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Does early monocular enucleation in a marsupial affect the surviving uncrossed retinofugal pathway?

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ABSTRACT

Monocular enucleations have been done during early stages (postnatal days 3 to 9) of visual system development of *Monodelphis domestica*, in order to determine whether in this marsupial, as in several eutherian mammals, there are any interactions between the pathways from the two eyes in establishing the uncrossed retinofugal projection. We have examined the distribution and the number of retrogradely labelled ganglion cells that project to the same side of the brain from the surviving eyes shortly after the uncrossed pathway is first formed in normal development (postnatal days 14 to 28). Even at these early stages of development the surviving uncrossed pathway shows no significant reduction, confirming earlier observations of adult marsupials and showing that at no stage in development is there any evidence that the crossed pathway from one eye influences the navigation of axons that will form the uncrossed pathway from the other eye. This is in sharp contrast to observations of mice, rats and ferrets and is in accord with expectations based on the difference of the chiasmatic structure in marsupials as compared with eutherians.

Key words: Eye; visual system; decussation; temporal crescent; axon guidance.

INTRODUCTION

The evidence that is currently available about the mammalian retinofugal pathways suggests that a very early monocular enucleation, done before a significant number of fibres have reached the optic chiasm, produces a marked reduction in the uncrossed pathway from the surviving eye in eutherian mammals (Godement et al. 1987; Guillery, 1989; Chan & Guillery, 1993; Taylor & Guillery, 1994*a*), but does not have this effect in marsupials (Mendez-Otero et al. 1986; Coleman & Beazley, 1989). The relationships that exist at the optic chiasm between the fibres from the two eyes correlate well with this experimental finding. In marsupials the uncrossed fibres form a distinct bundle and turn into the optic tract before they reach the optic chiasm and therefore before they encounter any of the fibres from the other eye (Bodian, 1936; Jeffery & Harman, 1992; Guillery & Taylor, 1993). In contrast, the developing retinofugal fibres of eutherians take an uncrossed course only after they have encountered the fibres from the other eye at the optic chiasm. The conclusion, that the anatomical relationships between the retinofugal pathways in marsupials and eutherians account for the different effects of the early enucleation, would appear to be natural and attractive, except for one difficulty, outlined below, which the present study is designed to address.

If a monocular enucleation in eutherian mammals is done postnatally, after the chiasm has formed, but before the terminal fields of the retinofugal fibres have been established, the surviving uncrossed pathway increases in size (Lund & Lund, 1973; Jeffery, 1984; Jen et al. 1984; Chan et al. 1989). One explanation for this phenomenon is that there are more target cells available for the uncrossed component from one eye when the crossed component from the other eye is removed, and consequently the naturally occurring cell death within the surviving uncrossed pathway is less than normal. The effect of an early and a later enucleation are thus opposite, and it is possible that in some species they might cancel each other. Chan & Guillery (1993) showed recently that in rats such an interaction between the two effects can be seen.

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Whereas the effects of a prenatal enucleation are relatively small when studied in the adult, they are much greater when studied in the neonate, after the fibres have all passed the chiasm, but before the period of stabilisation of the retinal terminals and the period of naturally occurring cell death.

The currently available evidence that an early enucleation in a marsupial does not produce any reduction in the uncrossed pathway (see above) is based on studies of the uncrossed pathway in the adult, done after the period of developmental cell death. In order to confirm that an early enucleation done in a marsupial has no effect on the development of the uncrossed pathway, and thus to establish that there is a genuine difference between eutherians and marsupials in the developmental interactions that occur between the two retinofugal pathways, we studied the effects of early monocular enucleations at relatively short survival times in the grey short-tailed opossum, *Monodelphis domestica*.

MATERIAL AND METHODS

The opossums were checked daily, and the date of birth of the pups was counted as postnatal day zero (d 0). At d 3, d 5, d 7 or d 9 the mothers were anaesthetised with halothane. Pups were individually anaesthetised using a series of puffs of dry ice dust until they showed no spontaneous movements. The eyelid was then incised and the eye removed using forceps. In each litter 2 of the pups were left as controls. 60 pups were enucleated monocularly in 9 litters. Many of the pups failed to survive to the next experimental stage, commonly because as their body weight increased they dropped off the mother's nipple and were either eaten, or died of neglect. This occurred for the normal and for the experimental pups.

At 14, 24 or 28 d (see Table) operated and control pups were anaesthetised with ether and the tract on the side of the remaining eye was filled with horseradish peroxidase (HRP). In addition 3 adult opossums were anaesthetised with halothane and in each, one optic tract was labelled with HRP. For HRP labelling, the skin was incised, the cranium windowed, and the cortex overlying the optic tract removed by suction. Multiple (usually 8–12) 0.01 µl injections of a mixture of 5% WGA-HRP and 40% HRP in 2% dimethylsulphoxide in saline were made into the optic tract. The cavity was plugged with haemostatic foam (Surgicel, Johnson & Johnson) and the skin was sutured. The pups recovered in a warm and moist environment, the adults in normal conditions. After 12–18 h survival, the animals were given an overdose of sodium pentobarbitone (Sagatal) and perfused with normal saline followed by 1% paraformaldehyde in 0.1 M PO_4 buffer. The dorsal pole of the eye was marked and the eye removed and placed in buffer. Perfusion was then continued with a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M PO_4 buffer. The brain was removed and placed in the same fixative for several hours and then transferred to 20% sucrose in 0.1 M PO_4 overnight.

The retina was removed from the eye and 3 radial cuts made, the dorsal cut extending to the disc. The retina was carefully flattened onto a cover slip which was inverted onto a drop of 2.5% glutaraldehyde on a glass slide. After 10–15 min the retina was washed in phosphate buffer and then reacted using the Hanker–Yates method (Hanker et al. 1977).

Coronal frozen sections of the brains were cut and alternate sections reacted free floating with cobalt–diaminobenzidine (Adams, 1981) and tetramethyl-

Table. *Cell counts in the temporal crescent of control and experimental retinas*

Age of animal at enucleation	Age of animal at death	No. of cells in temporal crescent	Mean ± S.E.M.
(Normal, no enucleation)	Adult	6214 4842 6190	5749 ± 555
(Normal, no enucleation)	28 d	6916 7021 5633	6523 ± 547
(Normal, no enucleation)	24 d	6504 6787 6538	6609 ± 110
(Normal, no enucleation)	14 d	7610 7271	7441 ± 240
9 d	24 d	8030 7062 7748 8393	7808 ± 325
5 d	24 d	8393 7941 6957 7393 8603	7857 ± 343
3 d	14 d	9233 7280 5302* 8022 10250 9265	8225 ± 478
3 d	28 d	6505 5681 6868 6779	6458 ± 312

* Low count possibly due to artifact.

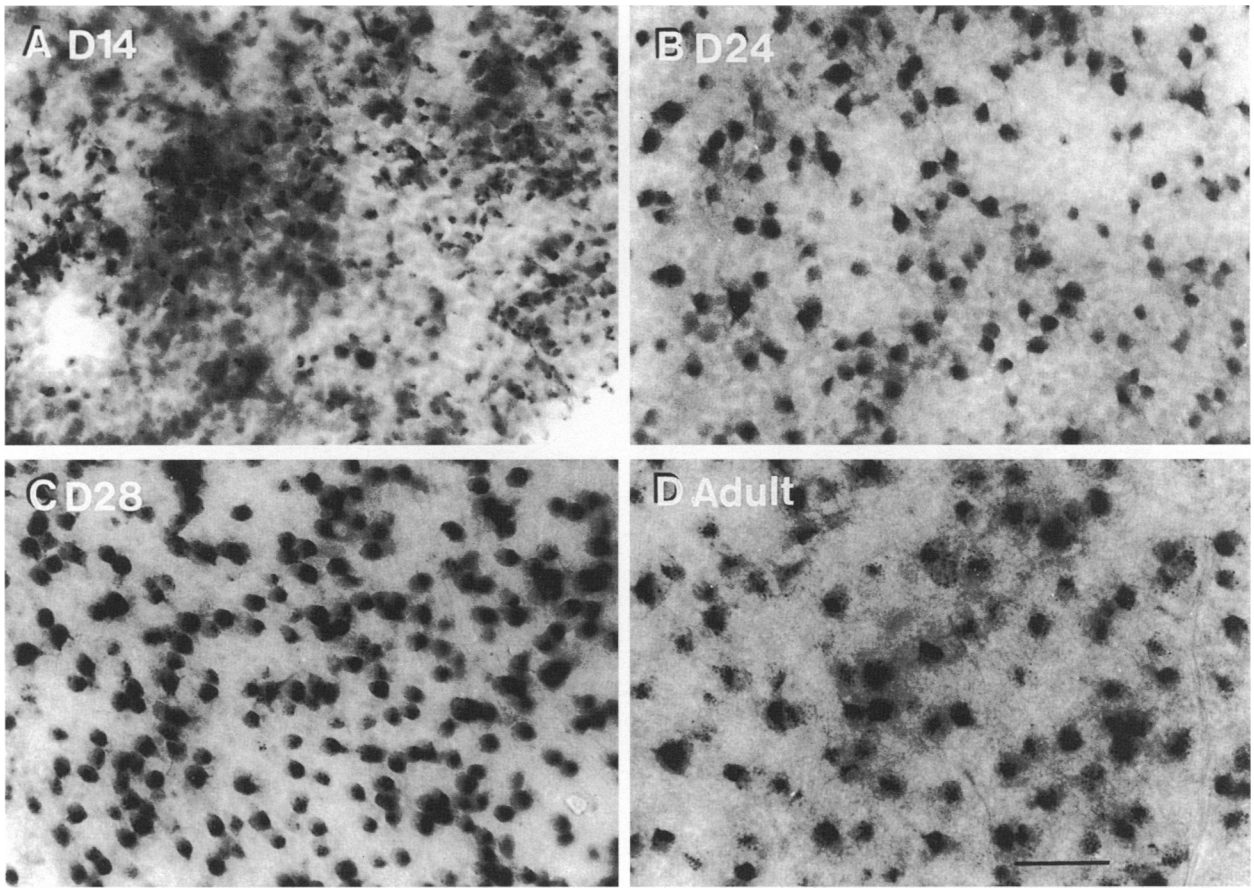

Fig. 1. Sample fields of retrogradely labelled ganglion cells in the temporal crescent of control retinae of (*A*) d 14, (*B*) d 24, (*C*) d 28, (*D*) adult, all taken at × 25 magnification, showing the changes in cell size and cell density with age, and the quantity of cell labelling. In (*A*) the soma size is small and the cell density great making identification of filled ganglion cells more difficult than at later stages. In the adult (*D*) the relative uniformity of soma size of the uncrossed retinal ganglion cells is apparent. Bar, 500 μm. Note that a higher magnification lens (× 100) was used for the cell counts (see Methods).

benzidine (Mesulam, 1978). Sections were mounted and checked to ensure that the injection site was restricted to one side of the brain.

For the cell counts, which were done without knowledge of the identity of the retinas, the outline of each retina was drawn and a regular square grid with intersections 250 μm apart was superimposed upon this drawing. The number of HRP filled cells in a square field with an 88 μm side was counted at each intersection. Cell outlines crossing the lower and right sides were counted, whilst those crossing the upper and left sides were not counted. An oil immersion objective (× 100) was used for the counts. The total number of cells counted in each retina was multiplied by 8.07076 ($250 \times 250/88 \times 88$) and these final totals are recorded in the results. Only cells clearly in ventrotemporal retina were counted. Since outside this area there were generally no cells or, rarely 1 or 2 cells in the outlined field, these nontemporal cells contributed less than 5% of the total number of labelled ganglion cells in any one retina. Sample micrographs were taken at × 25 magnification to allow comparison between the quality of filling and the variation in cell numbers and density in retinae at the various postnatal ages and between experimental and control retinae.

Student's t tests were used to evaluate the significance of differences between cell counts.

RESULTS

The size of the litters and the rates of growth of individuals within any one litter vary considerably. This is because the pups are simply attached to the teats and hang from the anterior abdominal wall with no pouch or other protection. As a consequence of these different growth rates, the visual system can show the same degree of development in pups which differ in age by up to 48 h. Taylor & Guillery (1994*b*) have shown that the first axons reach the optic chiasm at some time between d 3 and d 5, and that at this

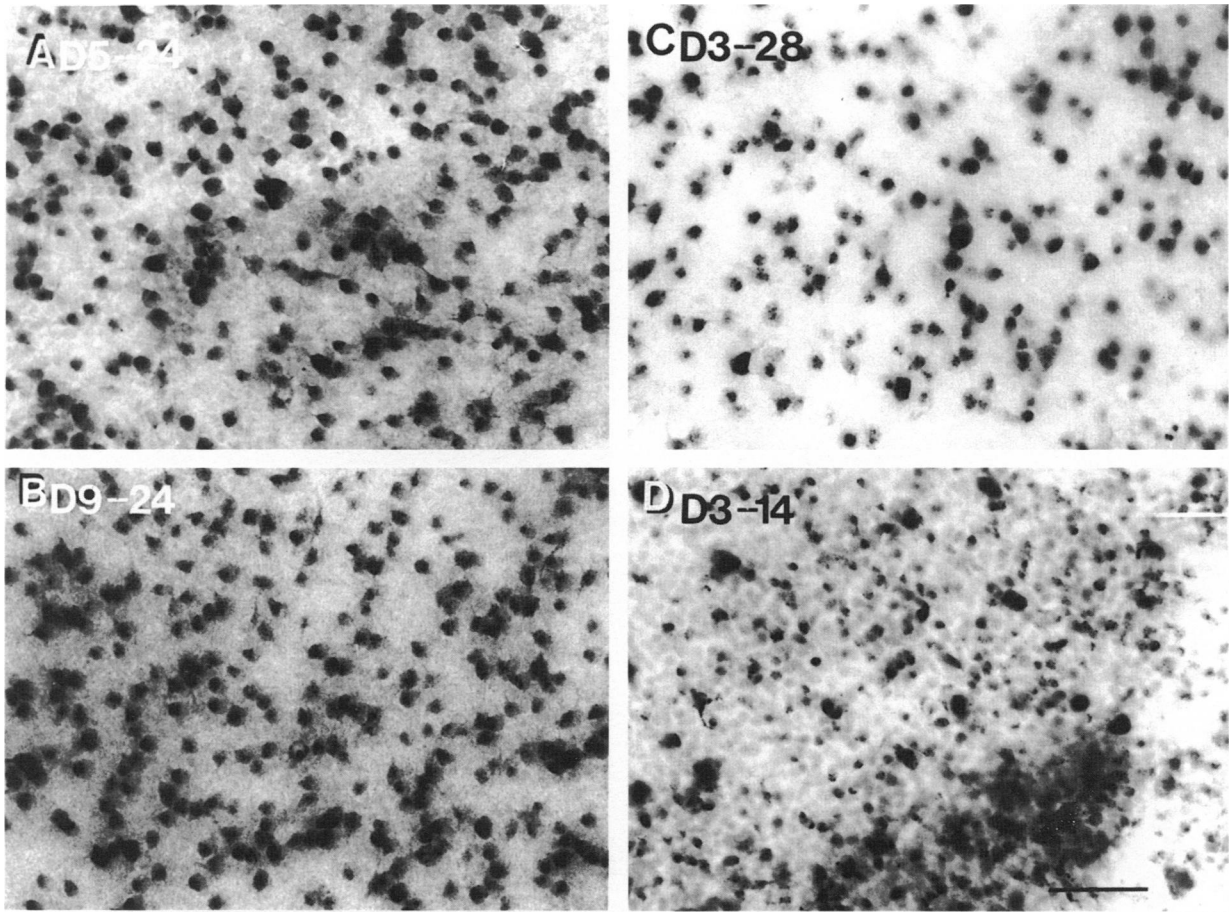

Fig. 2. Sample fields taken at × 25 from the temporal crescent of the intact retina of pups that had been enucleated on (*A*) d 5, (*B*) d 9, (*C*) d 3, (*D*) d 3 and allowed to survive to d 24 (*A*, *B*) d 28 (*C*), or d 14 (*D*). These show that there is little change in the numbers or density of ganglion cells when compared with similarly aged controls shown in Figure 1. Bar, 500 μm.

early stage the uncrossed fibres come from cells in the dorsocentral part of the retina. The earliest stage at which the uncrossed fibres from the temporal crescent of the retina could be retrogradely labelled from the upper part of the optic tract was d 9, indicating that they had grown through the optic chiasm on or about d 7. By varying the day on which surgery was done between d 3 and d 9, we have enucleated before retinal fibres reached the chiasm (d 3), during the stage when only the dorsocentral uncrossed component has formed (d 5), and during the stages when the first uncrossed fibres of the temporal crescent are growing (d 7 and d 9).

The numbers of cells filled and the ease with which the ganglion cells could be counted, depended upon a uniform and complete filling of the optic tract. In control animals assessment of the completeness of filling was made by examination of the contralateral retina, but for enucleates it was obviously not possible to use this method. For each retina, both control (Fig. 1) and experimental (Fig. 2) sample micrographs were taken to assess the uniformity of filling, and to compare the experimental retinas with control retinas from animals in which the contralateral retina showed even filling (Figs 1, 2). The age of the animal at filling also affected the results. At d 14, the small size of the pup meant that it was difficult to fill the optic tract and the flat mounted retinae were relatively small, with a high density of immature, small retinal ganglion cells (Fig. 1*A*). The d 14 preparations were not as clear as those from older animals and were more difficult to count. For this reason we did not study any animals younger than d 14, and are inclined to regard our d 14 results as possibly representing an underestimate (see below). At d 24 and d 28 the retinae were easier to count, and the filling better controlled (Fig. 1*B*, *C*).

The Table shows that in normal adults there were 5749 ipsilaterally projecting ganglion cells. These appeared to be of a relatively uniform size and density (Fig. 1*D*). At d 24 and d 28 the number of retrogradely labelled ganglion cells in the temporal crescent was slightly higher (d 24 = 6609; d 28 = 6523), suggesting that the process of death was not complete by d 24–d 28. At d 14 there were 7741 cells, demonstrating a significant cell loss during normal development from d 14 to adult (Fig. 3).

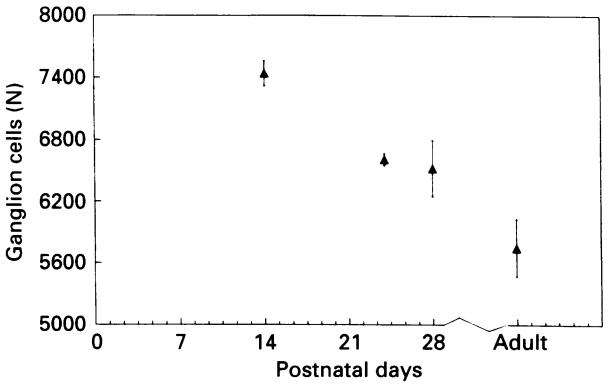

Fig. 3. Distribution of counts showing the decline in the number of cells in the temporal crescent over time for normal *Monodelphis*.

When animals were enucleated at d 5 or d 9 and allowed to survive to d 24, the density of cells showed little change when compared with controls (Figs 1 *B*, *C*, 2 *A*, *B*). In each of the retinae from enucleates there was a clear temporal crescent that showed no qualitative difference in cell distribution (Fig. 4 *A*, *B*, *C*). The numbers of ipsilaterally projecting cells were significantly higher than normal, being 7857 ($t = 3.9$, $P = 0.02$) and 7808 ($t = 4.1$, $P = 0.01$), respectively (Table). This would appear to confirm that the initial production of these cells was greater than the number surviving in the normal animals at d 24 and d 28. It is possible that enucleation of one eye produced an increase in cell production in the other eye, but we will discount this, since it is reasonable to expect a reduction in the normal cell loss after a unilateral enucleation (Jeffery, 1984; Jen et al. 1984; Chan et al. 1989). These counts demonstrate that there is an increase, not a decrease in the number of ipsilaterally projecting ganglion cells produced by the d 5 and d 7 enucleations at d 24 and d 28.

Since it can be argued that the earliest uncrossed

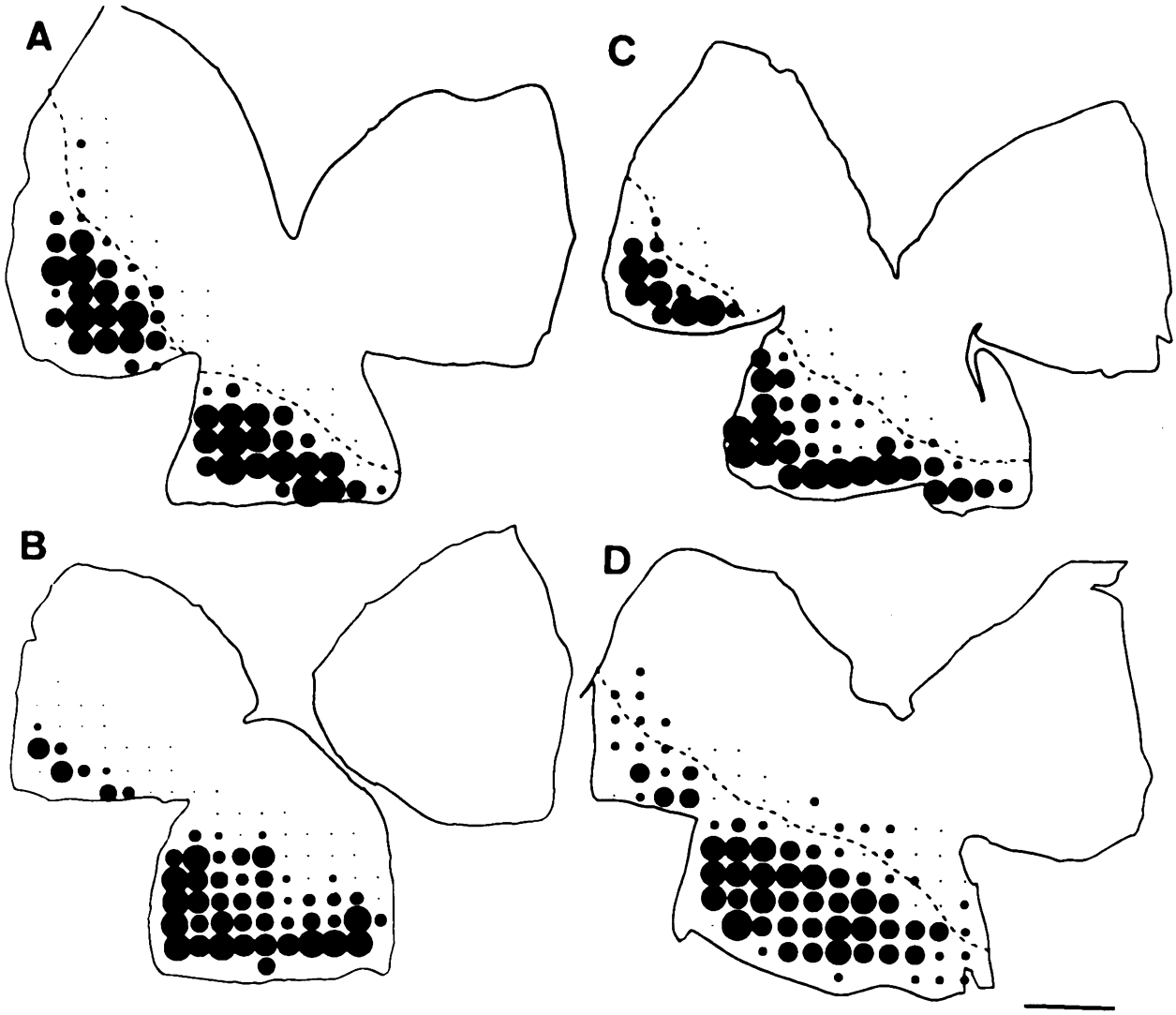

Fig. 4. Dot plots of retinal ganglion cell counts in the temporal crescent of retinae from: (*A*) d 5 enucleate that survived to d 24; (*B*) d 9 enucleate that survived to d 24; (*C*) d 24 control; (*D*) d 3 enucleate that survived to d 28. There is no decline in the numbers of filled cells nor change in their distribution in enucleates. Bar, 1 mm.

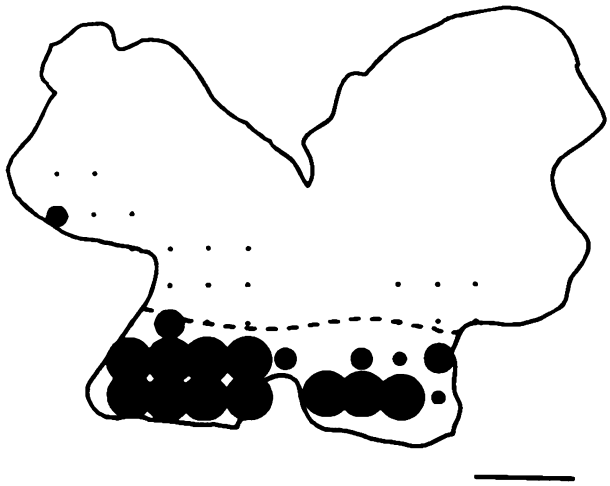

Fig. 5. Dot plot of a d 14 retina from an animal with monocular enucleation on d 3 showing the presence of a temporal crescent. Bar, 1 mm.

axons were already in the optic tract at d 5, and that possibly a relative cell loss might be apparent earlier than d 24, we did some further enucleations at d 3 and allowed some of the animals to survive to d 14 only, and others to survive to d 28 (see Table).

At d 14 there was a clear temporal crescent in the remaining retinae of the d 3 enucleates (Fig. 5). The cell counts at d 14 proved more difficult than those done later and for both the normal and the experimental animals we found a high variability. We are inclined to regard the lower numbers obtained as being artifactual due to incomplete filling. Again, the controls, giving a mean of 7441, were lower than the experimentals, mean 8225, and while not significantly different ($t = 1.55$, $P < 0.1$), provide no evidence that the enucleation produced any reduction in the uncrossed pathways at d 14. Omitting the lowest, possibly artifactual d 14 count (asterisk) would make no difference to this conclusion. The counts obtained at d 14 from the experimental animals provided the highest values obtained from both series of experiments, suggesting that peak cell numbers are reached before d 14 and that in normal development a significant cell loss has already occurred by d 14.

Finally, the d 3 enucleations that survived to d 28 had 6458 cells in the temporal crescent and again showed a clear temporal crescent (Fig. 4*d*). The numbers are significantly smaller than for the enucleations at d 5 ($t = 3.35$, $P = 0.02$) and d 9 ($t = 3.46$, $P = 0.02$) counted at d 24, but are very close to the control values ($t = 0.16$, $P > 0.1$). Again there is no decrease in the number of cells within the temporal crescent.

DISCUSSION

Our results show that a monocular enucleation done very early in *Monodelphis* does not produce the major loss of the uncrossed pathway seen in eutherian mammals. This finding suggests that the course of the uncrossed fibres from one eye is not influenced by the projection from the other eye and shows that the development of the uncrossed projection from the temporal crescent of the retina, must be governed by different mechanisms in marsupials and eutherians. The conclusion can most readily be interpreted as a consequence of the different structure of the marsupial optic chiasm.

In eutherian mammals the crossed and the uncrossed fibres are mingled with each other in individual small fascicles as they grow within the optic nerve (Colello & Guillery, 1990; Chan & Guillery, 1994; Baker & Colello, 1994). Just before the nerves join with the ventral diencephalon, the growing nerve fibres move to the pial surface, establishing an age-related order (Walsh & Guillery, 1986; Reese et al. 1994). This change in organisation occurs where the nature of the glial environment changes from the interfascicular glial cells that characterise the optic nerve to radial glial processes of the diencephalon. After the fibres advance beyond this glial border, the ones from the temporal or ventrotemporal retina behave as though they are repelled by the midline radial glia, whereas other fibres pass straight through this glial zone to enter the contralateral optic tract (Godement et al. 1990, 1994; Marcus et al. 1995). In normal eutherian development the ventrotemporal fibres, having turned away from the midline glia, join the crossed component from the other eye. After an early monocular enucleation they fail to do this, accumulating at the edge of the midline glial palisade (Godement et al. 1990; Taylor & Guillery, 1994; Marcus et al. 1995). This suggests that the uncrossed component requires the crossed component in order to enter the optic tract.

The structure of the optic chiasm has been shown to be radically different in marsupial and eutherian mammals (Bodian, 1936; Jeffery & Harman, 1992; Guillery & Taylor, 1993; see Introduction), and we have recently shown that the adult structure reflects the prechiasmatic segregation of the uncrossed projection during development (Taylor & Guillery, 1994). In the early development of *Monodelphis*, there is also a border between the interfascicular glia of the postoptic nerve and the radial glia of the juxtachiasmatic nerve, as in eutherians (Taylor & Guillery, 1994). Within much of the postoptic nerve, the

uncrossed axons are mingled with the crossed axons, as in the mouse and the ferret. However, when the axons reach the prechiasmatic border between the two glial cell types, the uncrossed axons from the ventrotemporal retina pass directly towards the pial surface, and the other fibres continue towards the midline. From the information available at present it would appear that the prechiasmatic glial border serves essentially different functions in marsupials and eutherians. It serves in marsupials to separate the uncrossed fibres from the crossed fibres before either component has encountered any of the fibres from the other eye, and this early segregation can be seen to account for the lack of any effect of monocular enucleation on the development of the surviving uncrossed projection.

We have done enucleations at times which are comparable to those used in eutherian mammals (Godement et al. 1987, 1990; Chan & Guillery, 1993; Sretavan & Reichard, 1993; Taylor & Guillery, unpublished). On d 3 the first retinal axons grow from the retina, but have not yet reached the chiasm (Taylor & Guillery, 1994). This is comparable to an E12 mouse or an E23 ferret (Cucchiaro & Guillery, 1984; Colello & Guillery, 1990). At d 5 in *Monodelphis* (E14 in mouse, and E28 in ferret), the early dorsocentral component has grown into the ipsilateral optic tract, but it is only at d 9 that the fibres of the temporal crescent are growing into the ipsilateral optic tract (Taylor & Guillery, 1994), and this is comparable to E16 in mouse (Colello & Guillery, 1990) or E30 in ferret (Baker & Reese, 1993). Our analysis of the effects of enucleation has been done at stages when we expected to see maximal effects, relatively unaffected by the process of cell death. Our results suggest that peak ganglion cell numbers are found before d 14. Further, it appears that this early loss is reduced after a d 3 enucleation.

After an enucleation at d 3, we have shown that there were significantly fewer cells in the temporal crescent at d 28, than were found at d 24 after enucleation at d 5 or d 9. Since in control retinas there is not a great difference between counts at d 24 and d 28, there is a possibility that there may be a small early component in *Monodelphis* that behaves like the eutherian uncrossed component in failing to reach the ipsilateral tract in the absence of the crossed component from the other eye. The relevant fibres would be the earliest to reach the chiasm and would be expected to mingle with the crossed component from the other eye before deviating into the uncrossed tract.

The earliest uncrossed component in *Monodelphis* and in mice, rats and ferrets, arises from the dorsocentral retina, and in *Monodelphis* these fibres are present between d 3 and d 5 (Taylor & Guillery, 1994). So far as we know, few if any of these dorsocentral fibres survive for long during development. We have found recently that these early fibres do not enter the ipsilateral optic tract through the independent prechiasmatic ipsilateral bundle as do the later fibres. Instead, they turn within the ventral diencephalon directly into the ipsilateral optic tract, where they join the crossed fibres from the other eye. In terms of the comparison with the eutherian model, it would be concluded that these fibres may well stall at the chiasm after an early (d 3) enucleation, and that these earliest fibres may follow the same developmental programme in all mammals. A comparable reduction of the early dorsocentral component has been noted after early monocular enucleations in ferrets (Taylor & Guillery, unpublished).

Apart from this slight puzzle, of the d 3 enucleation producing a smaller uncrossed bundle at d 28 than does a d 5 or a d 7 enucleation, the results show a clear difference between *Monodelphis* and 3 eutherian species studied earlier (see Introduction). The interactions that occur at the optic chiasm segregate ventrotemporal from nasal axons in all mammals, and the chiasmatic fibre arrangement also depends upon the glial environment. However, the nature of the glial interaction appears to be quite different and the extent to which the uncrossed fibres from one eye depend upon the crossed fibres from the other eye also differs critically. The comparative anatomy of chiasmatic development clearly merits further study and may in turn help to provide useful experimental models for understanding chiasmatic development in a broad perspective.

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REFERENCES

ADAMS JC (1981) Heavy metal intensification of DAB-based HRP reaction product. *Journal of Histochemistry and Cytochemistry* **29**, 775–778.

BAKER GE, REESE BE (1993). Chiasmatic course of temporal retinal axons in the developing ferret. *Journal of Comparative Neurology* **330**, 95–104.

BAKER GE, COLELLO RJ (1994) The origin and course of retinofugal axons during normal development of the ferret. *Society for Neuroscience Abstracts*, **20**, 449.9.

BODIAN D (1936) An experimental study of the optic tracts and

retinal projection of the Virginia opossum. *Journal of Comparative Neurology* **66**, 113–144.

CHAN SO, CHOW KL, JEN LS (1989) Postnatal development of the ipsilaterally projecting retinal ganglion cells in normal rats and rats with neonatal lesions. *Developmental Brain Research* **49**, 265–274.

CHAN SO, GUILLERY RW (1993) Developmental changes produced in the retinofugal pathways of rats and ferrets after early monocular enucleation: the effects of age and the difference between albino and normal animals. *Journal of Neuroscience* **13**, 5277–5293.

CHAN SO, GUILLERY RW (1994) Changes in fiber order in the optic nerve and tract of rat embryos. *Journal of Comparative Neurology* **344**, 20–32.

COLELLO RJ, GUILLERY RW (1990) The early development of retinal ganglion cells with uncrossed axons in the mouse: retinal position and axonal course. *Development* **108**, 515–523.

COLEMAN LA, BEAZLEY LD (1989) Retinal ganglion cell number is unchanged in the remaining eye following early unilateral eye removal in the wallaby *Setonix brachyurus*, quokka. *Developmental Brain Research* **48**, 293–307.

CUCCHIARO J, GUILLERY RW (1984) The development of the retinogeniculate pathways in normal and albino ferrets. *Proceedings of the Royal Society of London, Series B* **223**, 141–164.

GODEMENT P, SALAÜN J, MÉTIN C (1987) Fate of uncrossed retinal projections following early or late prenatal monocular enucleation in the mouse. *Journal of Comparative Neurology* **255**, 97–109.

GODEMENT P, SALAÜN J, MASON CA (1990) Retinal axon pathfinding in the optic chiasm: divergence of crossed and uncrossed fibers. *Neuron* **5**, 173–186.

GODEMENT P, WANG LC, MASON CA (1994) Retinal axon divergence in the optic chiasm: dynamics of growth cone behaviour at the midline. *Journal of Neuroscience*, **14**, 7024–7039.

GUILLERY RW (1989) Early monocular enucleation in fetal ferrets produces a decrease of uncrossed and an increase of crossed retinofugal components: a possible model for the albino abnormality. *Journal of Anatomy* **164**, 73–84.

GUILLERY RW, TAYLOR JSH (1993) Different rates of axonal degeneration in the crossed and uncrossed retinofugal pathways of *Monodelphis domestica*. *Journal of Neurocytology* **22**, 707–716.

HANKER JS, YATES PE, METZ CB, RUSTIONI AJ (1977) A new, specific, and non-carcinogenic reagent for the demonstration of horseradish peroxidase. *Histochemical Journal* **9**, 789–792.

JEFFERY G (1984) Retinal ganglion cell death and terminal field retraction in the developing rodent visual system. *Developmental Brain Research* **13**, 81–96.

JEFFERY G, HARMAN AM (1992) The distinctive pattern of organisation in the retinofugal pathway of a marsupial: II Optic chiasm. *Journal of Comparative Neurology* **325**, 57–67.

JEN LS, SO KF, WOO HF (1984) An anterograde study of the retinocollicular pathways in normal hamsters and hamsters with one eye enucleated at birth. *Brain Research* **294**, 169–173.

LUND RD, LUND JS (1973) Reorganisation of the retinotectal pathways in rats after neonatal retinal lesions. *Experimental Neurology* **40**, 377–390.

MARCUS RC, BLAZESKI R, GODEMENT P, MASON CA (1995) Retinal axon divergence within the optic chiasm: uncrossed axons diverge from crossed fibers within a midline glial specialization. *Journal of Neuroscience*, in press.

MENDEZ-OTERO R, ROCHA-MIRANDA CE, CARVALHO-DIAS E (1986) Effects of monocular enucleation at different stages of development on the uncrossed retinocollicular projection in the Opossum. *Developmental Brain Research* **27**, 101–108.

MESULAM M-M (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry. A non-carcinogenic blue reaction product of superior sensitivity for visualising neuronal afferents and efferents. *Journal of Histochemistry and Cytochemistry* **24**, 1273–1280.

REESE BE, MAYNARD TM, HOCKING DR (1994) Glial domains and axonal reordering in the chiasmatic region of the developing ferret. *Journal of Comparative Neurology*, **349**, 303–324.

SRETAVAN DW, REICHARDT LF (1993) Time lapse video analysis of retinal ganglion cell axon pathfinding at the optic chiasm: growth cone guidance using intrinsic chiasm cues. *Neuron* **10**, 761–777.

TAYLOR JSH, GUILLERY RW (1994*a*) The effect of a very early monocular enucleation upon the development of the uncrossed retinofugal pathway in ferrets. *Journal of Comparative Neurology* **350**, 109–121.

TAYLOR JSH, GUILLERY RW (1994*b*) The development of the uncrossed retinofugal projection in *Monodelphis domestica*. *Journal of Comparative Neurology*, **350**, 109–121.

WALSH C, GUILLERY RW (1986) Age related fiber order in the optic tract of the ferret. *Journal of Neuroscience* **5**, 3061–3059.