lateral geniculate nucleus in the macaque, with which the present observations on normal laminae may be compared, have been given by Le Gros Clark (1941) and by Chow, Blum & Blum (1950); those of Chow (1955) are also revelant.

The observations on the interaction of retrograde with transneuronal degeneration suggest that the effect on the neuron of combining the two processes is not much greater than the effect of either alone. There is no evidence, for example, of any

widespread death of neurons. The combined degeneration produces cytoplasmic pallor and general shrinkage of the neuron, as do the other two types of degeneration, but its effects do not seem to be those of a simple addition of transneuronal to retrograde degeneration. The neuronal changes are more severe than those of transneuronal degeneration, but rather closely resemble those produced by retrograde degeneration. The limited quantitative observations add little to the conclusions formed from the qualitative assessment, but they provide some guide to degrees of cell shrinkage, which cannot be estimated well from inspection alone. It would be interesting to apply other forms of analysis, such as electron microscopy and histochemical methods, to the interaction of these types of degeneration, in order to investigate further the nature and the modifications of the processes involved.

SUMMARY

1. Qualitative and quantitative studies have been made, in the lateral geniculate nucleus of the macaque monkey, of transneuronal cell degeneration 1 year after unilateral eye enucleation, and of the interaction of transneuronal with retrograde cell degeneration during the first 16 days after the simultaneous enucleation of one eye and production of a lesion in the visual cortex of one hemisphere.

2. The shrinkage of neurons produced by transneuronal degeneration after 1 year is more profound than that seen during the first 4 months, and there is evidence of loss of $\frac{1}{5}$ to $\frac{1}{7}$ of the neurons in most of the degenerate laminae.

3. When retrograde degeneration is combined with transneuronal degeneration the resulting changes in the neuron are little more severe than those caused by retrograde degeneration alone.

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