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Control of Cell Number in the Developing Visual System. I. Effects of Monocular Enucleation*

BARBARA L. FINLAY, DALE R. SENGELAUB and CLAIRE A. BERIAN

Department of Psychology, Cornell University, Ithaca, NY 14853 (U.S.A.)

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Monocular enucleation of hamsters on the day of birth caused an increase in cellular degeneration and a corresponding loss of cells in the dorsal lateral geniculate nucleus contralateral to the enucleation over the first 12 postnatal days. The superficial layers of the contralateral superior colliculus showed a similar increase in cell degeneration, except rostrally where the remaining ipsilateral projection is found. No changes in degeneration were found in either the ipsi- or contralateral ventral lateral geniculate nuclei, the intermediate and deep layers of the superior colliculus, or in the dorsal lateral geniculate and superficial superior colliculus ipsilateral to the enucleation, even though all were denervated to some degree. The disparities in the incidence of degenerating cells normally seen in the central and peripheral regions of the superior colliculus and dorsal lateral geniculate were preserved following the monocular enucleation. The incidence of degenerating cells in early development correlates well with known alterations in adult cell number. Only major denervations of retinal targets appear to be adequate to produce measurable changes in early cellular degeneration.

INTRODUCTION

Cell numbers in interconnecting neuronal populations are thought to be regulated in part by a normally occuring cell death during development in vertebrates whose nervous systems do not continuously grow or regenerate[2,32]. Studies of cell number following ablation or alteration of muscles or peripheral sense organs have established that the availability of both synaptic targets and synaptic input can be important for a neuron's survival. Target availability is essential for cell survival; for example, following removal of motoneuron targets, cell death in the appropriate motoneuron pools is increased markedly[3,4,18,23,27,35] while target addition decreases cell death[15,22,31]. Similarly, decreases in competition for targets by removal of a portion of the competing cells increases cell survival in the remaining population[34,37].

The role of afference in the regulation of cell survival appears less consistent over neuronal systems and is less well understood. For example, although several early studies showed no effect on motoneuron survival after deletion or addition of afference[16,19,29,42] a recent study[33] showed that deletion of either the neural crest or the descending inputs to the motoneurons, or both, would reduce motoneuron survival. The effects of these deletions were roughly additive, and in the cases where one source of afference was deleted, the motoneuron loss occurred after the period of normally occurring death. Other examples of such shifts in the time course of cell loss following deafferentation have been noted[5]. More consistent effects on cell survival following deletion of afference have been reported in studies of developing sensory systems. For example, ablation of the otocyst in chick increases cell death in the cochlear and ves-

* A preliminary report of these investigations has been published (ref. 40).

Correspondence: B.L. Finlay, Department of Psychology, Cornell University, Ithaca, NY 14853, U.S.A.

tibular nuclei[28] and removal of the retina increases cell death in the mouse lateral geniculate nucleus[21], superior colliculus[7] and the chick optic tectum[25]. However, all of the cell losses reported following deletions of afference are smaller (typically 10–50%) than those seen after deletions of targets, where cell loss can reach 100%.

In all of the studies cited, the objective has been to examine alterations in cell survival following a simple change in the amount of target or afference, which is most easily accomplished in the sensory or motor periphery. However, some of the features which make such systems excellent models for quantitative study of early cell loss also limit their applicability to other cases of central nervous system development. Using the visual system as an example, the multiplicity and bilaterality of retinal terminal sites and the number of other potential central targets suggest that the possibility of error is larger in retinal axon projection than in neuromuscular connectivity. As a result, an error correcting function for cell death might be more prominent for retinal ganglion cells than for spinal motoneurons. Similarly, mapping large numbers of retinal axons appropriately onto postsynaptic arrays might present a different set of problems than that which must be solved by smaller groups of motoneurons. Furthermore, the factors that control neuron survival may be specified differently for neuromuscular systems than for central neurons. While the muscle is only a recipient of innervation, central neurons both receive as well as establish such connections, and unlike motoneurons, axons of central neurons often branch and contact numerous targets. For all of these reasons it is necessary to investigate cell death in central nervous system development directly, even though the possibility of limiting an experimental manipulation to a simple deletion of afference or efference, or to absolutely quantify neuron numbers, may be lost. Several germinal papers have indicated that such an approach is feasible[2,7].

In each of the following 3 papers we examine the effect of deleting a single element in the hamster visual system on cell survival in other directly or indirectly connected structures. Because of the highly interrelated and reciprocal connectivity in the visual system, no single manipulation can conclusively distinguish the relative contributions of efference and afference to cell survival. However, over a number of such experiments, hypotheses to account for all experimental results become progressively constrained.

In the first study, we have replicated the experimental procedure used by numerous investigators, early monocular enucleation[7,21,23] to determine if measures of cell degeneration in thalamus and midbrain early in development relate consistently to the demonstrated changes in neuron number. We know that early monocular enucleation reduces cell degeneration in the remaining retina[37], resulting in more neurons surviving to adulthood[41]. We are interested in determining the magnitude, time course and spatial pattern of neuron loss both normally and after various manipulations. If measures of cell degeneration are reliable indicators of differences in cell loss, such measures could be particularly useful for central nervous system structures, because large areas may be sampled without the necessity of determining absolute changes in total neuron number. Furthermore, measures of cell degeneration could be used to determine differences in cell loss within identified areas of single structures.

MATERIALS AND METHODS

All of the hamsters (*Mesocricetus auratus*) in these studies were bred in our laboratory and only pups delivered after the standard 15.5–16 day gestation period were used in order to minimize variation in postconceptional age.

Neonatal surgery

Within 24 h of birth (day 1), the right eye of each pup was removed by the following method. Pups were anesthetized by induction of mild hypothermia and with the aid of a dissecting microscope a small incision was made under the line of the prospective eyelid and the eye, including all pigmented fragments, was withdrawn with fine forceps. The animal was then warmed and returned to its mother.

Neonatal histology

On each postnatal day, the required number of pups were removed from their mothers, overdosed with an intraperitoneal injection of urethane and perfused through the heart with saline followed by 4% formalin–45% alcohol. The brains were removed, dehydrated in alcohols, embedded in polyester wax,

cut coronally at 10 μm, and stained with cresylecht violet.

Counting procedures

For the lateral geniculate nuclei, 16 hamster pups from 5 litters were used, two each from postnatal days 2–8, 10 and 12. For each brain, 5 sections equally spaced through the rostrocaudal extent of the lateral geniculate nuclei ipsilateral and contralateral to the eye removed were analyzed at 500×. The dorsal and ventral lateral geniculate nuclei are distinguishable from the rest of the thalamus and from each other at postnatal day 1. The location and number of all degenerating cells in the dorsal and ventral lateral geniculate nuclei in each of the sections counted was noted. Normal cell counts of the same sections were obtained in order to compute the incidence of degenerating cells relative to normal cells. This counting procedure provides a good sample of normal and degenerating cells, with 0–24 degenerating cells and 2008–3193 normal cells counted for the dorsal lateral geniculate nucleus and 4–38 degenerating cells and 1220–3810 normal cells for the ventral lateral geniculate nucleus. Counts of both normal and degenerating cells were corrected for frequency of encounter by cell size by the method of Abercrombie[1].

For the superior colliculus, 16 hamster pups from 4 litters were used, two each for postnatal days 2–8, 10 and 12. For each brain, 3 sections, one each from the rostral, middle and caudal third of the superior colliculi, ipsilateral and contralateral to the removed eye were analyzed at 500×. The location and number of all degenerating cells in the superficial and deep laminae of the superior colliculus in each of the sections counted was noted. All normal cells in the superficial and deep laminae of the same sections were counted in order to compute the incidence of degenerating cells relative to normal cells. Counts of both normal and degenerating cells taken by this procedure ranged from 18 to 133 for degenerating cells and from 1084 to 1675 for normal cells counted per animal per side. Counts of both normal and degenerating cells were corrected as described above.

Degenerating cells were recognized by their darkly staining, shrunken and sometimes fragmented nuclei and their pale or absent cytoplasm[6,8,38]. Normal cells were counted if they contained a bounded nucleus and at least one nucleolus. It should be noted that we have deliberately not attempted to make a distinction between neurons and glia in our counts of total cells. In early development the absence of significant amounts of Nissl substance and changing nuclear morphology makes unambiguous identification of cell type quite problematic. Our interpretation of our data relies on other studies of early monocular enucleation in rodents which have traced the fate of these populations to adulthood, where cells can be more unambiguously identified[7,21].

The incidence of degenerating cells as a measure of neuronal loss

Previous studies have demonstrated that early eye removal results in a lower neuron number in various central visual structures. In this study, we evaluate the relationship of changes in early cell degeneration to these changes in cell numbers. We have expressed the incidence of degenerating cells as a ratio of degenerating cells to normal (non-degenerating) cells. This measure has been shown to be a sensitive predictor of normal and induced changes in eventual neuron numbers in the retina[24,37,41] and normal changes in cell number in the neocortex[9,20].

Alternative measures of cell loss include: (1) complete counts of all cells; (2) tracing the fate of an identified group, for example by using tritiated thymidine or long-term retrogradely transported tracers; or (3) counting absolute numbers of degenerating cells, rather than using ratios. All these measures have distinct uses, but each presents problems when applied to the analysis of cell loss in large central nervous system structures. In the case of complete cell counts, subtractive effects due to cell death can be completely obscured by the additive effects of continued cell generation and/or migration because these subtractive and additive processes often overlap in time. Progressive alteration in the criteria for assignment of borders over development, as neurons and fiber tracts differentiate, can completely compromise assessments of neuron number. In the case of areas with ambiguous borders, or where cell number is intrinsically variable, for example, in the remaining colliculus after a partial collicular lesion, cell counts are not informative. Autoradiographic labeling with thymidine requires a unique generation period for the cells of interest and quantification requires that special conditions apply for comparisons across ani-

mals. Long-term retrograde labels are relevant only for those cases where an isolable tract can be filled.

Counts of the total number of degenerating cells give results quite similar to degenerating/normal cell ratios in structures with defined borders (see Fig. 2 for comparison), but are subject to all of the problems of ambiguous borders and variable populations described for complete cell counts. Expressing cellular degeneration as a ratio of degenerating cells to normal cells is robust with respect to small alterations in the placement of borders; unambiguous areas can be sampled and variable lesions present no difficulty. Appropriate caution must be taken in interpretation of these ratios; both the numerator and the denominator of the ratio measure can change over development, either through changes in the number of degenerating cells or by addition and subtraction of normal cells. The proper use of this measure is comparative, to tell the direction of differences in cell degeneration in matched populations, get some indication of magnitude, and tell when in development alterations affecting degenerative processes occur.

While the number of degenerating cells observable at any one time is usually small, because of the rapid clearance of degenerating debris in developing systems[11,26] these small numbers can correspond to substantial cell losses. It should be emphasized that in order to convert the number of degenerating cells observed into an actual amount of cellular loss it would first be necessary to estimate the clearance time of degenerating debris, which requires the problematic assumption that it is constant over development or experimental condition.

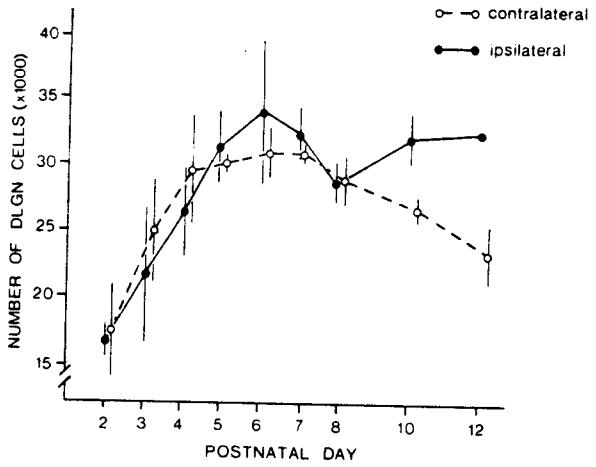

Fig. 1. Changes in the number of cells in the dorsal lateral geniculate nucleus (DLGN) over the first 12 postnatal days after monocular enucleation on the day of birth. Cell number is significantly lower contralateral to enucleation compared to cell number ipsilaterally. Points represent averaged values ± S.E.M.

RESULTS

Effects of monocular enucleation on the lateral geniculate nuclei

The dorsal and ventral lateral geniculate nuclei normally receive a dense innervation from the contralateral retina and a small amount of innervation from the ipsilateral retina. For the dorsal lateral geniculate, the retina is the principal source of innervation; for the ventral lateral geniculate, the retina is only one of several sources of input, including also the superior colliculi, the pretectum and the neocortex[14]. Thus, removal of one eye results in a major denervation of the contralateral dorsal lateral geniculate, a partial denervation of the contralateral ventral lateral geniculate and a still smaller denervation of both the ipsilateral dorsal and ventral lateral geniculate nuclei. Cell loss in these structures after enucleation can thus be related to the amount of denervation.

Following early monocular enucleation, cell number in the dorsal lateral geniculate ipsilateral to the enucleation increases through postnatal day 6, declines briefly, and then increases again through postnatal day 12. Cell counts in the contralateral geniculate begin to diverge from ipsilateral cell numbers at postnatal day 5. Over postnatal days 6–12, cell number in the dorsal lateral geniculate contralateral to the eye removed continues to decline and is significantly lower relative to the ipsilateral lateral geniculate ($t = 2.73$, $P < 0.05$; Fig. 1). This pattern of changes in cell number is quite similar to that reported in the mouse lateral geniculate nuclei by Heumann and Rabinowitz[21], where monocular enucleation first caused a neuronal loss contralaterally, followed by a secondary failure in glial proliferation.

As can be seen in Fig. 2, the incidence of degenerating cells was substantially higher in the dorsal lateral geniculate contralateral to the eye removed relative to the ipsilateral lateral geniculate ($F_{1,16} = 16.67$, $P < 0.01$). There was no contra-ipsilateral difference observed in degeneration for the ventral lateral ge-

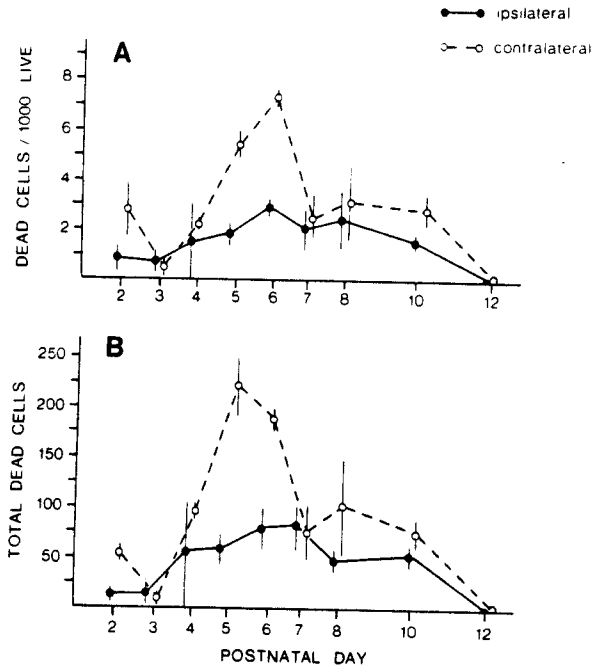

Fig. 2. Comparison of measures of cell degeneration in the dorsal lateral geniculate nucleus over the first 12 postnatal days after monocular enucleation on the day of birth, contralateral and ipsilateral to enucleation. A: incidence of degenerating cells as a ratio to the number of normal cells in the nucleus. B: estimate of total number of degenerating cells in the nucleus at each time point. Points represent averaged values ± S.E.M.

niculates ($F_{1,16} = 0.13$, $P > 0.25$; Fig. 3). The time course of cell degeneration for both the dorsal and ventral lateral geniculates was similar to normal[39], extending from postnatal days 2–10, peaking on days 5–6 with evidence of a secondary peak on day 8. In both the contralateral and ipsilateral lateral geniculates cell loss as indicated by degeneration is close to zero by postnatal day 12.

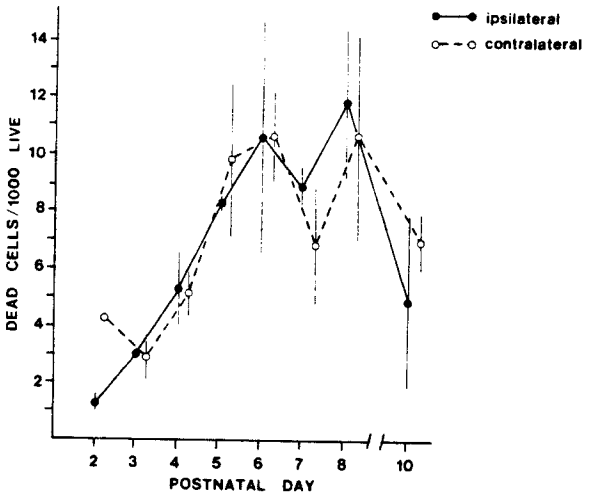

Fig. 3. Incidence of degenerating cells in the ventral lateral geniculate nucleus over the first 10 postnatal days after monocular enucleation on the day of birth. Cell degeneration in the nucleus contralateral does not differ from that ipsilateral to the enucleation. Points represent averaged values ± S.E.M.

Center/periphery degeneration differences in the lateral geniculate

Spatial inhomogeneities in normally occurring cell death have been reported in several structures including the retina, superficial layers of the superior colliculus and the dorsal lateral geniculate[8,38,39]. In each of these cases, the amount of degeneration in the periphery is greater than that observed in the center of these structures. If it were the case that this pattern was imposed by the retina on its two principal target structures, then removal of the retina should cause the center/periphery disparity in degeneration to disappear.

To compare the incidence of degenerating cells in central vs peripheral regions, each of the 5 sections analyzed for each animal were divided into sectors representing the central 90° of gaze and the peripheral annulus of the same angular extent according to the retinotopic map of the hamster lateral geniculate nucleus[12,39] and the incidence of degenerating cells determined for each region.

As seen in Fig. 4, the incidence of degenerating cells in the dorsal lateral geniculates was significantly higher in the periphery than in the center ($F_{1,32} = 7.86$, $P < 0.01$), as in the normal geniculate. The cen-

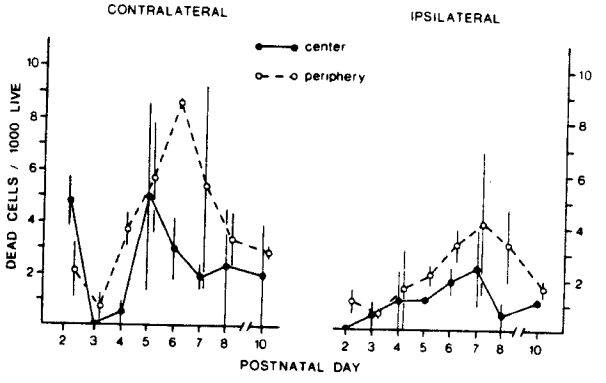

Fig. 4. Incidence of degenerating cells in the dorsal lateral geniculate nucleus contralateral and ipsilateral to the eye removed over the first 10 postnatal days. Cell degeneration is greater in the periphery than centrally. Points represent averaged values ± S.E.M.

ter/periphery difference was found in both the dorsal lateral geniculate contralateral ($F_{1,16} = 15.77$, $P < 0.01$) and ipsilateral to the eye removal ($F_{1,16} = 3.94$, $P < 0.05$), indicating that the center/periphery disparity in cell degeneration is independent of retinal innervation. As previously reported for normal animals[39], no center/periphery differences were observed in the ventral lateral geniculate following enucleation.

Effects of monocular enucleation on the superior colliculi

The superior colliculus normally receives a dense innervation from the contralateral retina, and a small amount of innervation from the ipsilateral retina. Following early monocular enucleation, the incidence of degenerating cells in the superficial layers of the superior colliculus (stratum griseum superficiale and stratum opticum) contralateral to the removed eye was near double that observed ipsilaterally. This difference was highly statistically significant ($F_{1,32} = 27.39$, $P < 0.0001$). The elevation in the incidence of degenerating cells is apparent at the onset of normally occurring cell death (Fig. 5A), but the peak in degenerating cell incidence is delayed from day 5 to day 7. The period of cell death shows evidence of a protraction past day 10, when cell death has essentially ceased in the normal colliculus[8].

In contrast, the incidence of degenerating cells in the deep collicular layers ipsilateral and contralateral to the eye removed are identical ($F_{1,16} = 0.075$, not significant; Fig. 5B), even in the late postnatal days when a transsynaptically induced degeneration caused by cell loss in the superficial gray might be expected. It is still possible that cell death was increased in later postnatal days that we did not examine.

Cell degeneration differences in ipsilaterally innervated collicular areas

The superficial layers of the superior colliculus can be divided into areas which receive only contralateral retinal innervation vs those which receive innervation from both eyes. (The lateral geniculate nucleus in rodents is too small for reliable quantification of cell degeneration ratios in the binocular area with these techniques.) The ipsilateral retinal projection to the superior colliculus in normal animals is a small and patchy projection confined to the deepest aspect

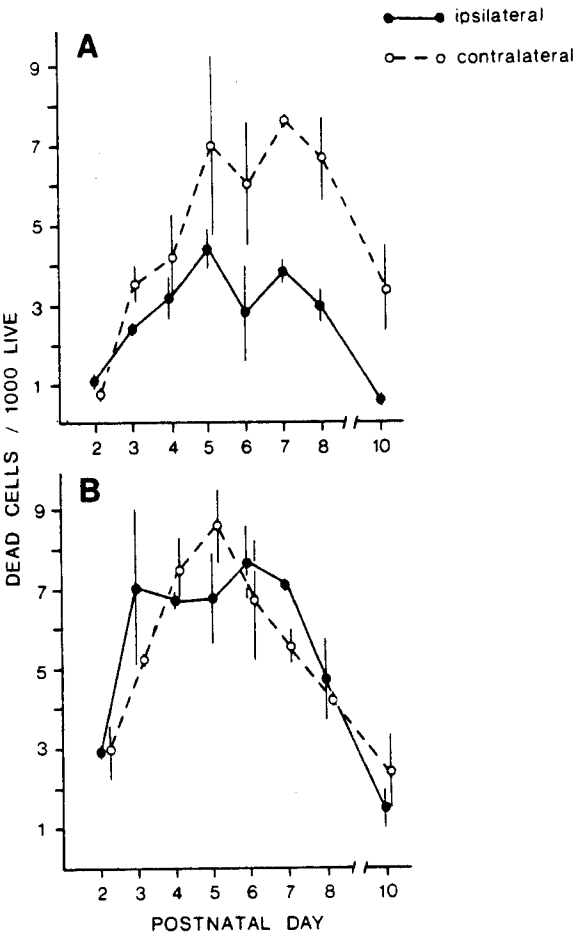

Fig. 5. Incidence of degenerating cells in the superficial layers (A) and deep layers (B) of the superior colliculus over the first 10 postnatal days following monocular enucleation on the day of birth. Cell degeneration is greater contralateral than ipsilateral to the eye removed in only the superficial colliculus. Points represent averaged values ± S.E.M.

of the superficial gray layer in the rostral colliculus[13].

After early enucleation of one eye, the ipsilateral retinal projection from the remaining eye becomes more dense, loses its patchiness, and extends to the collicular surface, but is still substantially smaller than the normal contralateral projection[10,30]. After monocular enucleation, only the caudal superior colliculus contralateral to the eye removed is completely deprived of its retinal input; the rostral part receives the expanded ipsilateral projection from the remaining eye described above. The rostral superior colliculus ipsilateral to the removed eye has lost its small ipsilateral retinal projection, but the retinal innervation of the caudal colliculus remains essentially normal.

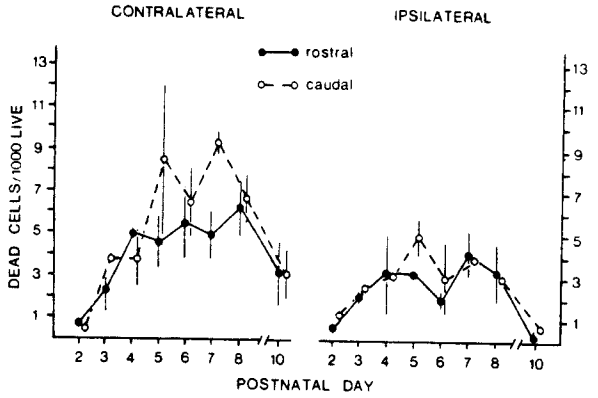

Fig. 6. Incidence of degenerating cells in the superior colliculus contralateral and ipsilateral to the eye removed over the first 10 postnatal days. Cell degeneration is greater in the caudal, monocular region than in the rostral, binocular region. Points represents averaged values ± S.E.M.

The pattern of cell degeneration in these subdivisions is shown in Fig. 6 and corresponds with the degree of denervation. There is no difference in the incidence of degenerating cells in the rostral vs caudal areas of the superior colliculus ipsilateral to the removed eye ($F_{1,16} = 1.08$, not significant). Cell degeneration in the contralateral, partially denervated rostral superior colliculus is significantly greater than in the ipsilateral rostral colliculus ($F_{1,16} = 10.32$, $P < 0.01$), and lower than the completely denervated contralateral caudal colliculus ($F_{1,16} = 2.91$, $0.10 > P > 0.05$).

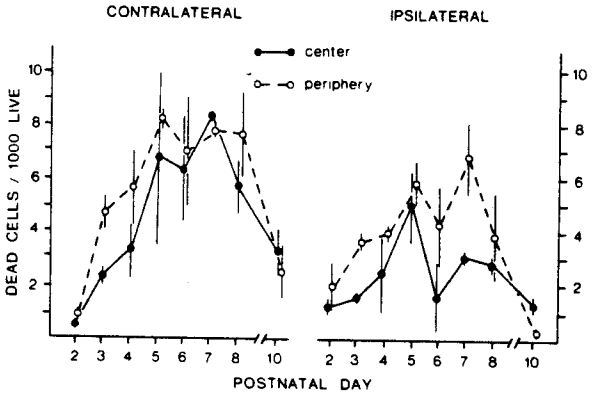

Fig. 7. Incidence of degenerating cells in the superior colliculus contralateral and ipsilateral to the eye removed over the first 10 postnatal days. Cell degeneration is greater in the periphery (broken line) than centrally (solid line). Points represent averaged values ± S.E.M.

Center/periphery degeneration differences in the superior colliculus

As described above, cell degeneration in the periphery of the superficial layer of the superior colliculus is normally elevated relative to the center[8]. We were interested to determine if this difference reflects some process intrinsic to the development of the superior colliculus or some interaction between it and the retina. Each section of colliculus examined was divided into its central and peripheral components according to the retinotopic map of the hamster superior colliculus[8,12] and the incidence of degenerating cells was determined for each region. As was found for the dorsal lateral geniculate, following monocular enucleation the normal difference between the incidence of degenerating cells peripherally and centrally was preserved ($F_{1,32} = 7.75$, $P < 0.01$; Fig. 7), indicating that the center/periphery difference in cell degeneration was independent of retinal innervation. The colliculus contralateral to the eye removed showed an elevation in the incidence of degenerating cells in the periphery as early as days 3–5. However, by days 6–10 the incidence of degenerating cells in central portions of the contralateral superior colliculus was not different from that found in the periphery.

DISCUSSION

Information contributed by analysis of degenerating cells

Alterations in the incidence of degenerating cells conform in detail to what is known about neuron loss in central visual system structures consequent to monocular enucleation. In the mouse lateral geniculate nucleus, neuron loss induced by monocular enucleation is small (ca. 10%) in the early postnatal period and there is a secondary failure of glial proliferation in later postnatal days[21]. Our counts of total cell number in the hamster geniculate replicate these findings, with evidence of a small cell loss apparent by postnatal day 5 and a subsequent failure of cell proliferation. Following enucleation, decreases in total cell number (Fig. 1) coincide with increases in the measures of cell degeneration; both the estimated total number of degenerating cells and the ratio of degenerating cells to normal cells are elevated in the geniculate contralateral to the eye removed (Fig. 2).

After early enucleation, there is a small change in neuron number but a large difference in the incidence of degenerating cells in the dorsal lateral geniculate contralateral to the eye removal. This result demonstrates the greater sensitivity for detecting cell loss achieved by direct assessments of cell degeneration rather than by counts of total cell number. For example, if a given population of cells has a normal cell loss of 10% and an induced loss of 20%, there is an eventual difference of only 10% in final cell numbers, while the incidence of cell degeneration is 100% higher in the induced condition.

To compare the incidence of degenerating cells across experimental conditions with confidence, induced variations in debris clearance time or rate of degeneration (which could artifactually alter counts of degenerating cells) must be ruled out. In this study, the levels of degeneration in structures ipsilateral to the eye removal were not different from normal levels, indicating that no such variations were induced by the enucleation.

DeLong and Sidman[7], in an autoradiographic analysis of changes in cell survival and proliferation in the superior colliculus of the mouse after monocular enucleation, found effects similar to those shown for the lateral geniculate. Following enucleation there was an increase in neuronal loss followed by a secondary failure of glial proliferation and the pattern of degeneration we observed is consistent with their observed neuronal loss. Neither the deep layers of the colliculus nor the ventral lateral geniculate nucleus show an alteration in cell degeneration, consistent with the degree of denervation produced by enucleation.

In summary, alterations in the incidence of degenerating cells after monocular enucleation conform to what is known about induced changes in eventual neuron numbers in visual system structures. The time course of the cell degeneration is consistent with previous studies, though these studies have looked at a more limited time sample. The fact that counts of degenerating cells predict final neuron numbers reliably in this and prior studies[9,17,23,37] has encouraged us to use the information from cell degeneration to analyze the time course and spatial pattern of cell degeneration in this study and in the two which follow.

Time course of normal and induced cell degeneration

The onset of the greater-than-normal cell degeneration seen after enucleation is coincident with the normal onset of degeneration in both the lateral geniculate and the superficial layers of the superior colliculus. There is evidence in the superior colliculus for protraction of the cell degeneration period past the time when cell degeneration has normally ceased, consistent with observations in the spinal cord[33]. It is of note that the multiple peaks in normal cell loss seem amplified after enucleation in both the lateral geniculate and the superior colliculus.

Spatial patterns in induced cell degeneration

In most of the structures we have examined for the incidence of degenerating cells, there have been spatial inhomogeneities such that more degenerating cells can be seen peripherally than centrally (retina, lateral geniculate, superficial colliculus) or centrally than peripherally (deep superior colliculus). In the retina, this differential degeneration is involved with the production of the increased central density of cells in the hamster retina[36]. We were thus interested to determine if the pattern of increased cell degeneration in the periphery of the geniculate and colliculus is induced by the similar pattern of cell degeneration in the retina. If so, the center/periphery disparity in the incidence of cell degeneration should disappear after enucleation but in both cases, the center/periphery disparity remains, amplified appropriately to the new higher level of induced cell loss. This disparity in degeneration might thus be dependent on some process endogenous to each structure, such as a privileged position with respect to acquisition of within-structure connectivity by central neurons, or some differential ability of central neurons to acquire target space. A possibility of interest is that these disparities in cell degeneration reflect the mechanism by which different visual field subareas are magnified or reduced in their representation in various central nervous system structures[36].

Afference and the control of cell number

The enucleation-induced cell losses of 10% in the lateral geniculate and 30–40% in the superior colliculus are small when compared to losses produced by target deletions of comparable magnitude, which can result in a total cell loss. In no case was the removal of afference complete — both the dorsal lateral geniculate and superior colliculus receive other innerva-

tion, including the projection from the remaining eye, the visual cortex and ascending monoaminergic input. However, as the retina is the primary source of input in both of these cases, the denervations produced by the enucleation must be considerable.

Furthermore, a minor denervation did not produce a measurable effect on cell degeneration in the rostral part of the ipsilateral superior colliculus, the ipsilateral dorsal lateral geniculate, the ventral lateral geniculate nuclei, or the deep layers of the superior colliculus. These neuronal populations thus appear relatively insensitive to small changes in afference. This relative insensitivity of central neurons to small denervations might be explained by the observation of early diffuse connectivity and potential hyperinnervation due to excess neurons. If afference is always present in excess during early development, then small deletions should have little effect on cell number. Only denervations in excess of the normal amount of cell loss in afferent populations might thus be expected to have an effect on cell survival.

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