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Cell Death in the Mammalian Visual System During Normal Development: II. Superior Colliculus

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ABSTRACT

Degenerating cells may be observed with light microscopy in the hamster superior colliculus during early postnatal development. In the superficial gray layer and stratum opticum, 1.8 degenerating cells for each 1,000 live cells could be seen on the first postnatal day. This rate increased to 5.6 degenerating cells per 1,000 live cells by postnatal day 5, and declined to 2.6 per 1,000 live cells by postnatal day 8. The rate of cell degeneration was consistently elevated at the medial, lateral, and caudal margins of the superficial gray layer relative to the center. In the intermediate and deep gray layers, the rate of cell death was consistently higher, starting at three degenerating cells per 1,000 on the first postnatal day, increasing to 15 per 1,000 on postnatal day 5, and declining to 4.7 per 1,000 by postnatal day 8. In contrast to the superficial gray layer, the number of degenerating cells in the central versus peripheral segments of the intermediate and deep gray layers was quite similar.

Although the rate of observable degeneration is low, the likely rapid clearance of degenerating cell debris indicates a substantial loss of cells from the midbrain tectum in early development. The time course of observable degeneration, the amount, and the distribution of degenerating cells are quite similar in the tectum, and its major innervating structure, the retina.

The two principal hypotheses advanced to account for the function of the overproduction and subsequent death of neurons in the nervous system of vertebrates are regulation of cell number in interconnecting populations of neurons (Cowan, '73) and correction of errors of connectivity (Clarke and Cowan, '76). These hypotheses address themselves to the total number of neurons and their relative locations. The temporal pattern of neuron generation and its relationship to neuron death has not been as widely considered. It is possible that neuron death in some populations may either be a functional or epiphenomenal consequence of asynchronies in generation of interconnecting populations. Temporal gradients of neuron generation within and between structures have been described in detail (Altman and Bayer, '78a,b, '79; Angevine and Sidman '62). Moreover, a critical period for neuron maintenance early in development has been shown for several populations by deletion of their normal target field (Delong and Sidman '62; Ostrach and Mathers, '79).

The retina and optic tectum present an interesting test case for the role of temporal factors in the control of cell death. The tectum, in all rodents studied, has a fairly synchronous generation of cells according to retinotopic location: no striking rostral to caudal or medial to lateral patterns of cell generation have been described (Bruckner et al., '76; Mustari et al., '79). In retina, by contrast, there is a general central-to-peripheral pattern of neurogenesis and differentiation (Sidman, '61; Morest, '70). The consequences of this asynchronous pattern of cell generation for the maintenance of retinal and tectal cells has not yet been examined.

Several observations of cell degeneration during development of the midbrain tectum have been made. In the chick, Cantino and Sisto-Daneo ('71) have observed a wave of cell degeneration beginning at the tenth day of incu-

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bation, which is coincident with the major phase of axon ingrowth from the retina. Cell death has also been observed in the optic tectum of the rat postnatally (Arees and Astrom, '77; Giordano and Cunningham, '78). In tree shrew, reductions in tectal size and cell density over development have led to the inference of cell death (Zilles, '78).

In addition, partial denervation of the tectum by removal of its retinal input early in development has been shown to increase cell loss (Delong and Sidman, '62; Kelly and Cowan, '72; Ostrach and Mathers, '79). In rat, an increase of neuron numbers in tectum by early removal of visual cortex has been reported, interpreted as a reduction of normally occurring cell death in tectum by maintenance of tectal cells by a supernormal retinal input (Cunningham et al., '79).

These observations show that cell death during development of the optic tectum is characteristic of both nonmammals and mammals and that it plays a role in at least degenerative changes in development. Cell death may thus be involved in both the normal organization and plastic reorganizations of the visual system during development. However, no systematic study of the dimensions and distributions of normally occurring cell death in the mammalian midbrain has as yet been reported. In this study, we report on the amount, variability, and particularly the spatial and temporal distribution of degenerating cells in the dorsal midbrain of the hamster during normal development, with particular regard to its relationship to retinal development. A preliminary report of these investigations has been published (Berg and Finlay, '79).

METHODS

Twenty-five hamster pups *(Mesocricetus auratus)* of both sexes from nine litters were used for this study. Only pups delivered in the standard 15.5–16-day gestational period were used to minimize variability in postconceptional age. Assessments of rate and distribution of cell degeneration were made for postnatal days 1, 2, 3, 4, 5, 6, 7, 8, and 10 (where 1 is the day of birth) for three hamsters at each postnatal day 1 to 8, and one at day 10.

Histology and counting procedures

Hamster pups were taken from their mothers at the desired postnatal day, overdosed with urethane, and perfused through the heart with 4% formalin/45% alcohol. Brains were dissected out, dehydrated with alcohols, embedded in paraffin, cut in 10-μm coronal sections, and stained with cresylecht violet. From each brain, three sections were taken for analysis, one each from the rostral, middle, and caudal third of the superior colliculus. At all postnatal ages, the superior colliculus can be clearly delimited from surrounding structures. Discrimination of the boundaries of collicular laminae is more difficult at the earliest postnatal days because of incomplete development of the white laminae, although a gross division into superficial and deep colliculus is possible.

A drawing and complete count of all degenerating cells visible in each coronal section was made at 500 ×, including all collicular laminae and the dorsal aspect of central gray, as in Figure 1. Degenerating cells were recognized by their liquefied, darkly staining, shrunken, and sometimes fragmented nuclei, and their pale or absent cytoplasm (Fig. 2; Sengelaub and Finlay, '82; Chu-Wang and Oppenheim, '78). The relative density of degenerating cells to normal cells was computed from normal cell counts of the same section. A ten by ten grid was superimposed on the microscope field and ten squares in a standard pattern were counted for all cells, including both neurons and glia. A normal cell was counted if it contained a bounded nucleus and at least one nucleolus. Counts of both normal and degenerating cells were corrected for frequency of encounter by size and section thickness by the method of Abercrombie ('46).

RESULTS

Maturation and degeneration

Degenerating cells may be seen in the optic tectum in the first 8 postnatal days; after that, they are encountered only rarely (Figs. 1, 2, and 3). Prenatal days were not examined. The appearance of degenerating cells conforms closely to that described for other species and structures (Glücksmann, '61; Chu-Wang and Oppenheim, '78). Figure 2 shows representative sections of the superficial gray and intermediate gray layers of the superior colliculus in which at least one degenerating cell could be found for postnatal days 2, 5, 8.

At the point of maximum observable degeneration, day 5, surrounding neurons have not yet reached full cytoplasmic differentiation or mature nuclear organization. Some Nissl substance is apparent, though mature Nissl development has not been reached. Although the intermediate and deep gray layers lead the superficial gray in maturation, average day of generation (Mustari et al., '79), initial differentiation of nuclear structure (day 2), reduction of multiple nucleoli (days 5–8), development of cytoplasmic processes (day 5–8), and assumption of mature size, the peak of degeneration is the same for both areas, postnatal day 5.

Up to day 3, radial glia are still in evidence, as well as mitotic figures in the ependymal zone. It is thus possible that some neurons are being added to the superior colliculus in the first 3 postnatal days. In other rodents, it has been demonstrated that substantial glia are added in the first postnatal days (Delong and Sidman, '62; Bruckner et al., '76).

Spatial distribution of degenerating cells

Drawings of the distribution of degenerating cell profiles in coronal sections appear in Figure 1. These formed the basis for our analysis. We considered the distribution of individual degenerating cells within a structure, compared the total amount across structures, and looked for inhomogeneities in degeneration within structures.

On visual inspection, degenerating cells appear somewhat patchy or clumped in their distribution, as in the retina (Sengelaub and Finlay, '82). However, since the retinal nearest-neighbor analysis demonstrated that this type of distribution was indistinguishable from a random

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Abbreviations

CG, central gray
SGI, stratum griseum intermediale
SGP, stratum griseum profundum
SGS, stratum griseum superficiale
SO, stratum opticum.

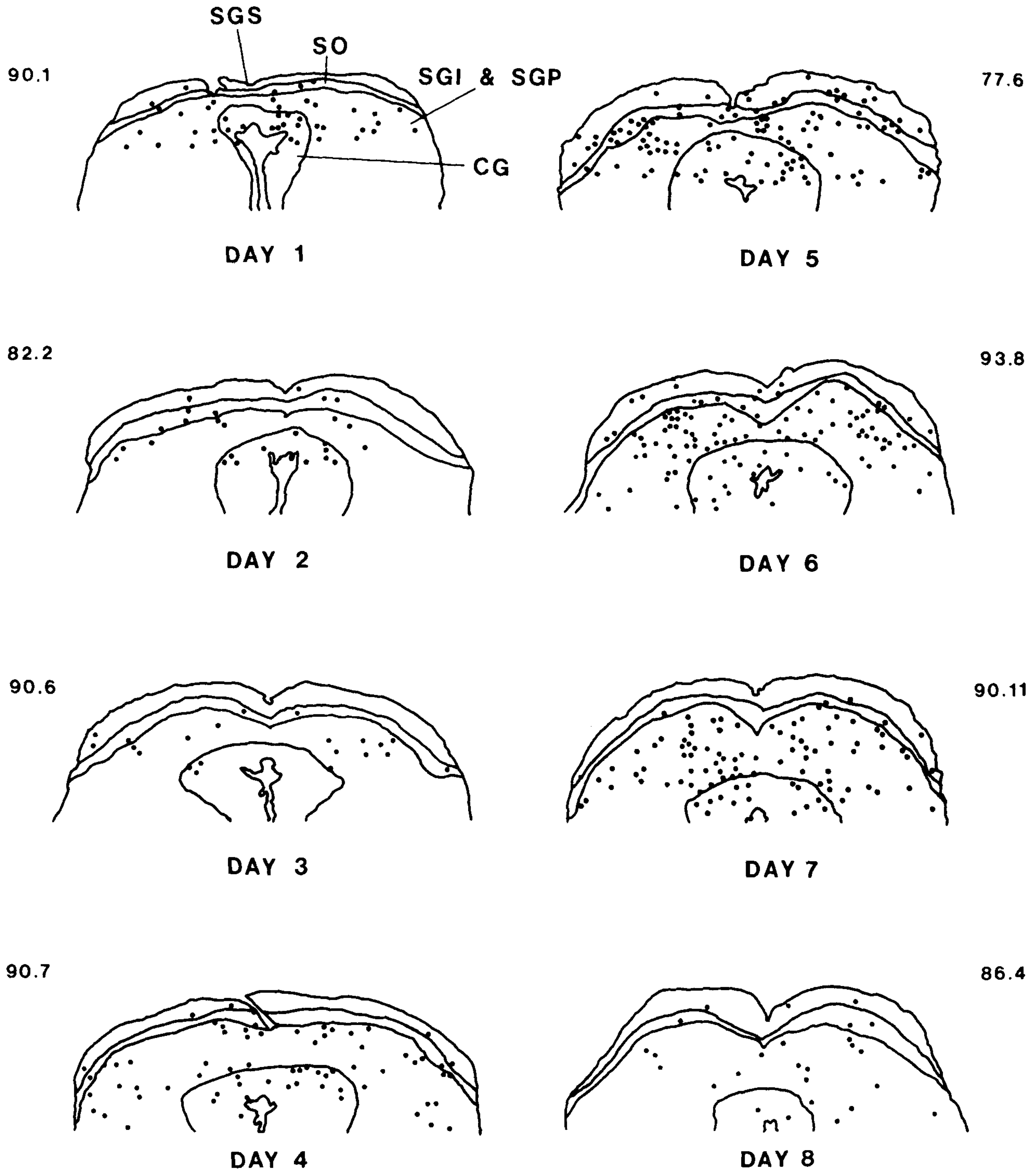

Fig. 1. Drawings of representative coronal sections through the middle of the superior colliculus for postnatal days 1 through 8. Dots indicate the number and location of degenerating cells. For this figure all drawings have been standardized in size to correct for differential shrinkage.

Fig. 2. Photomicrographs of coronal sections through 2 layers of the developing hamster superior colliculus. Superficial gray (A) and intermediate gray (B) at postnatal day 2; superficial gray (C) and intermediate gray (D) at postnatal day 5: superficial gray (E) and intermediate gray (F) at postnatal day 8. Arrows indicate degenerating cells. cresylecht violet stain, × 400.

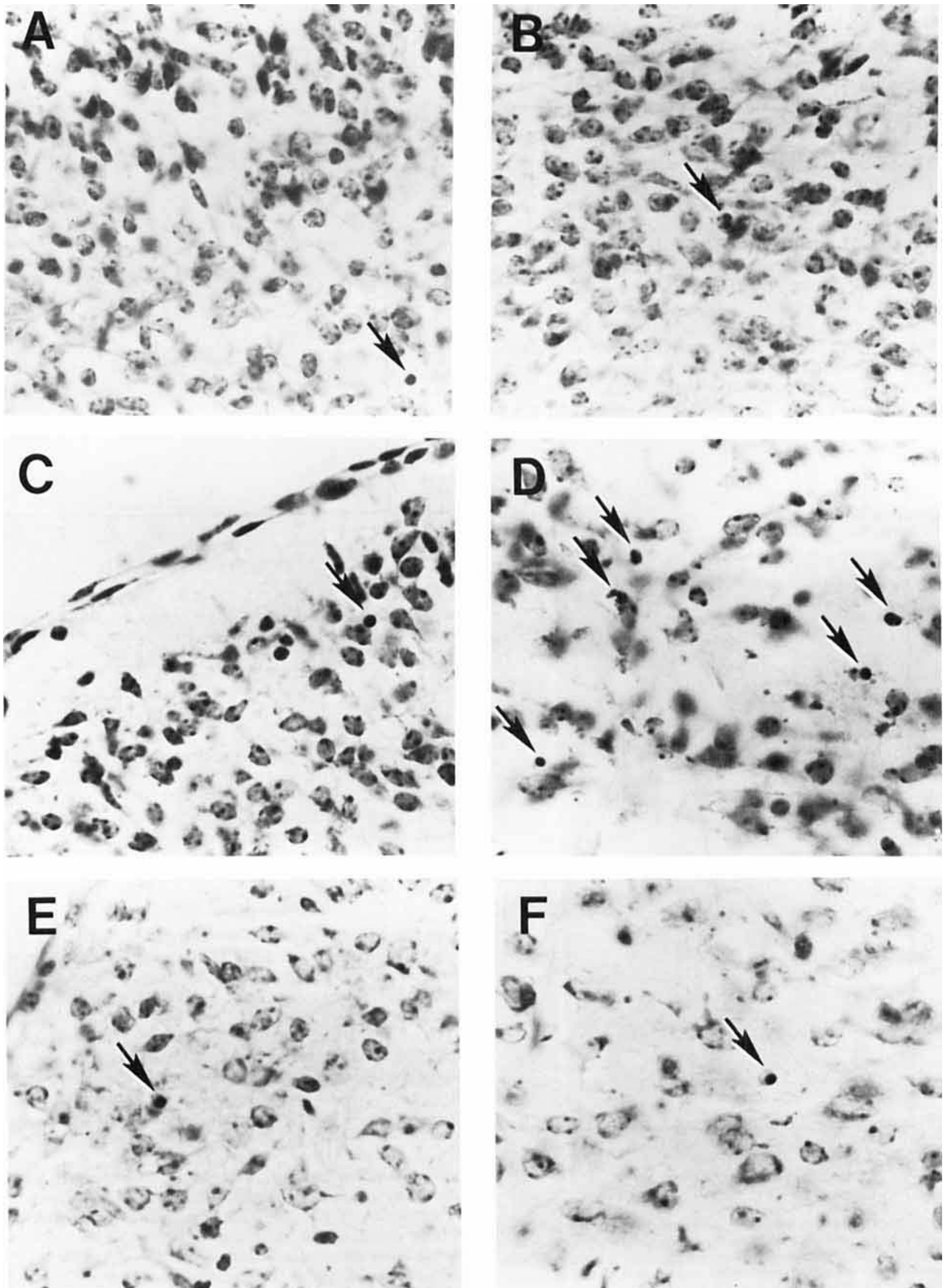

Figure 2

distribution, we did not submit this data to a laborious nearest-neighbor analysis, but instead assume there is no compelling evidence to suggest that these cells are other than randomly distributed. This observation refers only to the columnar assemblies of cells that might be seen in coronal section and not to the distribution of cells across the tectal surface, since we have only assessed the coronal dimension.

Cell death rate was markedly different between collicular laminae (Fig. 3) and was always lower in superficial gray and stratum opticum than in the intermediate and deep layers. The cell death rate for central gray (Fig. 4) was also high, and did not show a clear peak. Variability of cell death rates was not markedly different across structures (48% of mean, superficial laminae; 56%, deep laminae; 33%, central gray).

A striking within-lamina difference in cell death rate was apparent (Fig. 5). In superficial gray and stratum opticum, the rate of cell death was consistently elevated at the medial and lateral margins of the superior colliculus relative to the center. This elevation was statistically significant ($F = 6.14$; $df = 1, 32$; $P < 0.025$). In contrast, the cell death rates for central and peripheral areas of intermediate and deep gray layers showed considerable overlap (Fig. 5B). A high cell death rate centrally on postnatal day 7 produced a significant difference in cell death rates between central and peripheral areas ($F = 4.22$; $df = 1, 32$; $P < 0.05$).

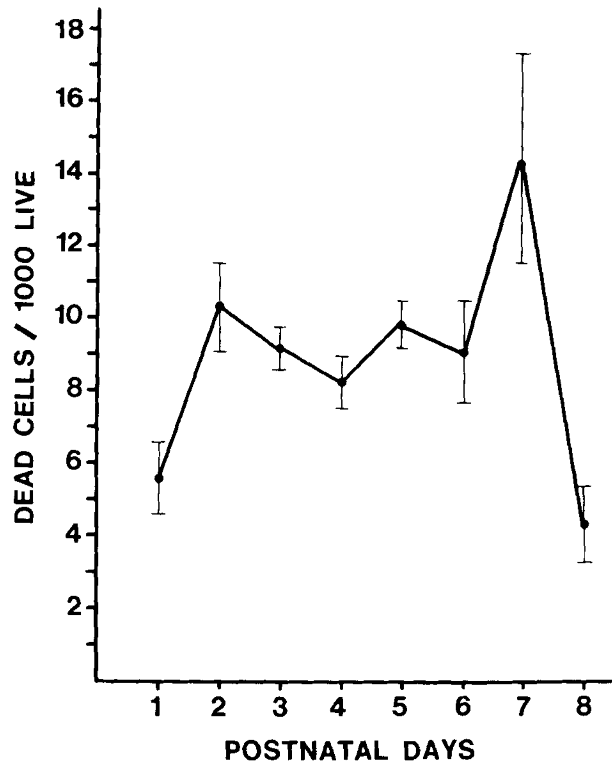

Fig. 4. Ratio of degenerating cells to normal cells in the central gray layer of the superior colliculus over the first 8 postnatal days. Points represent averaged values ± standard error of the mean.

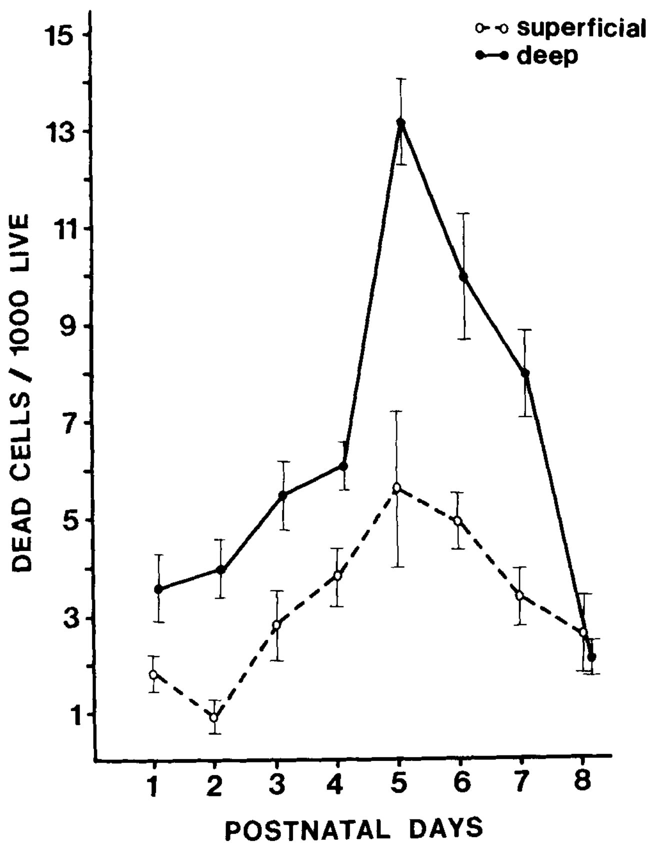

Fig. 3. Ratio of degenerating cells to normal cells over the first 8 postnatal days for superficial (broken line) and deep (solid line) laminae of the superior colliculus. Points represent averaged values ± standard error of the mean. While the peak degeneration rate for both the superficial and deep laminae coincide, cell degeneration is higher in the deep laminae.

Magnitude of cell death

In the preceding paper, we estimated that normal cell loss in the retinal ganglion cell layer was approximately 49%, based on calculations from changes in cell number in the remaining retina after early monocular enucleation (Sengelaub and Finlay, '81, '82). In order to estimate cell loss in the tectum, we compared the sum of the rates over the first 8 postnatal days for tectum and retina. Assuming that the clearance rate for cell debris is similar in the two structures, we estimate that 19% of the cells in the superficial gray die during normal development as compared to 38% of the cells in the deep and intermediate gray layers.

Glial addition in the first postnatal weeks has been demonstrated in rodent tectum (Delong and Sidman, '62; Bruckner et al., '76; Mustari et al., '79). This would artificially decrease the degenerating cell/live cell ratio and result in an underestimation of cell loss. From Delong and Sidman's estimate of thymidine-labeled cells observed in mouse tectum in the first postnatal week, it would appear

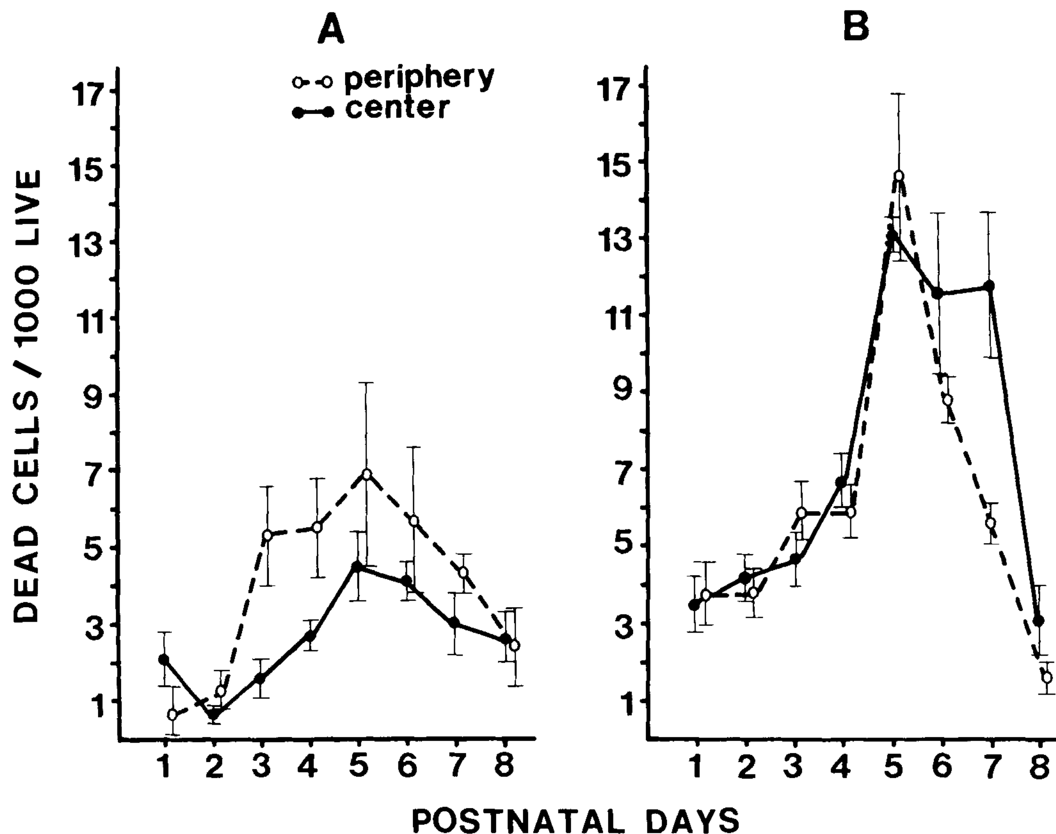

Fig. 5. Ratios of degenerating cells to normal cells over the first 8 postnatal days for superficial gray and stratum opticum (A), intermediate and deep gray (B). Points represent averaged values ± standard error of the mean. Within the superficial layers (A), cell degeneration rates were significantly higher at the periphery (broken line) with respect to the center (solid line). Within the deep layers (B), cell degeneration rates were quite similar.

that up to 20% of the total cell number could be added in that time. If glia degenerate at the same rate as neurons, no error would be introduced.

DISCUSSION

Magnitude and variability

Our estimates of general magnitudes of cell loss (19% loss in superficial gray and 38% loss in intermediate and deep gray), though subject to the difficulties in estimation described in the prior paper, conform well in general magnitude to values reported for other structures. For example, Chu-Wang and Oppenheim ('78) report losses of 40% in the anterior horn of the spinal cord of chick; Hughes and McLoon ('79) report a 20% retinal ganglion cell loss in chick; Cowan ('73) reports a 60% loss of cells in the trochlear nucleus and a similar reduction in the isthmooptic nucleus.

Cowan has proposed that one of the functions of cell death is to regulate normal variability in numbers of cells generated in interconnecting populations ('73). If this is the case, the variability in cell death rates between animals should give an indication of the range of variability in cell generation that might be compensated for by differential cell death. We compared the difference in total cell loss using the mean rate of the lowest individual animal and the highest individual animal summed over all postnatal days. In the superficial gray, at the low rate there is a 12% loss of cells, and at the high rate, a 25% loss of cells. In intermediate and deep gray, at the low rate there is a 31% loss of cells, and at the high rate, a 46% loss of cells. Thus, if all the variability we observe is due to actual individual differences in cell death rate, this variability could compensate for 13 to 15% deviations in cell numbers in interconnecting populations. A study of actual variability in cell numbers generated would clearly be of high interest.

Spatial distribution of degenerating cells

The observation of consistent elevation of the cell death rate in peripheral superficial gray relative to central superficial gray is an interesting one. In other structures such as the spinal cord and sympathetic ganglia, variations in cell death rate have provided insight into the mechanisms of mapping of neuron density onto peripheral muscular and glandular mass. In this case, however, no functional argument to account for greater cell loss in the tectal periphery is obvious; if anything, the tectum is thought to be specialized for information arising from the visual periphery (Tiao and Blakemore, '76; Finlay et al., '80).

The most compelling hypothesis to account for the differential cell death rate in tectum arises from the differential patterns of neurogenesis in retina and tectum. In rodent, the retina is generated in a diffuse central to peripheral gradient (Morest, '70; Bruckner et al., '76) and

the peripheral tectal margins are contacted correspondingly last by incoming retinal fibers (Frost et al., '79). However, no such central to peripheral gradient is seen in the generation of tectal neurons (Lund and Lund, '72; Mustari et al., '79). It is thus possible that the peripheral tectal margins are underinnervated relative to central tectum in early development and show a "compensatory" cell loss. In support of this hypothesis, the cell death rate in peripheral tectum is significantly elevated early on, in postnatal days 3 to 4. Cell death rate in the retinal periphery is elevated later, on postnatal days 5 to 7.

Though this differential loss of neurons might well be an accidental result of the differential timing of neurogenesis in tectum and retina and the rather brief critical period for neuron maintenance prior to synaptogenesis, it could also be that alterations in the timing of the generation of neurons could be employed for morphogenesis. For example, this could be used to enhance a specialization for neuron density in central retina. Other cases of differential timing of cell generation in interconnecting structures should be studied for their morphogenetic consequences.

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