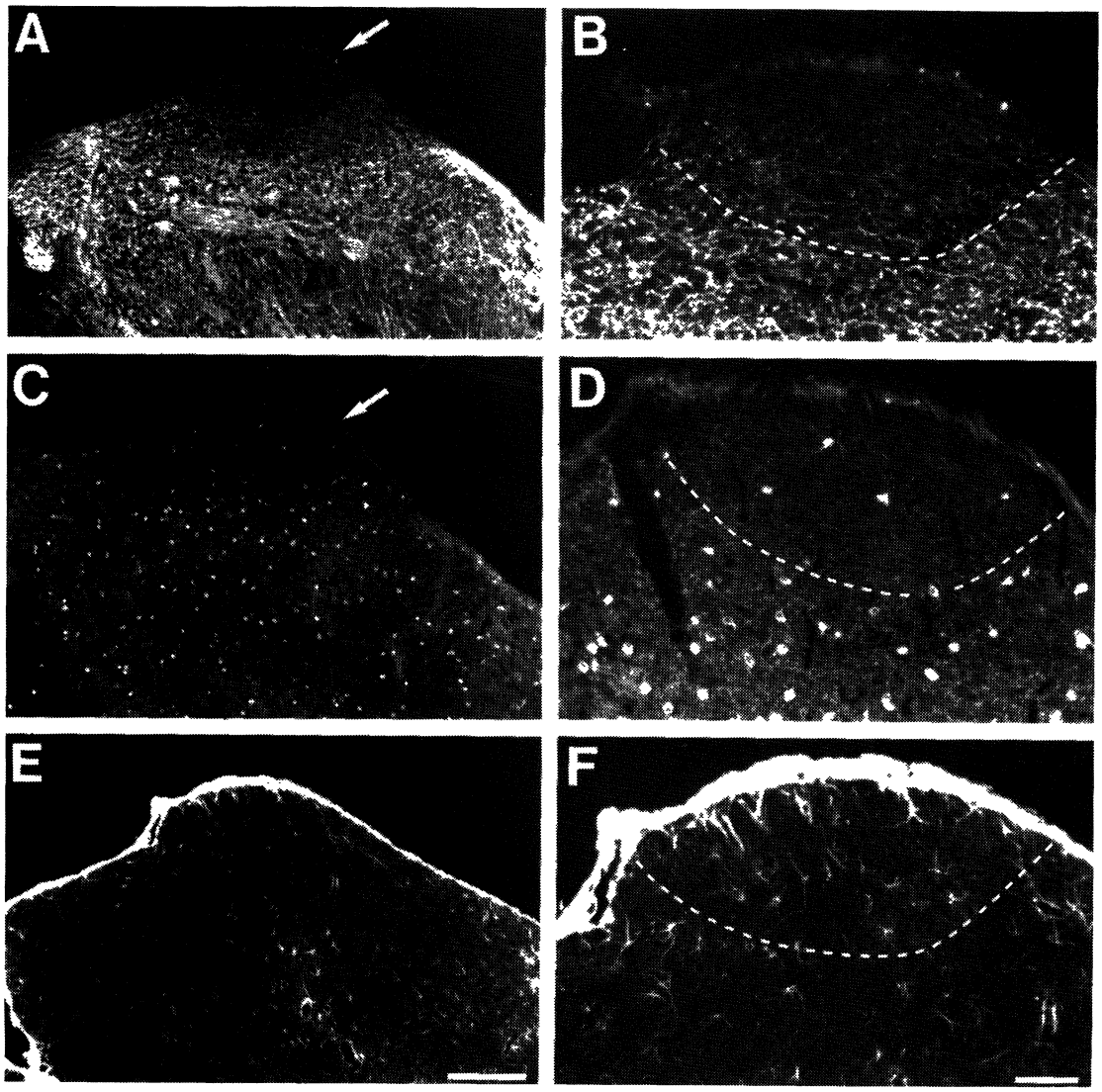

Fig. 4: A tectal graft examined 10 months after transplantation. Adjacent sections through the graft were immunostained for PLP (A,B), CAII (C,D) and GFAP (E,F). The dorsal myelin deficient patch arrowed in A is outlined in the high power fluorescence photomicrographs in B,D and F. Note the paucity of 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 μm; B,D,F = 50 μm.

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.

ACKNOWLEDGEMENTS

We are indebted to Dr. N. A. Gregson for the generous gift of the polyclonal PLP and CAII antibodies, and for his comments on the manuscript. ARH wishes to thank Professor M. Berry for making facilities available during his period of study leave at UMDS. We thank Lazlo Bubrick for his assistance with photography and Rita Bonjour for her secretarial help. This work was supported by grants from NH and MRC, International Spinal Research Trust, and Paraplegic-Quadriplegic and Civilian Maimed and Limbless Associations.

REFERENCES

1. Agrawal HC, Harman BK. Proteolipid protein and other proteins of myelin. In: Bradshaw RA, Schneider DM, eds, Proteins of the Nervous System, 2nd Ed. New York: Raven Press, 1980; 145-169.
2. Armstrong RC, Harrath L, Dubois-Dalcq, ME. Type I astrocytes and oligodendrocyte-type 2 astrocyte glial progenitors migrate toward distinct molecules. J Neurosci 1990; 27: 400-407.
3. Bandtlow C, Zachleder T, Schwab ME. Oligodendrocytes arrest neurite growth by contact inhibition. J Neurosci 1990; 10: 3837-3848.
4. Barradas PC, Cavalcante LA, Mendez-Otero R, Vieira AM. Astroglial differentiation in opossum superior colliculus. Glia 1989; 2: 103-111.
5. Barres BA, Chun LLY, Corey DP. Ion channel expression by white matter glia: 1. Type 2 astrocytes and oligodendrocytes. Glia 1988; 1: 10-30.
6. Berry M. Post injury myelin breakdown products: a possible hypothesis to explain the failure of axonal regeneration in the mammalian CNS. Bibl Anat 1982; 23: 1-11.
7. Berry M, Hall S, Follows R, Wyse JPH. Defective myelination in the optic nerve of the Browman-Wyse (BW) mutant rat. J Neurocytol 1989; 18: 141-159.
8. Bignami A, Dahl D. The astroglial response to stabbing. Immunofluorescence studies with antibodies to astrocyte-specific protein (GFA) in mammalian and submammalian vertebrates. Neuropathol Appl Neurobiol 1976; 2: 99-111.
9. Bignami A, Eng LF, Dahl D, Uyeda CT. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res 1972; 43: 429-435.
10. Björklund H, Dahl D. Glial disturbances in isolated neocortex: Evidence from immunohistochemistry of intraocular grafts. Dev Neurosci 1982; 5: 424-435.
11. Björklund H, Dahl D, Haglid K, Rosengeren L, Olson L. Astrocytic development in fetal parietal cortex

grafted to cerebral and cerebellar cortex of immature rats. Dev Brain Res 1983; 9: 171-180.
12. Björklund H, Dahl D, Olson L. Morphometry of GFA and vimentin positive astrocytes in grafted and lesioned cortex cerebri. Int J Dev Neurosci 1984; 2: 181-192.
13. Björklund H, Eriksdotter-Nilsson M, Dahl D, Rose G, Hoffer B, Olson L. Image analysis of GFA-positive astrocytes from adolescence to senescence. Exp Brain Res 1985; 58: 163-170.
14. Bovolenta P, Liem RKH, Mason CA. Glial filament protein expression in astroglia in the mouse visual pathway. Dev Brain Res 1987; 33: 113-126.
15. Bray GM, Aguayo AJ. Exploring the capacity of CNS neurons to survive injury, regrow axons, and form new synapses in adult mammals. In: Seil FJ, ed, Neural Regeneration and transplantation. New York: Alan. R. Liss, 1980; 67-78.
16. Cammer W. Glutamine synthetase in the central nervous system is not confined to astrocytes. J Neuroimmunol 1990; 26: 173-178.
17. Cammer W, Tansey FA. The astrocyte as a locus of carbonic anhydrase in the brains of normal and dysmyelinating mutant mice. J Comp Neurol 1988; 275: 65-75.
18. Cammer W, Tansey FA. Carbonic anhydrase immunostaining in astrocytes in the rat cerebral cortex. J Neurochem 1988; 50: 319-322.
19. Cammer W, Tansey FA, Brosnan CF. Gliosis in the spinal cords of rats with experimental allergic encephalomyelitis: Immunostaining of carbonic anhydrase and vimentin in reactive astrocytes. Glia 1989; 2: 223-230.
20. Cammer W, Zhang H. Comparison of immunocytochemical staining of astrocytes, oligodendrocytes and myelinated fibres in the brains of carbonic anhydrase II-deficient mice and normal littermates. J Neuroimmunol 1991; 34: 81-86.
21. Cammer W, Zhang H. Carbonic anhydrase in distinct precursors of astrocytes and oligodendrocytes in the forebrains of neonatal and young rats. Dev Brain Res 1992; 6: 257-263.
22. Caroni P, Schwab ME. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. Neuron 1988; 1: 85-96.
23. Cavalcante LA, Barradas PC, Martinez AMB. Patterns of myelination in the opossum superior colliculus with additional reference to the optic tract. Anat Embryol 1991; 183: 273-285.
24. Crutcher KA. Tissue sections from the mature rat brain and spinal cord as substrates for neurite outgrowth in vitro: Extensive growth on gray matter but little growth on white matter. Exp Neurol 1989; 104: 39-54.
25. Doering LC, Eriksdotter-Nilsson M, Olson L. Spatial distributions of cytoskeletal proteins and the nerve growth factor receptor in septal transplants in oculo: Protection from abnormal immunoreactivity by hippocampal co-grafts. Neuroscience 1991; 44: 381-392.
26. Dyson SE, Harvey AR, Stone AF, Bell G. Metabolic activity in rat tectal grafts is influenced by host sensory innervation. J Neurosci 1988; 8: 1822-1829.
27. Eriksdotter-Nilsson M, Olson L. Growth of brain tissue grafts is dependent upon host age. Mech Ageing and Dev 1989; 49: 1-22.
28. Ghandour MS, Langley OK, Vincendon G, Gombos G, Filippi D, Limozin N, Dalmasso C, Laurent G. Immunochemical and immunohistochemical study of carbonic anhydrase II in adult rat cerebellum: A marker for oligodendrocytes. Neuroscience 1980; 5: 559-571.
29. Ghandour MS, Skoff P. Double-labeling in situ hybridization analysis of mRNAs for carbonic anhydrase II and myelin basic protein: Expression in developing cultured glial cells. Glia 1991; 4: 1-10.
30. Ghandour, MS, Skoff RP, Venta PJ, Tashian RE. Oligodendrocytes express a normal phenotype in carbonic anhydrase II-deficient mice. J Neurosci Res 1989; 23: 180-190.
31. Hajos F, Kalman M. Distribution of glial fibrillary acidic protein (GFAP) - immunoreactive astrocytes in the rat brain. II. Mesencephalon, rhombencephalon and spinal cord. Exp Brain Res 1989; 78: 164-173.
32. Hall SM, Kent AP. The response of regenerating peripheral neurites to a grafted optic nerve. J Neurocytol 1987; 16: 317-331.
33. Hartman BK, Agrawal HC, Agrawal D, Kalmbach S. Development and maturation of central nervous system myelin: Comparison of immunohistochemical localization of proteolipid protein and basic protein in myelin and oligodendrocytes. Proc Natl Acad Sci USA 1982; 79: 4217-4220.
34. Harvey AR, Gan SK, Pauken JM. Fetal tectal or cortical tissue transplanted into brachial lesion cavities in rats: Influence on the regrowth of host retinal axons. J Comp Neurol 1987; 263: 126-136.
35. Harvey AR, Golden GT, Lund RD. Transplantation of tectal tissue in rats III. Functional innervation of transplants by host afferents. Exp Brain Res 1982; 47: 437-445.
36. Harvey AR, Lund RD. Transplantation of tectal tissue in rats. II. Distribution of host neurons which project to transplants. J Comp Neurol 1981; 202: 505-520.
37. Harvey AR, Lund RD. The host retinal projection to tectal transplants in rats. In: Stone J, Dreher B, Rapaport DH, eds, Development of Visual Pathways in Mammals. New York: Alan R. Liss, 1984; 303-314.
38. Harvey AR, MacDonald AM. The development of acetylcholinesterase activity in normal and transplanted superior colliculus in rats. J Comp Neurol 1985; 240: 117-127.
39. Harvey AR, Plant GW, Kent AP. The distribution of astrocytes, oligodendroglia and myelin in normal and transplanted superior colliculus. Proc Aust Neurosci Soc 1992; 3: 200.

40. Harvey AR, Rush RA, Keating PJ. Cultured fetal tectal tissue grafted to the midbrain of newborn rats: morphology of grafts and innervation by host retinal and cortical axons. Brain Res 1988; 462: 89-98.
41. Harvey AR, Tan MML. Spontaneous regeneration of adult rat retinal ganglion cell axons in vivo. NeuroReport 1992; 3: 239-242.
42. Harvey AR, Warton SS. The morphology of neurons in rat tectal transplants as revealed by Golgi-Cox impregnation. Anat Embryol 1986; 174: 361-367.
43. Hatten ME. Riding the glial monorail: A common mechanism for glial-guided neuronal migration in different regions of the mammalian brain. TINS 1990; 13: 179-184.
44. Hatten ME, Liehm RKH, Shelanski ML, Mason CA. Astroglia in CNS injury. Glia 1991; 4: 233-243.
45. Huerta MF, Harting JK. The mammalian superior colliculus: studies of its morphology and connections. In: Vanegas H, ed, Comparative Neurology of the Optic Tectum. New York: Plenum Press, 1984; 687-773.
46. Jaeger CB. Cytoarchitectonics of substantia nigra grafts: A light and electron microscopic study of immunocytochemically identified dopaminergic neurons and fibrous astrocytes. J Comp Neurol 1985; 231: 121-135.
47. Jaeger CB, Lund RD. Influence of grafted glial cells and host mossy fibers on anomalously migrated host granule cells surviving in cortical transplants. Neuroscience 1982; 7: 3069-3076.
48. Janeczko K. The proliferative response of astrocytes to injury in neonatal rat brain. A combined immunocytochemical and autoradiographic study. Brain Res 1988; 456: 280-285.
49. Janzer RC, Raff MC. Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 1987; 325: 253-257.
50. Jhaveri S, Erzurmlu RS, Friedman B, Schneider GE. Oligodendrocyte and myelin formation along the optic tract of the developing hamster: An immunohistochemical study using the rip antibody. Glia 1992; 6: 138-148.
51. Kapfhammer JP, Schwab ME, Schneider GE. Antibody neutralization of neurite growth inhibitors from oligodendrocytes results in expanded pattern of postnatally sprouting retinocollicular axons. J Neurosci 1992; 12: 2112-2119.
52. Karnovsky MJ, Roots L. A "direct-coloring" thiocholine method for cholinesterases. J Histochem Cytochem 1964; 12: 219-221.
53. Kierstead SA, Rasminsky M, Fukuda Y, Carter DA, Aguayo AJ, Vidal-Sanz M. Electrophysiological response in hamster superior colliculus evoked by regenerating retinal axons. Science 1981; 246: 255-257.
54. Killackey HP, Erzurumlu RS. Trigeminotectal projections to the superior colliculus in the rat. J Comp Neurol 1981; 201: 221-242.
55. Kimelberg HK, Stieg PE, Mazurkiewicz JE. Immunocytochemical and biochemical analysis of carbonic anhydrase in primary astrocyte cultures from rat brain. J Neurochem 1982; 39: 734-742.
56. Konola JT, Tyler BM, Yamamura T, Lees MB. Distribution of proteolipid protein and myelin basic protein in cultured mouse oligodendrocytes: Primary vs secondary cultures. J Neurosci Res 1991; 28: 49-64.
57. Kruger S, Sievers J, Hansen C, Sadler M, Berry M. Three morphologically distinct types of interface develop between adult host and fetal brain transplants: Implications for scar formation in the adult central nervous system. J Comp Neurol 1986; 249: 103-116.
58. Kumpulainen T, Dahl D, Korhonen LK, Nystrom SHM. Immunolabelling of carbonic anlydrase isoenzyme C and glial fibrillary acidic protein in paraffin-embedded tissue sections of human brain and retina. J Histochem Cytochem 1983; 31: 879-886.
59. Landry CF, Ivy GO, Brown IR. Developmental expression of glial fibrillary acidic protein mRNA in the rat brain analyzed by in situ hybridization. J Neurosci Res 1990; 25: 194-203.
60. Lund RD, Harvey AR. Transplantation of tectal tissue in rats. I. Organization of transplants and pattern of distribution of host afferents within them. J Comp Neurol 1981; 201: 191-209.
61. Martin DL. Synthesis and release of neuroactive substances by glial cells. Glia 1992; 5: 81-94.
62. Meshul CK, Seil FJ, Herndon RM. Astrocytes play a role in regulation of synaptic density. Brain Res 1987; 402: 139-145.
63. Muller CM. A role for glial cells in activity-dependent central nervous plasticity? Review and Hypothesis. Int Rev Neurobiol 1992; 34: 215-281.
64. Paulson OB, Newman EA. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science 1987; 237: 896-898.
65. Prochiantz A. Mallat M. Astrocyte diversity. In: Raine CS, ed, Advances in Neuroimmunology. Ann NY Acad Sci 1988; 540: 52-63.
66. Reier PJ, Perlow MJ, Guth L. Development of embryonic spinal cord transplants in the rat. Dev Brain Res 1983; 10: 201-219.
67. Roussel G, Delounoy J-P, Nussbaum J-L, Mandel P. Demonstration of a specific localization of carbonic anhydrase C in the glial cells of rat CNS by an immunohistochemical method. Brain Res 1979; 160: 47-55.
68. Rousselet A, Antilio-Tovati A, Avaud D, Prochiantz A. In vitro regulation of neuronal morphogenesis and polarity by astrocyte derived factors. Dev Biol 1990; 137: 33-45.
69. Rubel EW, MacDonald GH. Rapid growth of astrocytic processes in N. magnocellularis following cochlea removal. J Comp Neurol 1992; 318: 415-425.

70. Schwab ME. Myelin-associated inhibitors of neurite growth and regeneration in the CNS. TINS 1990; 13: 452-456.
71. Shehab SAS, Cronly-Dillon JR, Nona SN, Stafford CA. Preferential histochemical staining of protoplasmic and fibrous astrocytes in rat CNS with GFAP antibodies using different fixatives. Brain Res 1990; 518: 347-352.
72. Shiota C, Miura M, Mikoshiba K. Developmental profile and differential localization of mRNAs of myelin proteins (MBP and PLP) in oligodendrocytes in the brain and in culture. Dev Brain Res 1989; 45: 83-94.
73. Silver J, Lorenz SE, Wahlsten D, Coughlin J. Axonal guidance during development of the great cerebral commissures: Descriptive and experimental studies, in vivo, on the role of preformed glial pathways. J Comp Neurol 1982; 210: 10-29.
74. Silver J, Sidman RL. A mechanism for the guidance and topographic patterning of retinal ganglion cell axons. J Comp Neurol 1980; 189: 101-111.
75. Sorenson T, Zimmer J. Ultrastructural organization of normal and transplanted fascia dentata: 1. A qualitative analysis of intracerebral and intraocular grafts. J Comp Neurol 1988; 267: 15-42.
76. Steedman HF. Polyester wax: A new ribboning embedding medium for histology. Nature 1957; 179: 1345.
77. Sternberger NH. Patterns of oligodendrocyte function seen by immunocytochemistry. In: Norton WT, ed, Oligodendroglia-Advances in Neurochemistry, Vol 5. New York: Plenum Press, 1984; 125-174.
78. Tan MML, Harvey AR. The development and distribution of α-bungarotoxin binding sites in rat tectal transplants. Dev Brain Res 1987; 36: 293-298.
79. Tardy M, Faces C, Riol H, Leprince G, Ratosonl P, Charriere-Bertrand C, Ninez J. Developmental expression of the glial fibrillary acidic protein mRNA in the central nervous system and in cultured astrocytes. J Neurochem 1989; 52: 162-167.
80. Vidal-Sanz M, Bray GM, Villegas-Perez MP, Thanos S, Aguayo AJ. Axonal regeneration and synapse formation in the rat superior colliculus by retinal ganglion cells in the adult rat. J Neurosci 1987; 7: 2894-2909.
81. Vidal-Sanz M, Bray GM, Aguayo AJ. Regenerated synapses persist in the superior colliculus after the regrowth of retinal ganglion cell axons. J Neurocytol 1991; 20: 940-952.
82. Walz W, Hertz L. Functional interactions between neurons and astrocytes. II. Potassium homeostasis at the cellular level. Prog Neurobiol 1987; 20: 133-183.
83. Warton SS, Jones DG. Postnatal development of the superficial layers in the rat superior colliculus: a study with Golgi-Cox and Kluver-Barrera techniques. Exp Brain Res 1985; 58: 490-502.
84. Zimmer J, Sunde N. Neuropeptides and astroglia in intracerebral hippocampal transplants. An immunohistochemical study in the rat. J Comp Neurol 1984; 227: 331-347.