

# The Distribution of Astrocytes, Oligodendroglia and Myelin in Normal and Transplanted Rat Superior Colliculus: An Immunohistochemical Study

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## SUMMARY

Immunohistochemical methods have been used to determine the distribution of macroglia and myelin in the normal rat superior colliculus (SC) and in grafts of fetal tectal tissue. The fetal tissue was derived from 15 day-old (E15) rat embryos and was transplanted onto the midbrain of newborn host rats of the same (PVG/c) strain. Antibodies to glial fibrillary acidic protein (GFAP) and carbonic anhydrase II (CAII) were used to visualize astrocytes and oligodendroglia respectively. Myelin was immunostained with antibodies to either proteolipid protein (PLP) or myelin basic protein (MBP). In the normal SC, GFAP positive astrocytes were found scattered throughout the SC, particularly in the superficial layers. They were especially prominent at the pial surface, around major blood vessels and at the midline between the two colliculi. CAII immunoreactive oligodendroglia and associated myelin were also found throughout the SC; by far the lowest density was seen in the stratum griseum superficiale (SGS). Both types of macroglial cell were found in abundance in tectal transplants, indicating that the precursors of these glial types were present in the E15 rat mesencephalon. In mature grafts, large numbers of fibrous astrocytes were found throughout the neuropil and the level of GFAP immunoreactivity was consistently greater than

in host SC. Astrocytes seemed to be maintained in a reactive, perhaps immature state within the grafted tissue. Tectal transplants possessed large numbers of fully differentiated CAII-positive oligodendroglia and the grafts contained a dense network of myelinated axons. However the distribution of CAII and PLP immunoreactivity was not homogeneous; there were localized, well-defined regions that contained few oligodendroglia and relatively little myelin. These areas stained intensely for acetylcholinesterase (AChE) and were almost certainly homologous to the SGS of normal SC. The relative lack of oligodendroglia in the AChE stained patches in grafts and in SGS *in situ* suggests that local factors influencing the proliferation and distribution of oligodendroglia in normal SC may have been operating in a similar manner within the tectal transplant neuropil.

## KEY WORDS

glia, GFAP, carbonic anhydrase II, proteolipid protein, myelin basic protein, neural grafting, development

## INTRODUCTION

In recent years, considerable attention has been directed towards an understanding of the function and physiological importance of macroglia in the central nervous system (CNS). Astrocytes and oligodendroglia are now implicated in an ever increasing range of roles — during development, in the normal CNS, during ageing, and in the response

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of CNS tissue to traumatic injury (see /43,44,61,63, 65,70/ for recent reviews). In development, for example, astrocytes appear to act as guides for axonal growth /73,74/ and influence neuronal morphogenesis /68/ as well as the number and distribution of synapses that are established on neurons /62,63/. They also participate in the induction of the blood-brain barrier /49/. In the normal mature CNS, astrocytes appear to be involved in: (i) potassium buffering and homeostasis within the neuropil /82/; (ii) the uptake, storage and release of neuroactive substances /61/, and (iii) the regulation of blood flow through capillary networks /64/. Their role in the long-term modulation of electrical activity may prove to be of great importance in the CNS /63,69/.

Major functions of oligodendroglia in the normal CNS include the myelination of axons and the homeostatic regulation of potassium /5/. In addition, oligodendroglia and associated myelin have recently been shown to possess molecules which inhibit growth cone motility and axon growth /3,24/. These inhibitory molecules may play a role in: (i) channelling fibre growth in developing white matter tracts /70/, and (ii) limiting axonal regeneration after injury to the mature mammalian CNS /6,22,70/.

Studies on the morphology, connectivity and functional efficacy of fetal neural grafts in neonatal and adult hosts have mostly concentrated on the neuronal organization of the transplants. Given the diverse roles of astrocytes and oligodendroglia during development and in the normal mature CNS neuropil, it is pertinent to examine how these macroglia develop and differentiate within fetal brain grafts. Such information will increase our understanding of the factors that influence the maturation of grafts and the eventual physiological integration of the grafted tissue with the host brain (or spinal cord).

Over the past decade or so we have studied in detail the neuronal architecture and connectivity of fetal tectal transplants /36,37,40,42,60/ and functional analyses of host/graft connections have also been undertaken /26,35/. In the present report we set out to examine the distribution of astrocytes, oligodendroglia and myelin in tectal grafts and to compare their distribution with the neuronal

architecture described in earlier studies. To visualize the two major macroglial cell types we used immunohistochemical procedures. Fibrous astrocytes were identified on the basis of GFAP expression /8,9/. Oligodendrocytes were visualized using an antibody directed towards carbonic anhydrase II (CAII) /28-30/ and myelin was identified using antibodies directed against two of the major protein constituents of CNS myelin: (i) proteolipid protein (PLP), and (ii) myelin basic protein (MBP) /1,33,77/. For comparison, we also used these antibodies to examine the distribution of astrocytes, oligodendroglia and myelin in normal rat superior colliculus (SC). A preliminary account of this work has been presented in abstract form /39/.

#### MATERIALS AND METHODS

Fifteen day-old rat embryos (PVG/c strain, day after mating = E0) were removed from time-mated mothers by Caesarian section. The pregnant female rats were anaesthetized with halothane. Tectal tissue was dissected out from the E15 embryos, transferred to ice-cold Hams F10 medium and then transplanted onto the midbrain of ether-anaesthetized newborn host rats of the same strain /60/.

At various times after transplantation (between 6 to 18 months), host rats were deeply anaesthetized with sodium pentobarbitone (i.p. injection) and perfused with 1% sodium nitrite wash followed by 4% paraformaldehyde in Sorensons buffer (pH 7.4). Normal rats which had not received tectal grafts at birth were perfused in the same manner. All brains were post-fixed for 3 hours. Brains were then dehydrated through a graded series of alcohols and embedded in polyester wax /76/ at 38-40°C. Serial coronal sections (10 µm) of the SC and/or tectal grafts were cut on a microtome with a cooled chuck. Sections were then floated out onto alcohol cleaned slides using a 1% gelatin solution and allowed to dry overnight at room temperature.

Sections were dewaxed through a graded series of alcohols to Dulbecco's "A" phosphate buffered saline (DPBS) and standard procedures for indirect immunofluorescence were applied using the

following polyclonal antibodies: anti-GFAP and anti-MBP (DAKO); anti-CAII and anti-PLP (both antibodies prepared by Dr. N.A. Gregson, Guy's Hospital, London). The specificity of the CAII and PLP antibodies was determined using Western blots and ELISA (N.A. Gregson, personal communication). ELISAs against isoenzymes purified from rat blood showed reactivity only with CAII, not CAI. On Western blots, the PLP antibodies reacted only with PLP and DM<sub>20</sub> (a small gene product from alternatively spliced PLP gene). Primary antibodies were diluted in DPBS containing 0.1% bovine serum albumin (BSA) at the following dilutions: GFAP (1/200), CAII (1/250), PLP (1/250), MBP (1/25). Secondary antibody was goat anti-rabbit TRITC (Sigma) used at 1/100 and diluted in 0.1% BSA/PBS solution. In all brains, control sections were prepared as above except that the primary antibodies were omitted during the first incubation. Sections were coverslipped using an anti-quenching medium and sealed with nail varnish.

The use of polyester wax methods allows relatively thin (10 µm) sections to be cut and results in excellent tissue and antigen preservation and well-defined immunofluorescent labelling /7/. However, in the present study it was also important to examine the relationship between antibody staining and acetylcholinesterase (AChE) staining patterns in the tectal grafts. AChE cannot be visualized in alcohol treated, polyester wax embedded material, thus two brains containing tectal grafts were cryoprotected with 30% sucrose and 40 µm frozen coronal sections were cut. Adjacent sections were immunostained for GFAP, PLP, CAII and one series of sections was stained histochemically for AChE /38,52/.

## RESULTS

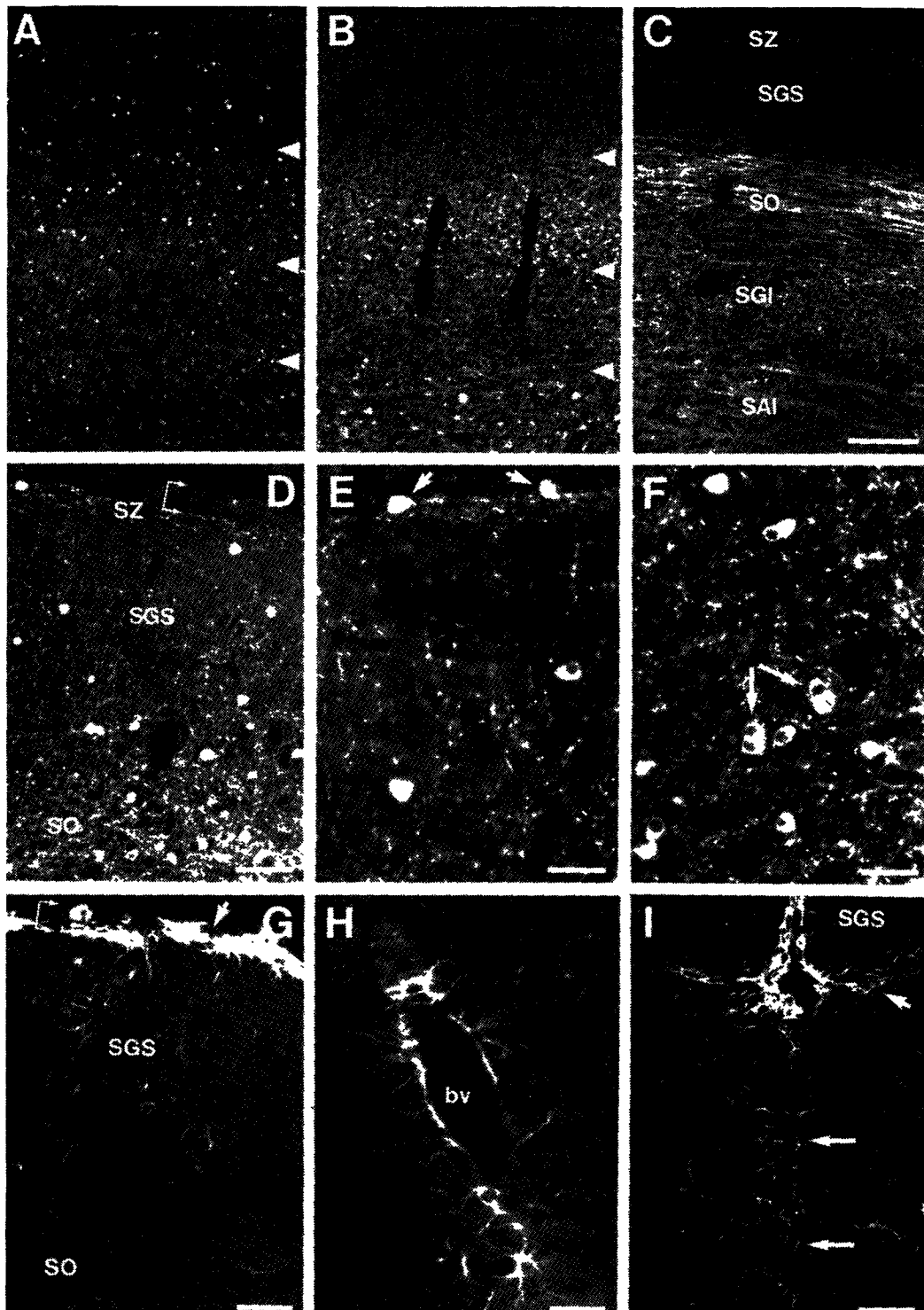
The data described below were obtained from 4 normal rats and 8 rats which had received E15 tectal grafts at birth. The nomenclature used for describing the SC layers is similar to that used by Killackey and Erzurumlu /54/.

### Normal SC

*Oligodendroglia and myelin.* The pattern of CAII and PLP immunoreactivity in the intermediate and superficial layers of the rat SC is shown in Fig. 1A-F. There was a general relationship between the number of CAII immunopositive cells and the density of myelin in the various collicular layers. Thus CAII containing glia were most numerous in the stratum opticum (SO) and stratum album intermediale (SAI), were slightly less common in stratum griseum intermediale (SGI) and were least numerous in the stratum griseum superficiale (SGS) (Fig. 1A). oligodendroglia were about three times more frequent in SO compared to SGS (Fig. 1A,D).

Intense CAII immunoreactivity was seen in the cytoplasm of cell bodies (note the weak or absent immunoreactivity in the nucleus) as well as on oligodendrocyte derived processes and myelin (Fig. 1E,F). The CAII staining on these processes was discontinuous, resulting in a punctate staining pattern within the SC neuropil (Fig. 1D-F). CAII immunoreactive cells were commonly seen in the SZ, immediately below the external glial limitans and pia (Fig. 1D, arrows in Fig. 1E). A thin stratum of CAII stained profiles that ran parallel to the surface was also evident in the dorsal part of stratum zonale (SZ1) /45/ (Fig. 1E). This corresponded to the location of a band of myelin seen in both PLP (Fig. 1B,C) and MBP immunoreacted sections. Note the occasional presence of paired CAII positive cells, particularly in SO (arrows in Fig. 1F).

Examples of PLP staining midway through the SC and in caudal colliculus are shown in Figure 1B,C respectively. Note the increase in immunofluorescence in the predominantly white matter layers (SO and SAI), associated with the large numbers of CAII positive oligodendroglia. The lowest density of PLP immunoreactivity was found in the SGS (Fig. 1B,C). This marked decrease in myelinated fibres was also evident in the MBP immunoreacted material (data not shown). Myelinated axons showed no consistent or characteristic orientation within the SGS; profiles were often found running vertically, but horizontal and obliquely oriented myelinated axons were also



**Fig. 1:** Immunofluorescence photomicrographs showing the distribution of CAII (A,D-F), PLP (B,C) and GFAP (G-I) positive profiles in the superficial and intermediate layers of the normal rat SC. The arrowheads in A and B show the approximate borders between the SC laminae. SZ, stratum zonale; SGS, stratum griseum superficiale; SO, stratum opticum; SGI, stratum griseum intermediale; SAI, stratum album intermediale; bv, blood vessel. Calibration bars: A-C = 150  $\mu$ m; D,G,I = 50  $\mu$ m; E,F,H = 25  $\mu$ m.

commonly seen. No cell body staining was evident in the SC using either the PLP or MBP antibodies.

**Astrocytes.** The external glial limitans stained intensely for GFAP (arrowed in Fig. 1G). The width of this zone of intense GFAP immunoreactivity is indicated by the joined double arrows (Fig. 1G). Note the relative lack of CAII immunoreactivity in this same region (double arrows in Fig. 1D). Within the superficial layers, GFAP positive astrocytes were scattered throughout the SGS and SO; in well-stained sections the number of astrocytes was, if anything, higher in SGS than in SO. GFAP immunoreactive astrocytes were prominent around major blood vessels (Fig. 1H). Intense GFAP immunofluorescence was also seen medially at the border between SGS and SO (short arrow in Fig. 1I) and there was a midline septum running vertically from the SC to the cerebral aqueduct (long arrows in Fig. 1I).

#### Tectal transplants

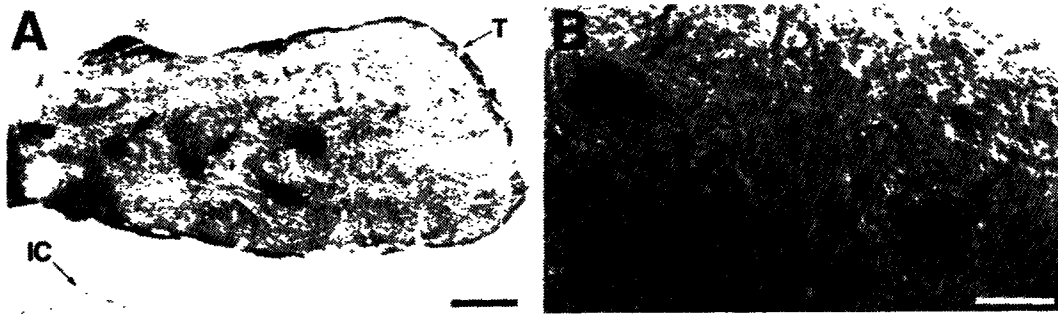
**AChE histochemistry and PLP immunoreactivity.** A characteristic feature of mature tectal transplants is the presence of localized regions or patches which contain intense, homogeneous AChE activity and relatively few stained fibres in neurofibrillar silver preparations /37,38,40/. These AChE-dense, relatively fibre-free patches can be found superficially or deep within the transplants. In the present study, two of the tectal grafts were frozen sectioned to permit the histochemical visualization of AChE within the graft neuropil and to relate this staining to the immunohistochemical staining patterns. An AChE stained section from one of the frozen sectioned grafts is shown in Fig. 2A. Note the localized AChE-dense patches located both superficially (asterisk) and deep (arrows) within the transplant. An adjacent section immunostained for PLP is shown in Fig. 2B. The two circular areas arrowed in Fig. 2B, containing very low PLP immunoreactivity, correspond to the two AChE-dense patches arrowed in Fig. 2A. This clear and consistent association between increased AChE activity and decreased PLP staining was important because it meant that the relatively myelin (PLP) free areas in tectal grafts could be

used as an alternative marker for AChE-dense patches in the polyester wax embedded material.

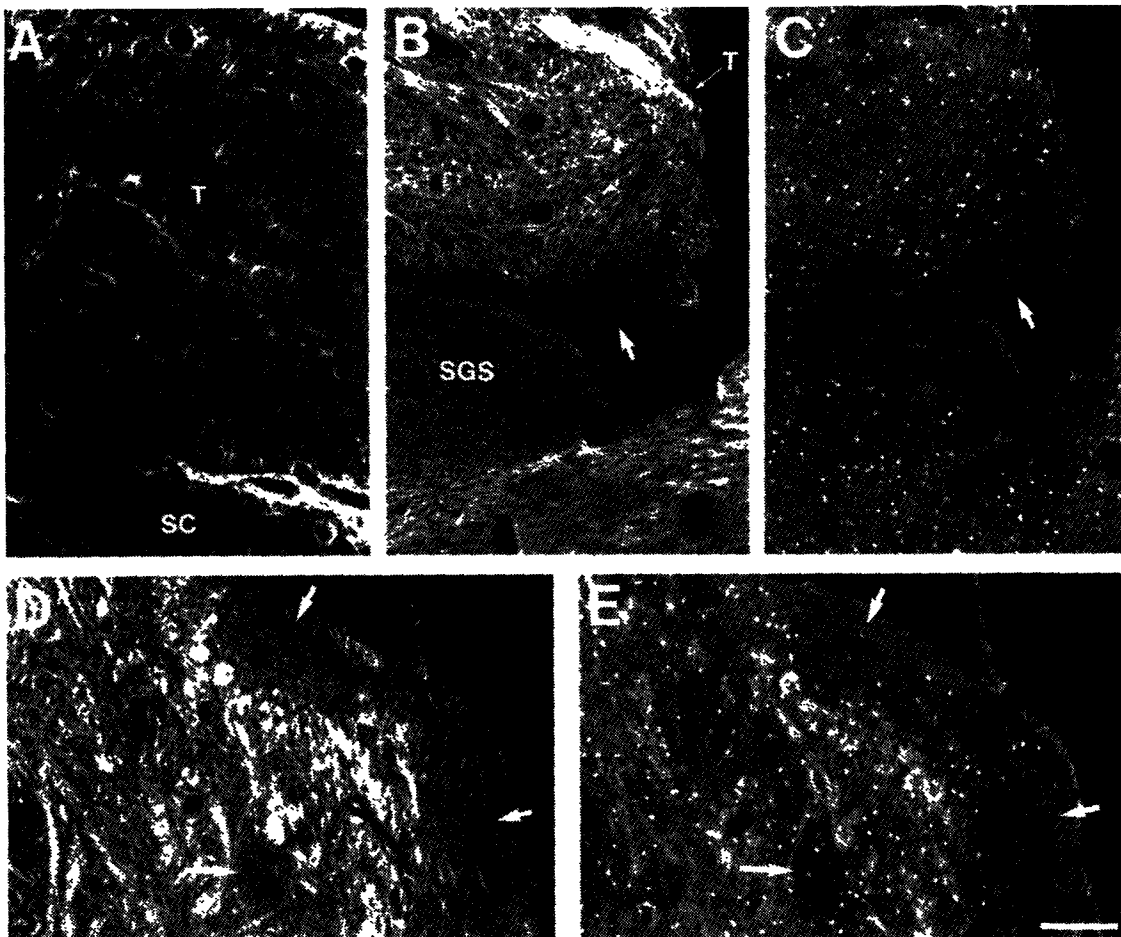
**GFAP, PLP and CAII immunoreactivity in grafts.** In all transplants the level of GFAP immunofluorescence was clearly higher than in the host SC. In addition, the number of GFAP positive astrocytes appeared to be greater in grafts that had no obvious tissue continuity with the underlying host midbrain. Examples of tectal grafts with relatively high GFAP immunoreactivity are shown in Figs. 3A, 4E, F. These transplants had no or only minimal contact with the host SC. Astrocytes were scattered throughout the grafts, but they were particularly prominent in the external glial limitans at the graft surface (Fig. 4E,F) and around major blood vessels.

Sections of tectal grafts immunostained for either PLP or CAII are shown in Fig. 3B,C and 3D,E. In Fig. 3B,C a tectal transplant can be seen lying over the caudal aspect of host SC. The majority of the graft contained considerable PLP immunoreactivity, with bundles of myelinated axons coursing in different directions within the transplant neuropil. A superficial, localized region containing very few PLP immunopositive profiles is arrowed at the ventral margin of the graft. The relative lack of myelin in this patch is markedly similar to that seen in the underlying host SGS. Large numbers of CAII immunoreactive glia were scattered throughout the graft (Fig. 3C) but none was found within the myelin-free patch. Again note the similarity with the subadjacent host SGS. In the example shown in Fig. 3D,E, both a large superficial area (short arrows) and a smaller region located deep within the graft (long arrow) displayed a clear decrease in PLP immunoreactivity. In an adjacent section (Fig. 3E), the density of CAII positive cells was significantly reduced in these areas compared to surrounding myelin-rich tissue.

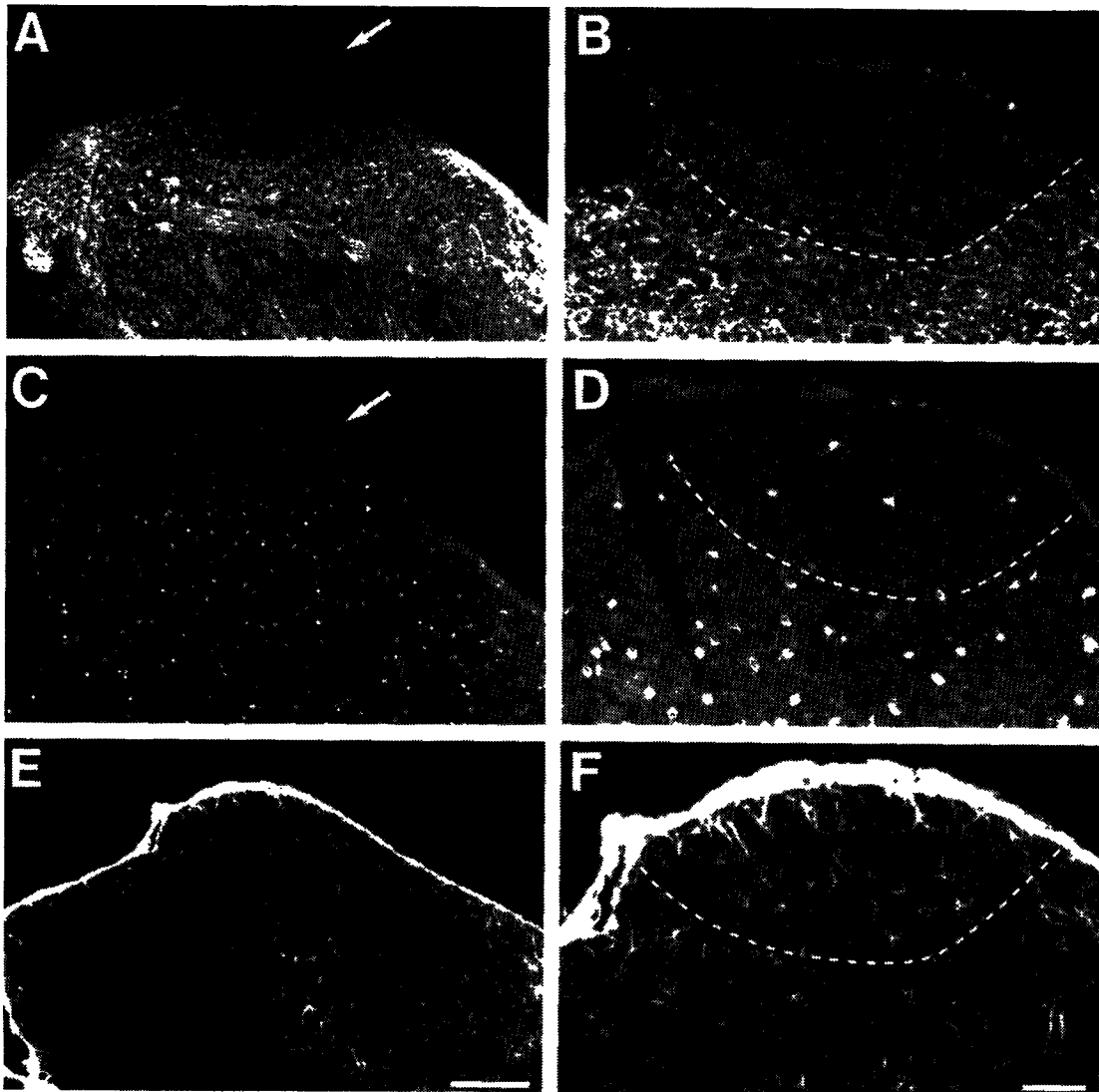
A final example is shown in Figure 4. In this graft, nearly adjacent 10  $\mu$ m sections were immunoreacted for PLP (A,B), CAII (C,D) and GFAP (E,F). This graft contained a high density of myelinated fibres (Fig. 4A), oligodendrocytes (Fig. 4C) and astrocytes (Fig. 4E) throughout most of the neuropil. The density of CAII positive cells was similar to that of the SO and SAI of normal SC. A



**Fig. 2:** The association between AChE-dense patches within a tectal graft (T) (arrows in A) and localized areas lacking myelin (PLP immunoreactivity) (arrows in B). This rat received a graft at birth and was perfused 18 months later. A superficial AChE-dense patch is asterisked in A. IC, host inferior colliculus. Calibration bars: A = 500  $\mu$ m; B = 150  $\mu$ m.



**Fig. 3:** Mature tectal grafts (T) examined 10 months (A-C) and 9 months (D,E) after transplantation. A, GFAP immunofluorescence. B-C, sections adjacent to A, immunostained for PLP (B) or CAII (C). D-E, adjacent sections from another graft showing PLP (D) and CAII (E) immunofluorescence. Myelin free areas (arrows in B,D) contained no or relatively few oligodendroglia (arrows in C,E). SC, host superior colliculus. Calibration bars: A-E = 150  $\mu$ m.



**Fig. 4:** A tectal graft examined 10 months after transplantation. Adjacent sections through the graft were immunostained for PLP (A,B), CAII (C,D) and GFAP (E,F). The dorsal myelin deficient patch arrowed in A is outlined in the high power fluorescence photomicrographs in B,D and F. Note the paucity of CAII positive cells in this localized region (C,D). This area does however contain large numbers of fibrous astrocytes (E,F). In this particular graft, the thin band of faint immunofluorescence seen at the surface of the PLP and CAII immunoreacted sections (A-D) was also present in control sections processed without primary antibodies and does not reflect specific immunostaining. Calibration bars: A,C,E = 150  $\mu$ m; B,D,F = 50  $\mu$ m.

localized area contiguous with the dorsal surface of the tectal graft and containing little PLP and CAII immunoreactivity is arrowed in Figure 4A,C. This region is shown at high power in Fig. 4B,D,F and is highlighted by the dashed line. Although there was a clear decrease in PLP staining and in the number of CAII immunoreactive oligodendrocytes, the number of GFAP positive astrocytes was not obviously different within or outside the patch area. Note also the lack of a band of myelinated fibres and CAII immunoreactive cells at the graft surface just below the external glial limitans (Fig. 4B,D), a feature typical of the SZ of normal SC (Fig. 1B-E). The pattern of CAII and PLP staining was similar in all grafts, irrespective of whether they had physical continuity with the underlying host SC. As in normal SC, no cell body staining was evident in sections of tectal grafts immunoreacted with PLP or MBP antibodies.

## DISCUSSION

### Technical considerations

In the CNS, the use of antibodies to GFAP permits the identification of astrocytes — but only that part of the astrocytic population expressing immunohistochemically detectable levels of the intermediate protein /8,9/. Injury causes a rapid increase in the level of GFAP immunoreactivity and in the number of astrocytes seen to express GFAP /8,44,48/, suggesting that in the normal CNS there may be many astrocytes which go undetected. Indeed, it was recently reported that the pattern of GFAP immunoreactivity seen in normal brain tissue is dependent upon the type of fixation used /71/. The formaldehyde-based fixatives used by most workers resulted in GFAP staining in the cortical gray matter, but very few GFAP-positive astrocytes in white matter. This pattern was completely reversed after acid-alcohol fixation, the predominant immunoreactivity now being found in white matter. In the present study para-formaldehyde was the only fixative used in both the normal rats and rats that received tectal transplants at birth. It is therefore valid to make direct comparisons between the levels of GFAP immunofluorescence seen in the tectal grafts and in

the host SC. However, it would be of interest to determine the staining pattern of astrocytes in normal and grafted colliculus using the acid-alcohol fixation protocol /71/.

Antibodies to PLP and MBP (major constituents of CNS myelin) have been well characterized /7,33,56,77/. There has, however, been disagreement in the literature concerning the immunostaining characteristics of antibodies to the enzyme carbonic anhydrase (CA). Antibodies to CA have been reported to stain astrocytes *in vivo* /67/, and *in vitro* /55/. More recently, Cammer and co-workers reported that antibodies to CA stained some GFAP positive astrocytes in developing and mature cortical gray matter /17,18,20/. They also reported the co-existence of CA and glutamine synthetase in CNS glia, although this enzyme has now been found in oligodendroglia as well as in astrocytes /16/. After EAE sensitization, Cammer *et al.* /19/ described CA immunoreactivity in GFAP positive reactive astrocytes, particularly in those regions most severely affected by the injury.

Others have reported that the CAII isoenzyme is found only in oligodendroglia in the CNS /e.g. 28, 30,58/. Using the electron microscope, immunostaining was located on oligodendroglial cell bodies, their processes and peripheral lamellae of myelin. Recently, tissue culture studies utilizing a cDNA CAII probe and *in situ* hybridization have demonstrated that CAII is exclusively restricted to oligodendroglia /29/. These authors suggested a number of reasons why CA immunoreactivity might apparently be associated with astrocytes, including the possibility of antisera contamination or poor fixation of non-bound enzyme released from CAII-rich oligodendroglia. However, more recent studies by Cammer argue against these factors being important /21/. A further possibility may be that, given the acquisitive nature of astroglia, a proportion of these cells may normally accumulate and store low levels of CAII *in vivo* and thus the enzyme may, under certain conditions, be detected immunohistochemically within astrocytes.

The specificity of the polyclonal antibody used to identify CAII in our present work has been described in the Methods section. It has not previously been reported to stain astrocytes /7,32/



and although we did not undertake double-staining analysis, the disposition of GFAP and CAII immunoreactive cells was clearly different, both in normal SC and in tectal transplants. In our hands, using this antibody and polyester wax embedding techniques, the oligodendrocyte appeared to be the primary locus for detectable CAII immunostaining. Thus the observed pattern of CAII immunofluorescence is interpreted as showing the distribution of oligodendroglia and their processes in the rat midbrain and within fetal tectal grafts.

#### Normal superior colliculus

The distribution of CAII immunoreactive oligodendroglia in the rat SC has not previously been described. Oligodendroglia were found in relatively high density in most SC laminae, especially in SO and SAI. In these layers, paired CAII positive cells were seen; these oligodendroglial couplets were always in the same focal plane and the cells appeared to be in close contact with each other /58/. Similar profiles have been described in other parts of the CNS using different oligodendrocyte-specific markers /30/. Oligodendroglia were least numerous in the SGS. Punctate CAII staining associated with cell processes /28,56/ was also at its lowest level in this superficial layer.

The pattern of PLP and MBP immunostaining in the rat SC was similar to the myeloarchitectural organization described previously /45,54,83/. In accord with the pattern of CAII staining, the lowest level of PLP and MBP immunoreactivity was found in the SGS. Interestingly, levels of PLP mRNA and MBP mRNA have also been shown to be lowest in this tectal lamina /72/. As described in the Introduction, oligodendroglia and central myelin have been shown to possess molecules which inhibit axonal growth *in vitro* and which may act to prevent CNS regeneration *in vivo* /3,6,22,24,70/. The relative lack of myelinating cells in the SGS is thus of particular relevance to studies aimed at promoting axonal regrowth and target reinnervation in the rodent visual system /15, 34,41,53,80,81/. In adult rat and hamster, at least some retinal ganglion cell axons regenerating through peripheral nerve autografts can grow out into a deafferented host SC and form functional

synaptic contacts with tectal neurons /15,53,80,81/. The limited outgrowth is mostly restricted to SGS and it may be that the relative paucity of oligodendroglia and myelin in this layer is a factor that contributes to this pattern of axonal growth. Indeed, recent studies on retinotectal growth after unilateral tectal ablation in the neonatal hamster have shown that neutralization of oligodendrocyte-derived neurite growth inhibitors results in an expanded retinal projection /51/.

With regard to the disposition of GFAP positive astrocytes in the rat SC, we observed intense immunoreactivity associated with the external glial limitans, around major blood vessels and at the midline between the two colliculi. GFAP-positive astrocytes were also prominent at the medial aspect of the SGS/SO border. These data are in accord with those described previously by Hajos and Kalman /31/. In addition, however, and unlike Hajos and Kalman /31/, who used a paraformaldehyde and picric acid fixative, we also observed many GFAP immunoreactive astrocytes scattered throughout the SC, particularly in SGS.

#### Tectal transplants

Compared to host SC, tectal grafts possessed greater GFAP immunoreactivity and contained larger numbers of GFAP positive astrocytes. This increase in GFAP expression was seen in all grafts, irrespective of whether they had tissue continuity with the underlying host brain. Increased levels of GFAP appear to be a characteristic feature of fetal brain grafts, whether the tissue is transplanted into the brain parenchyma or into the anterior chamber of the eye. A higher than normal density of GFAP-positive cells and processes has been described in intracranial grafts of fetal hippocampus /84/, cerebellum /12,57/, cortex /11,47,57/ and substantia nigra /46/, and in intraspinal grafts of fetal spinal cord /66/. In the present study, as in hippocampal grafts /84/, the increase in fibrous astrocytes appeared to be greatest in transplants physically isolated from the host brain. Increased GFAP expression has also been observed in intraocular grafts of fetal cortex, hippocampus, cerebellum and septum /10,25,27,75/. There is less of an increase in GFAP immunoreactivity when tissue is co-grafted with other fetal brain regions /10,25,27/.

In grafts of fetal neural tissue, the level of GFAP increases over a period of time and is comparable or higher than that of the host 30 days post-transplantation /57/. These authors showed that GFAP expression was temporally advanced and greater in grafts compared to normal development. Why is there this gliotic reaction in fetal brain grafts? Many of the GFAP positive astrocytes resemble the reactive astrocytes seen in injured or metabolically compromised brain tissue /44/. In normal brain there is a gradual increase in GFAP content and in the size and number of GFAP positive astrocytes with age /13/; however, even in old host rats (some of the host rats in the present study were 18 months of age) the level of GFAP expression in transplants is still higher than in comparable regions of the host brain. Thus ageing alone is not sufficient to explain the reactive appearance of astrocytes in fetal grafts.

It has been suggested /57/ that the gliosis may be a general reaction to the transplantation procedure and the disruption of the glial scaffold within the grafted tissue. Other evidence suggests that astrocytes do indeed participate in the formation of cytoarchitectonic boundaries and neuronal topographies during normal development /63/. Certainly, the imparting of mechanical strength and stability to the developing graft may be a factor in the gliotic response; however, the relation between GFAP expression and neuronal connectivity should also be taken into consideration. GFAP levels in intracranial or intraocular grafts are lower in grafts connected to the host or co-grafted with other parts of the developing neuraxis. This has led authors to propose that the glial reaction in grafts may, in part, be the result of a lack of appropriate afferent innervation and/or efferent interactions with specific targets /10,11,57/.

During development there is a transient increase in GFAP mRNA in rats in the second and third postnatal weeks /59,79/. A similar transient increase in immunohistochemically detectable GFAP has been described in the mouse visual pathway /14/ and in the opossum SC /4/. The decrease in GFAP after this peak appears to correlate with a number of developmental events, including interaction with growing axons /44/ and

the onset of synaptogenesis /4/. In the opossum SC, the transiently high expression of GFAP in the SGS is "temporally correlated with late transformations of the retino-collicular projections" /4/ — after the period of maximal elimination of optic axons and before the onset of myelination. Perhaps in tectal grafts, as in other fetal CNS grafts, a reduction in the normal complement of afferent input (and perhaps lack of appropriate targets for efferent connections) may influence the differentiation state of astrocytes. They may remain relatively immature, similar to the transiently reactive glia seen in normal development. Whatever the cause of the gliosis, it is clear that many transplanted astrocytes are in an abnormal state of differentiation. Given the diverse functional roles proposed for astrocytes in the normal CNS (reviewed in the Introduction), it would seem likely that neuronal activity in grafts may in some way be affected by the altered phenotype of at least some of these glia, and this should be taken into consideration when studying graft function and the morphological and physiological integration of grafts within the host brain.

To our knowledge the organization and distribution of oligodendroglia has not been systematically studied in fetal neural grafts. The development of myelin in the normal SC has been described in the rat /83/, hamster /50/ and opossum /23/. It has been suggested that oligodendroglial precursors may colonize the SC by migrating from the optic tract along ingrowing retinal axons /50/ and that the rate of myelination in the SC is related to the onset and refinement of visual input /23,50/. In the present study considerable PLP and MBP immunoreactivity was seen in most parts of the tectal grafts, even in grafts *physically isolated* from the host brain. Previous experience has shown that such grafts receive little or no host afferent input /36,37,60/; thus in these isolated grafts myelin must have been associated with intrinsic axons and must have developed independently of any specific functional innervation from the host. In addition, these data clearly demonstrate that E15 rat mesencephalon (which has not yet been innervated by retinal axons) already contains oligodendrocyte precursor cells and that the transplanted tectal tissue can provide all the signals necessary for

oligodendrocyte proliferation, differentiation and myelination.

Unlike astrocytes, which seemed to be randomly scattered in tectal transplants, CAII immunoreactive oligodendroglia were not distributed homogeneously throughout the graft neuropil. Many oligodendroglia were found in heavily myelinated (PLP,MBP positive) regions of the grafts and their density appeared similar to that seen in most layers of the SC. However these glia were considerably reduced in number in the relatively myelin free patches. These localized areas, found superficial or deep within the grafts, corresponded to AChE-dense patches in adjacent histochemically stained material. AChE-dense, fibre-free patches in tectal grafts are believed to be homologous to the superficial gray layers of normal SC and to contain presumptive SGS neurons /37,38,40,60,78/. The low density of CAII immunoreactive glia in the patches in transplants corresponds to their low density in the SGS of the SC *in situ* and thus whatever local factors control the disposition of oligodendroglia in the normal tectum also appear to be operating within the graft neuropil. The nature of these factors is unknown at present, although oligodendrocyte-type 2 astrocyte progenitors have been shown to migrate towards specific molecules, such as platelet derived growth factor /2/. Other factors may also be involved and it is interesting to speculate that the distribution of oligodendroglia may in some way be influenced by the neurons with which they are associated.

Finally, it is important to note that in all grafts the PLP and MBP antibodies stained only myelinated processes, not oligodendroglial cell bodies. This is similar to the immunostaining pattern seen in normal adult brain. In immature brains, however, antibodies to either MBP or PLP stain oligodendroglia during the period before and during myelinogenesis /e.g. 33,77/. These observations provide further evidence that the oligodendroglia in tectal grafts were indeed mature and in a relatively normal state of differentiation. This is in contrast to the transplanted astrocytes, which seemed to possess a reactive, perhaps immature phenotype.

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