PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage

NIGEL P. PRINGLE, HARDEEP S. MUDHAR, ELLEN J. COLLARINI and WILLIAM D. RICHARDSON

Department of Biology (Medawar Building), University College London, Gower Street, London WC1E 6BT, UK

Summary

Using in situ hybridization, we have visualized cells in the rat central nervous system (CNS) that contain mRNA encoding the platelet-derived growth factor alpha receptor (PDGF-αR). After embryonic day 16 (E16), PDGF-αR mRNA appears to be expressed by a subset of glial cells, but not by neurons. The temporal and spatial distribution of PDGF-αR$^+$ cells, together with ^{125}I-PDGF binding studies on subsets of glial cells in vitro, suggests that PDGF-αR may be expressed predominantly, or exclusively, by cells of the oligodendrocyte-type-2 astrocyte (O-2A) lineage. This conclusion is supported by the fact that the numbers of PDGF-αR$^+$ cells in developing and adult optic nerves correlate well with independent estimates of the number of O-2A progenitor cells in the nerve at equivalent ages. Small numbers of PDGF-αR$^+$ cells are present in the brain at E16, at which time they are found outside the subventricular germinal zones, suggesting that these cells do not express PDGF-αR until after, or shortly before they start to migrate away from the subventricular layer towards their final destinations. Reduced numbers of PDGF-αR$^+$ cells persist in the adult CNS. PDGF-αR is also expressed strongly in the meningeal membranes and choroid plexus, and in the inner limiting membrane of the retina.

Key words: PDGF receptors, rat CNS, glial cells, development, in situ hybridization.

Introduction

Polypeptide growth factors mediate a variety of cell-cell interactions in the developing central nervous system (CNS). They can signal cells to proliferate, migrate, differentiate or survive. One of the challenges of developmental neurobiology is to determine how the multitude of extracellular signals is coordinated, and their information interpreted, so as to generate the enormous variety of cell types and their complex interrelationships in the mature CNS. To make progress in this area we need to identify the signalling molecules involved, and define their cellular sources and targets during development.

Many characterized growth factors are expressed in the CNS during development and in the adult. These include members of the family of fibroblast growth factors (FGFs) (Gospodarowicz, 1984; Grothe et al., 1991; Eckenstein et al., 1991), the transforming growth factor-β (TGF-β) family (Millan et al., 1991), the insulin-like growth factors (IGF-I and IGF-II) (Ayer-le Lievre et al., 1991; Bartlett et al., 1991), the neurotrophins (Ernfors et al., 1990; Thoenen, 1991), and platelet-derived growth factor (PDGF) (Richardson et

al., 1988; Yeh et al., 1991; Sasahara et al., 1991). The function of many of these polypeptides in the CNS is still unclear. However, a specific role in the regulation of glial cell development has been proposed for PDGF (see Richardson et al., 1990 for a review). In the developing rat CNS there are glial progenitor cells (O-2A progenitors) that are capable of giving rise to either oligodendrocytes or type-2 astrocytes in vitro (Raff et al., 1983). O-2A progenitors are known to express PDGF receptors (Hart et al., 1989; McKinnon et al., 1990), and can be stimulated to divide in culture by PDGF (Noble et al., 1988; Richardson et al., 1988). PDGF mRNA and protein are present in the developing CNS (Richardson et al., 1988; Pringle et al., 1989; Yeh et al., 1991; Sasahara et al., 1991), so it is likely that PDGF is involved in the development of the O-2A lineage in vivo. When O-2A progenitors are cultured in the absence of growth factors, they stop dividing and differentiate rapidly into oligodendrocytes (Raff et al., 1983). When cultured in medium containing PDGF, O-2A progenitors continue to divide (Noble et al., 1988; Richardson et al., 1988), and oligodendrocytes are produced over a period of several weeks in vitro, just as in vivo (Raff et al., 1985, 1988). Thus, PDGF may be

important for controlling the final number of oligodendrocytes, and the time and rate of their production during development. PDGF is expressed widely in the developing CNS by neurons (Yeh et al., 1991; Sasahara et al., 1991) and by glial cells, possibly type-1 astrocytes (Richardson et al., 1988; Pringle et al., 1989; Yeh et al., 1991). Either of these cell types might supply PDGF to proliferating O-2A progenitors in vivo. The fact that PDGF is present in many mature neurons, including presumptive nerve terminals (Sasahara et al., 1991), raises the possibility that PDGF may also participate in some aspect of neuronal metabolism. It is therefore of interest to determine whether neurons as well as glia possess PDGF receptors and might be capable of responding to PDGF.

PDGF is a dimer of A and B chains with the structure AA, BB or AB, depending on its source (see Heldin and Westermark, 1990 for a review of PDGF). PDGF from human platelets, for example, consists mainly of PDGF-AB, while some tumour cell lines express predominantly PDGF-AA or PDGF-BB. In common with many other growth factors, PDGF elicits its biological effects by binding to transmembrane receptors with extracellular ligand-binding domains and intracellular tyrosine kinase domains. There are two PDGF receptors with different ligand specificities; PDGF alpha-receptors (PDGF-αR) can bind both A and B chains of PDGF whereas beta-receptors (PDGF-βR) bind only B chains. The unoccupied receptors are monomeric and inactive, but PDGF binding induces receptor dimerization and activates their tyrosine kinase domains. Thus, the response of a given cell to PDGF depends on the PDGF receptor type(s) that it expresses as well as the PDGF isoform(s) that it encounters. O-2A progenitor cells express predominantly PDGF-αR (Hart et al., 1989; McKinnon et al., 1990), and are stimulated to divide in vitro by all three dimeric isoforms of PDGF, although PDGF-AA is more effective than PDGF-BB (Pringle et al., 1989) because of the higher affinity of PDGF A chains for PDGF-αR (Heldin et al., 1988). It is not known whether any other cell types in the CNS express PDGF-αR. It was recently reported, however, that many CNS neurons express PDGF-βR (Smits et al., 1991).

The aim of the in situ hybridization studies reported here was to obtain information about the types of cells that express PDGF-αR in the developing and mature rat CNS. We used a probe, corresponding to the extracellular domain of PDGF-αR, that unambiguously distinguishes PDGF-αR mRNA from PDGF-βR mRNA. Our in situ hybridization results, together with ^{125}I-PDGF-AA binding studies on subsets of CNS glial cells in vitro (see below), lead us to speculate that PDGF-αR may be restricted to O-2A lineage cells in the late embryonic and postnatal CNS. If so, PDGF-αR might be a useful marker of this lineage in vivo and, in combination with other putative lineage markers, might help us reconstruct the developmental history of O-2A progenitor cells and their forebears (pre-progenitors) in situ. The ultimate origin of the O-2A lineage, in common with many other neural lineages, is presum-

ably in the subventricular germinal zones, some parts of which remain mitotically active after birth (Altman, 1966). Our observation that PDGF-αR$^+$ cells apparently arise in the embryonic CNS outside the subventricular layer suggests that PDGF-αR is not turned on until after these cells migrate away from the germinal zone toward their final destinations.

Materials and methods

Northern blot analysis

Total cellular RNA was prepared from freshly dissected rat brains by the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987), and poly(A)-containing RNA was selected on oligo(dT)-cellulose. Poly(A) RNA (10 μg per lane, estimated by absorbance at 260 nm) was denatured and electrophoresed on a 1% agarose gel containing formaldehyde (Sambrook et al., 1989). After transfer to Zetaprobe nylon membrane (Biorad) the RNA was hybridized with a DNA probe labelled with ^{32}P to a specific activity of ~2 × 10^8 cts/minute/μg by random priming (Feinberg and Vogelstein, 1984). The cDNA probe was a ~1.5 kb *Sac*I-*Pvu*II fragment containing most of the coding sequence for the extracellular domain of rat PDGF-αR. Membranes were washed under conditions of high stringency (0.1 × SSC at 53°C) according to the manufacturer's instructions, and exposed to Hyperfilm-MP (Amersham) at −70°C with an intensifying screen.

Preparation of tissue sections

Sprague-Dawley and Wistar rats were employed interchangeably for this study; no differences were observed between these strains. Whole heads of E16, E18 and P0 rats, and the brains of P3, P6, P10, P21 and adult (six-month-old) rats were processed for sectioning and in situ hybridization as follows. Tissues were fixed for 24 hours in ice-cold 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) and cryoprotected by immersion in 0.5 M sucrose in PBS for 24 hours at 4°C. Tissues were immersed in OCT embedding compound (BDH) and frozen in aluminium foil boats placed onto dry ice. Tissue was kept at −70°C until required. Frozen sections were cut (10 μm nominal thickness) on a cryostat and were collected on 3-aminopropyltriethoxysilane (APES)-coated glass microscope slides. Sections were briefly heated at 55°C, air dried for ~2 hours at 20°C and postfixed in 4% paraformaldehyde in PBS for 15 minutes at room temperature. After two 5 minute washes in PBS, the sections were dehydrated in increasing concentrations of ethanol (30, 60, 80, 95 and 100% for 1 minute each) and stored at −70°C.

In situ hybridization

Our in situ hybridization procedure, based on that of Lawrence and Singer (1985), has been described previously (Pringle et al., 1989), except that we omitted the preincubation in non-radioactive α-thio UTP, and we included an extra wash in 4×SSC for 1 hour at room temperature immediately following the hybridization step. Our ^{35}S-labelled RNA probe was generated as described previously by in vitro transcription from a ~1.5 kb *Sac*I-*Pvu*II fragment encompassing most of the extracellular domain of the rat PDGF-αR cloned into pGEM1. For autoradiography, the slides were coated with Ilford K5 nuclear emulsion, exposed for 1-2 weeks in the dark at 4°C, and developed in Kodak D-19. Some sections were lightly counterstained with Hematoxylin (Gills No.3, Sigma).

Cell counts

The proportion of cells in the CNS that are PDGF-αR$^+$ was estimated from pairs of bright- and dark-field micrographs of lightly stained sections of anterior hypothalamus or optic nerves. Micrographs were printed at approximately 50× final magnification. PDGFαR$^+$ cells were counted in dark field, and the total number of cell nuclei in bright field. At least three pairs of micrographs from different sections of one animal were counted at each age. Approximately 500 total cells were counted in each section and the proportion of PDGF-αR$^+$ cells (mean percent ± standard deviation) tabulated (Table 1). These numbers are likely to be minimum estimates, as PDGF-αR$^+$ cells frequently occur in pairs and it is difficult to resolve these pairs under dark-field illumination. Neither the density of PDGF-αR$^+$ cells nor total cell density varied significantly throughout the hypothalamus at each age, so the figures we obtained were not critically dependent on the precise region of hypothalamus examined. Nevertheless, the numbers in Table 1 are only approximate estimates, intended merely to illustrate how the PDGF-αR$^+$ cell population increases in the hypothalamic region during development. The estimates of cell numbers in optic nerve have a higher confidence level because counts were made at different positions along the length of the nerve in order to minimise systematic errors that might be caused by regional variations in cell density, which at any rate appeared to be insignificant after P2.

Primary glial cell cultures

Cortical astrocytes

Primary cultures of astrocytes from neonatal rat cerebral cortex were established by a modification of the method of McCarthy and de Vellis (1980). Briefly, neonatal rat pups (~2 days old) were killed and the cerebral cortex removed. The cortex was dissociated with trypsin and the cells cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (DMEM/FCS) until confluent. The cultures were vigorously agitated overnight at 37°C to remove cells growing on top of the monolayer of astrocytes. Rapidly dividing cells were subsequently eliminated by addition of cytosine arabinoside (10^{-5} M) for 24 hours. The remaining cells were trypsinized, plated into 24-well tissue-culture dishes and grown in DMEM/FCS until confluent and before being used for ^{125}I-PDGF binding studies (see below). The purity of astrocyte cultures was assessed by staining with antibodies to glial fibrillary acidic protein (GFAP). Cultures were routinely greater than 98% GFAP$^+$ astrocytes.

O-2A progenitors

Purified O-2A progenitor cultures were established by a modification of a method developed by B. Barres and E. Collarini (unpublished). Cultures of cells from neonatal rat cerebral cortex were established as described above. Instead of agitating the cultures as above, they were trypsinized and the cell suspension depleted of astrocytes and macrophages by incubating on Petri dishes coated with anti-RAN-2 antibody (Bartlett et al., 1981). Oligodendrocytes were removed on dishes coated with anti-galactocerebroside (GC) (Raff et al., 1978; Ranscht et al., 1982). O-2A progenitors were positively selected on dishes coated with antibody A2B5 (Eisenbarth et al., 1979). O-2A progenitors were trypsinized from the A2B5 dishes and grown in 24-well dishes in our modification of Bottenstein and Sato's (BS) defined medium (Richardson et al., 1988), supplemented with 0.5% FCS, PDGF-AA and bFGF (Peprotech recombinant growth factors, 10 ng/ml each). O-2A progenitors were grown until confluent and used

for ^{125}I-PDGF binding studies as described below. The final cultures were more than 96% A2B5$^+$GC$^-$ O-2A progenitors.

Microglial cells

Cultures of microglial cells (the macrophages of the CNS) were established from rat brains by a modification of the method of Giulian and Baker (1986). Neonatal rat brains were dissociated with papain and the cell suspension plated onto Petri dishes coated with IgM (to retain cells carrying Fc receptors). The adherent microglia were trypsinized from the dishes and grown in 24-well dishes in DMEM/FCS supplemented with 25% mouse L-cell conditioned medium (which contains CSF-1, a macrophage mitogen). When the cells were semi-confluent they were used for ^{125}I-PDGF binding studies as described below. The purity of the microglial cultures was assessed by staining with Bandeiraea simplicifolia B$_4$-lectin (BL) (Sigma), which labels microglia in the CNS (Chugani et al., 1991). Cultures were greater than 98% BL$^+$.

^{125}I-PDGF binding

^{125}I-labelled PDGF-AA was a generous gift from C.-H. Heldin and had a specific activity of ~3.9×10^4 cts/minute/ng. Confluent cultures of astrocytes, microglia and NIH 3T3 cells were incubated in DMEM containing 0.5% FCS overnight prior to assaying for PDGF binding. O-2A progenitors were incubated in BS medium containing 0.5% FCS and 10 ng/ml bFGF for 16 hours prior to the binding assay. Cultures were washed in HEPES-buffered Minimal Essential Medium (MEMH) before incubating for 6 hours at 4°C with ^{125}I-PDGF-AA (2 ng/ml) in MEMH containing 1% bovine serum albumin (binding buffer), with or without a 100-fold excess of unlabelled PDGF-AA or PDGF-BB. The cells were washed three times in binding buffer, and bound ^{125}I-PDGF-AA was measured by lysing the cells in PBS containing 1% Triton X-100 (v/v) and counting in a γ-counter.

Results

To map the distribution of PDGF-αR mRNA in the developing rat CNS, we performed in situ hybridizations with a ^{35}S-RNA probe corresponding to part of the extracellular domain of the rat PDGF-αR. This probe recognized a single ~7 kb mRNA on northern blots of poly(A)-containing RNA from perinatal rat brain (Fig. 1). A PDGF-αR transcript of 6.8 kb was previously described (Lee et al., 1990). Thus, our probe
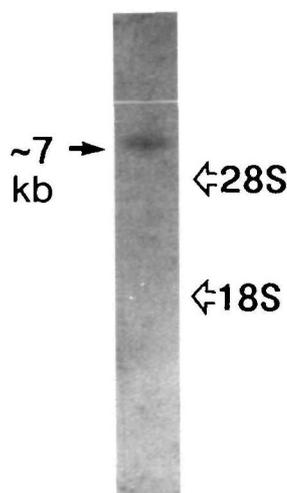

~7 →
kb

⟵28S

⟵18S

Fig. 1. Northern blot analysis of PDGF-αR mRNA in P13 rat brain. Poly(A)-containing mRNA (10 μg) was electrophoresed on a 1% agarose gel containing formaldehyde, transferred to nylon membrane and hybridized with a ^{32}P-labelled cDNA probe coding for the extracellular domain of PDGF-αR. Our probe recognises a single mRNA species at ~7 kb. The positions of the ribosomal 18S and 28S subunits are shown for comparison.

specifically recognizes PDGF-αR mRNA and does not cross-hybridize with PDGF-βR mRNA, a 5.3 kb species (Matsui et al., 1989) that is also present in the rat CNS (Sasahara et al., 1991). Cryostat sections (10 μm nominal thickness) of embryonic and postnatal rat brains (E16, E18, P0, P2, P6, P10, P20 and adult) were subjected to in situ hybridization with the PDGF-αR probe, as described in Materials and methods. Subsequently, sections were subjected to autoradiography, lightly stained with hematoxylin and examined in the microscope under bright- and dark-field illumination.

Small numbers of PDGF-αR+ cells are present in the rat forebrain by E16

Fig. 2 shows a coronal section through an E16 rat head,

at the level of the anterior forebrain. PDGF-αR is expressed strongly in most craniofacial tissues, the anterior proliferating lens epithelium and in the meningeal cell layer that surrounds and bisects the forebrain; there is very little PDGF-αR signal in the forebrain itself at E16. The few isolated PDGF-αR+ cells that are present in the E16 brain lie outside of the halo of densely packed cells that surrounds the ventricles (corresponding to the densely stained region delineated by dotted lines in the inset to Fig. 2). This region comprises the ventricular and subventricular zones, which are the major mitotically active areas and the primary source of new neurons and glia. It is noteworthy that at E16 most PDGF-αR+ cells lie inferior (ventral) to the lateral ventricles; few if any are present in the developing cerebral cortex at this age,

Fig. 2. Distribution of PDGF-αR mRNA in E16 rat head, visualized by in situ hybridization. Illustrated is a coronal section at the level of the anterior forebrain. Cryosections (10 μm nominal thickness) were subjected to in situ hybridization and autoradiography, using 35S-labelled single-stranded RNA probes specific for PDGF-αR. The exposed silver grains were developed and the sections photographed in dark-field illumination. Many craniofacial structures are strongly labelled as are the anterior proliferating cells of the lens. A few PDGF-αR+ cells are seen in the developing forebrain, away from the germinal zones surrounding the lateral ventricles. Scale bar, 100 μm. Inset: LV, lateral ventricle; SVZ, subventricular zone; Men, meningeal membrane; L, lens; RET, retina.
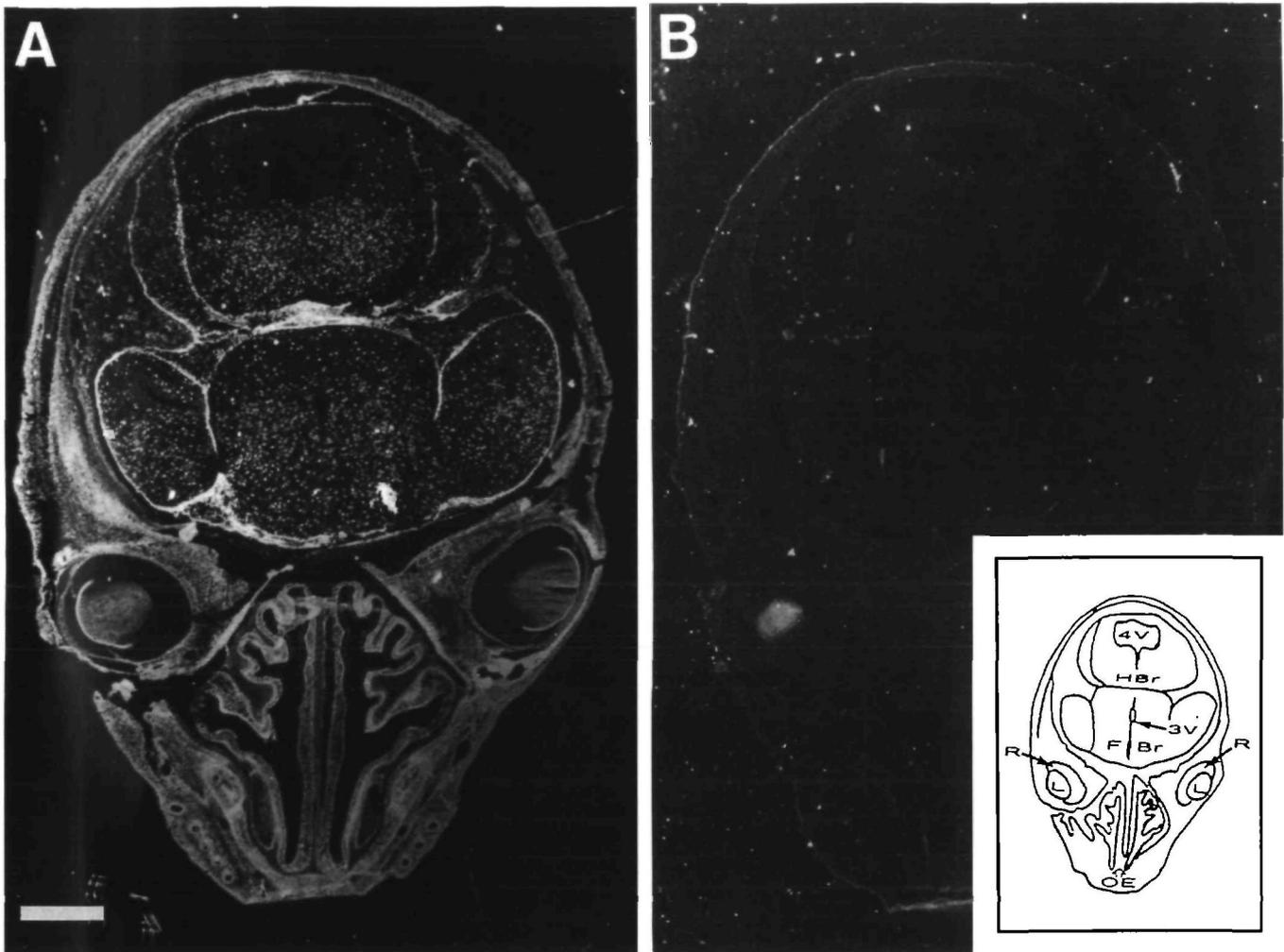
Fig. 3. PDGF-αR mRNA in the E18 rat head. Panel A, Horizontal section through the inferior (ventral) region of the brain, hybridized with the ^{35}S-labelled PDGF-αR "antisense" probe, autoradiographed and photographed under dark-field illumination. There are significantly more PDGF-αR^{+} cells in the brain at E18 than at E16. Panel B, adjacent section hybridized with the ^{35}S-labelled PDGF-αR "sense" probe shows no specific labelling. Scale bar, 100 μm. Inset: FBr, ventral forebrain; HBr, hindbrain; 3V and 4V, third and fourth ventricles; R, retina; L, lens; OE, olfactory epithelium.

even though E16-E17 is the peak time of production of postmitotic neurons in the rat cortex (see Jacobson, 1970 for review).

PDGF-αR^{+} cells proliferate during late embryonic and early postnatal brain development

Fig. 3 shows a pair of consecutive horizontal sections through an E18 rat head, one hybridized with the PDGF-αR antisense probe (i.e. complementary to the mRNA) and the other with the sense probe. Only the antisense probe hybridizes, providing further verification of the specificity of the signal. Fig. 4 shows a coronal section through the anterior forebrain at E18. PDGF-αR^{+} cells are scattered throughout the thalamus and hypothalamus. Some PDGF-αR^{+} cells are present at the lateral tips of the subcortical white matter at E18, but not in the more medial white or grey matter of the cortex. There are significantly more PDGF-αR^{+} cells in the E18 forebrain than at E16 (compare Fig. 2 with

3 and 4), but most of these cells still lie outside of the subventricular zones.

The number of PDGF-αR^{+} cells increases further by the day of birth (E21/P0) (Table 1). Individual PDGF-αR^{+} cells are apparent throughout the brain; their distribution is relatively uniform in many areas but, in general, they are less numerous in peripheral areas such as the inferior and superior colliculi, pons and the ventral-most hypothalamus (not shown). PDGF-αR^{+} cells are abundant in the cerebral cortex at P0, especially in the subcortical white matter, including the cingulum (Fig. 5). Relatively weakly labelled PDGF-αR^{+} cells are detected in the developing cerebellum at P0, mainly in the internal granule layer (Fig. 6A,B).

PDGF-αR^{+} cells multiply still further after birth (Table 1). By P3, there are large numbers of strongly labelled cells in the internal granule layer of the cerebellum, for example (Fig. 6C,D). Fig. 7 shows coronal and sagittal sections through a P10 rat brain. At
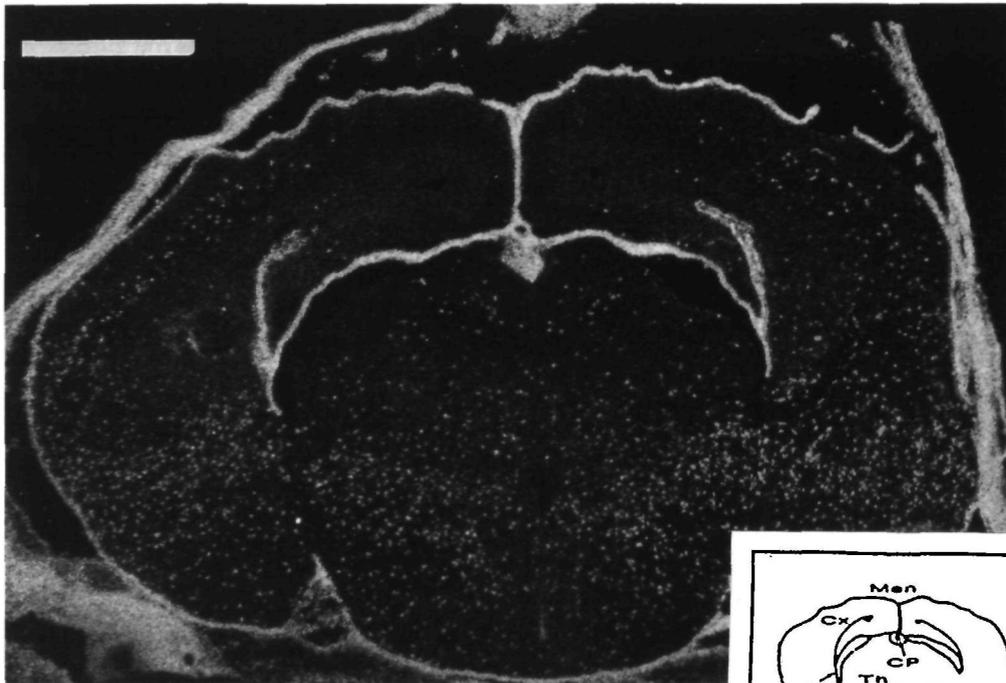
Fig. 4. PDGF-αR mRNA in the E18 forebrain (coronal section). PDGF-αR⁺ cells are more concentrated in the lower (ventral) half of the brain and at the lateral tips of the lateral ventricles. PDGF-αR⁺ cells are also present in the more lateral regions of the cortical intermediate zone (developing subcortical white matter), but not in the more medial white or gray matter of the cortex. Scale bar, 100 μm. Inset: LV, lateral ventricle; 3V, third ventricle; Th, thalamus; Hypoth, hypothalamus; Cx, cerebral cortex; CP, choroid plexus; Men, meningeal membrane.
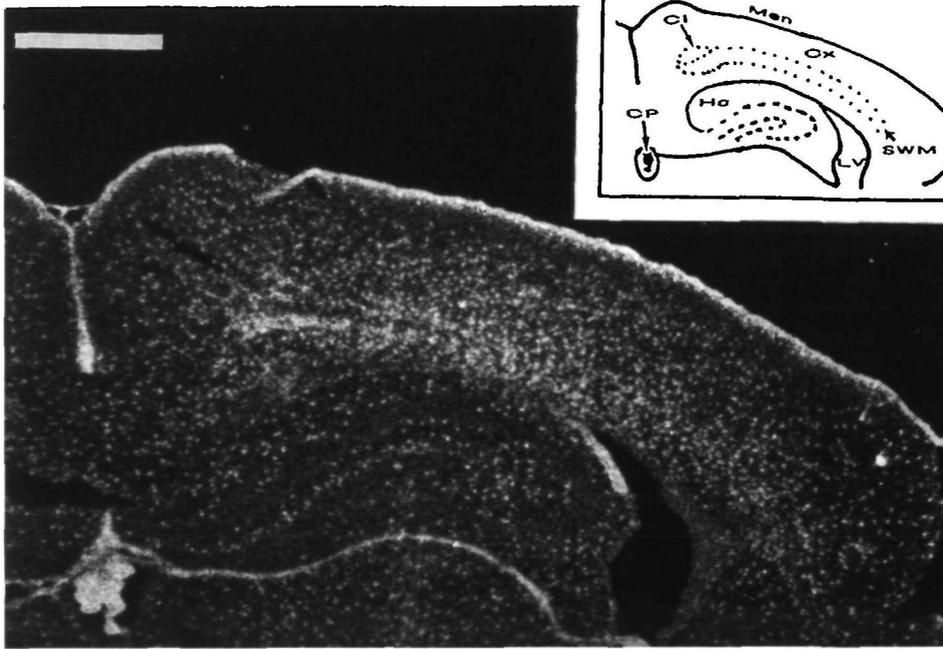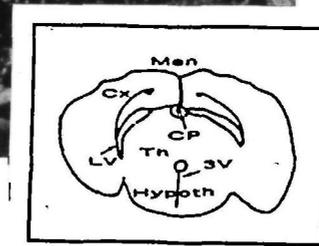

Fig. 5. PDGF-αR⁺ cells in the P0 cerebral cortex and developing hippocampus (coronal section). PDGF-αR⁺ cells are present throughout the cortex up to the pial surface, but are more concentrated in the subcortical white matter, including the cingulum. Scale bar, 50 μm. Inset: Cx, cerebral cortex; SWM, subcortical white matter; Ci, cingulum; LV, lateral ventricle; Hc, hippocampus; CP, choroid plexus; Men, meningeal membrane.
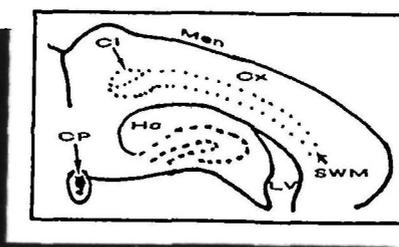
Fig. 6. Distribution of PDGF-αR⁺ cells in the developing rat cerebellum. Panels A and B, bright- and dark-field images of the P0 cerebellum and adjacent brain regions. A few weakly-positive cells are visible in the internal granule layer of the cerebellum (IGL), but almost none in the superior medullary vellum (SMV) or inferior colliculus (IC). Panels C and D, the P3 cerebellum. PDGF-αR⁺ cells are more numerous and appear more intensely labelled than before; they are distributed throughout the IGL, but are absent from the molecular layer (ML) and external granule layer (EGL). Panels E and F, the P10 cerebellum.

PDGF-αR⁺ cells in the IGL are concentrated in the developing foliar white matter (WM). The ML and EGL are still negative for PDGF-αR. Panels G and H, the adult rat cerebellum. PDGF-αR⁺ cells are now present in the (much expanded) ML and the IGL. The intense scattering from the foliar white matter tracts obscures the in situ hybridization signal there. At all ages examined, the choroid plexus (CP) and meningeal membranes (MEN) are strongly positive for PDGF-αR. The Purkinje cells (P, which lie at the boundary between the ML and IGL) do not express detectable PDGF-αR. Scale bars, 100 μm.
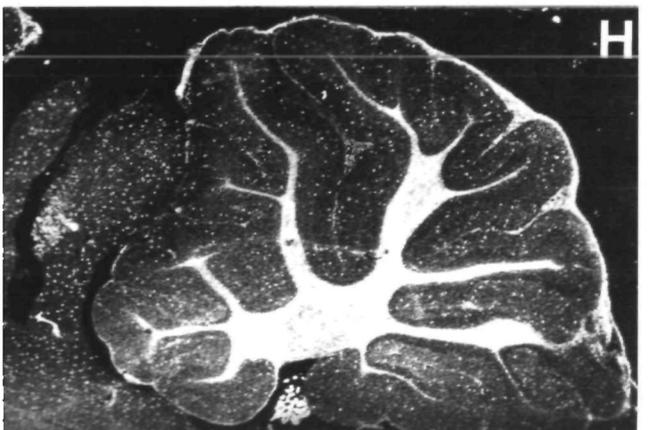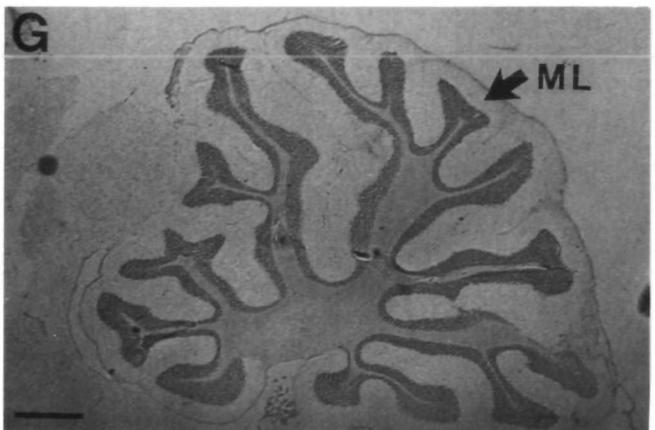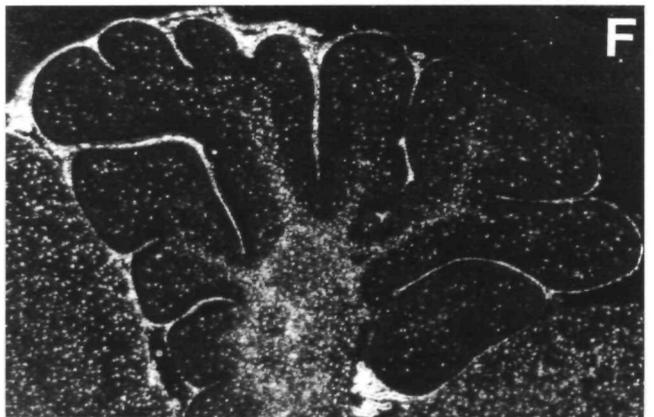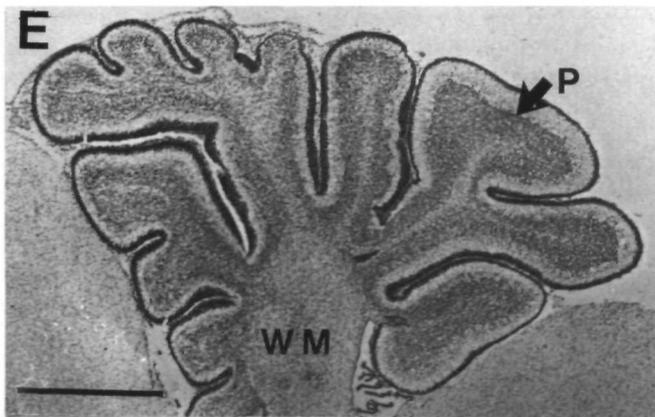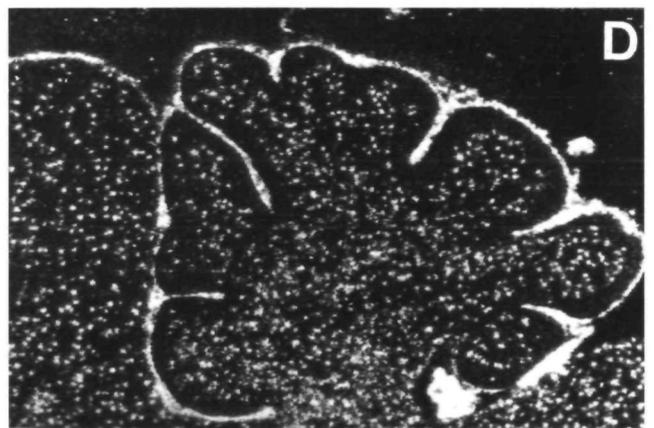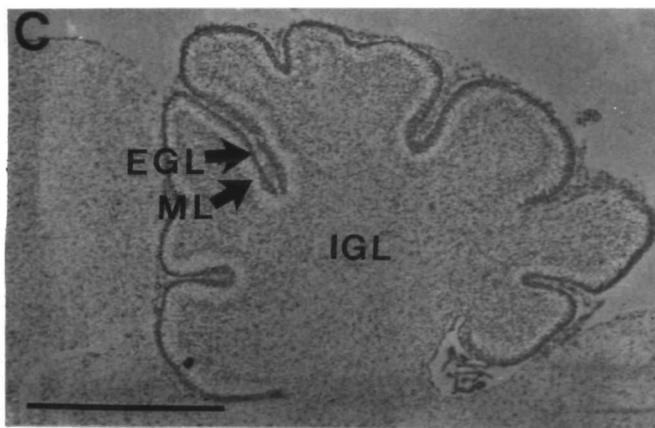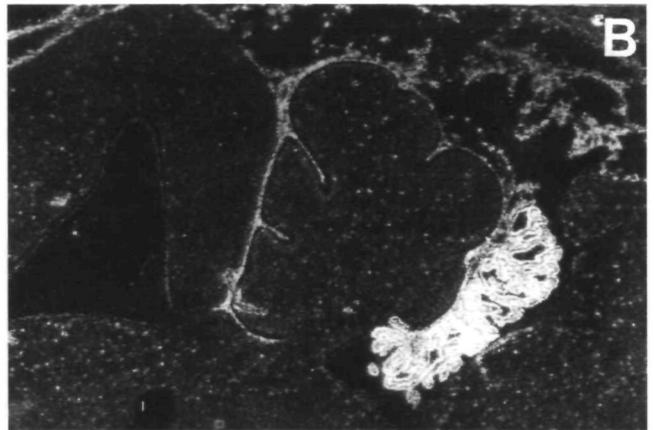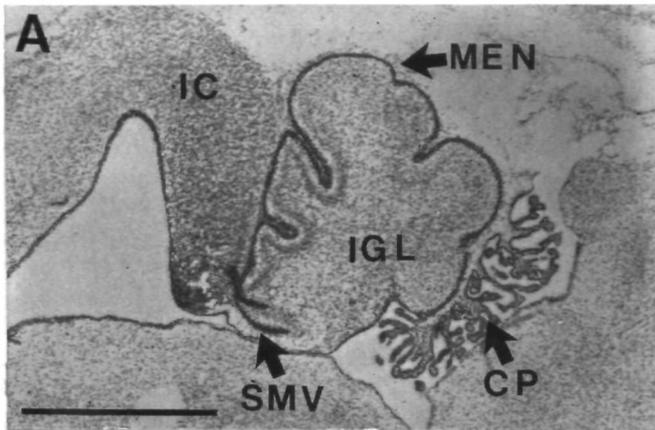
Table 1. *Numbers of PDGF-αR⁺ cells in the developing rat CNS*

Age	% PDGFαR⁺ cells	Total PDGFαR⁺ cells per optic nerve
Hypothalamus		
E16	0.15±0.03	–
E18	3.2 ±0.5	–
P0	4.8 ±0.2	–
P10	7.8 ±0.1	–
P20	6.5 ±0.14	–
ADULT	4.3 ±0.35	–
Optic nerves		
P7	11.1±0.1 *(~11.5%)**	1.6×10^4 *(~1.2×10⁴)†*
P15	9.3±0.7 *(~11.5%)**	2.4×10^4 *(~2.2×10⁴)†*
ADULT	3.6±0.7 *(~4%)**	1.9×10^4 *(~1.8×10⁴)†*

*Data taken from Vaughn (1969).
†Data taken from Fulton et al. (1992).

PDGF-αR⁺ cells were counted in dark-field micrographs of in situ autoradiographs of the hypothalamus and optic nerve. Total cell numbers were estimated from bright-field micrographs of the same sections, which had been lightly stained with hematoxylin. Between 1000 and 5000 total cells in at least three different fields of view were scored at each age, and the number of PDGF-αR⁺ cells expressed as a percentage of the total. In the hypothalamus the proportion of PDGF-αR⁺ cells increases from E16 to P10, when they account for ~8% of the total cells, and drops to ~4% of cells in the adult. In the optic nerve, the proportion of PDGF-αR⁺ cells is ~11% at P7, and ~4% in the adult. Numbers of PDGF-αR⁺ cells in whole optic nerves were calculated from these percentages and independent measurements (from DNA content) of the total number of cells in the nerve at each age (Barres et al., 1992). For comparison, we have listed Vaughn's (1969) figures for the proportions of "small glioblasts", counted in electron micrographs of rat optic nerves. From their ultrastructure, these cells probably correspond to O-2A progenitors (Fulton et al., 1992). We have also listed numbers of presumptive O-2A progenitors visualized in wholemount preparations of rat optic nerves by quisqualate-stimulated cobalt uptake (Fulton et al., 1992). Numbers of O-2A progenitors in vivo have also been estimated by counting A2B5⁺GC⁻ cells in suspensions of dissociated optic nerve cells (assuming complete recovery after the dissociation procedure). In this way, Miller et al. (1985) estimated 6 × 10³ O-2A progenitors in the P7 nerve; Small et al. (1987) estimated 9 × 10³ at P5 and 1.6 × 10⁴ at P9.

this age PDGF-αR⁺ cells are prominent in all regions of the brain including the outermost regions of the cerebral cortex. At P10 the density of PDGF-αR⁺ cells is noticeably higher, however, in developing white matter tracts such as the subcortical white matter (Fig. 7) and the foliar white matter of the cerebellum (Fig. 6E,F). At P20, the distribution and density of PDGF-αR⁺ cells in all regions of the brain (not shown) was very similar to the picture at P10.

The nuclear morphology of PDGF-αR⁺ cells in the forebrain is indicative of glial cells, not neurons

Fig. 8 shows an enlarged view of part of the P10 cerebral cortex corresponding to the area within the frame in the inset in Fig. 7A. Developed silver grains are associated with individual cells (or small groups of cells) possessing small, spherical nuclei that stain intensely with haematoxylin (small arrows). This nuclear morphology is usually regarded as a hallmark of glial cells, and oligodendroglia especially (e.g. see Bunge, 1968).

Often, the labelled cells are in pairs, suggesting that they may have divided locally. Neurons with large, lightly-staining nuclei (large arrows) never labeled for PDGF-αR, either in the cerebral cortex (Fig. 8) or in any other brain region that we examined.

PDGF-αR⁺ cells accumulate in the developing optic nerve in a wave starting from the optic chiasm

We find PDGF-αR⁺ cells in the rat optic nerve, from around birth (Fig. 9) through to adulthood (Fig. 10). The optic nerve carries axons from ganglion neurons in the retina to the brain, but does not contain any neuronal cell bodies. Hence, the PDGF-αR⁺ cells in the nerve correspond either to glial cells or, less likely, non-neural cells such as capillary endothelial cells or fibroblasts.

At P0, there are relatively few PDGF-αR⁺ cells in the optic nerve; these are more numerous towards the optic chiasm, being almost absent from the retinal end of the nerve (Fig. 9A,B). Two days later, at P2, PDGF-αR⁺ cells occupy the optic nerve uniformly, except for a short region at the extreme retinal end (Fig. 9C). The progressive appearance of PDGF-αR⁺ cells from the chiasmal to the retinal end of the optic nerve is similar to the way that O-2A progenitor cells accumulate in the nerve. It has been suggested that this results from a wave of migration of O-2A progenitors from a germinal zone, presumably around the third ventricle, into the developing nerve *via* the optic chiasm (Small et al., 1987).

There is a narrow band of PDGF-αR⁺ cells at the inner face of the retina from P0 (Fig. 9) through to adulthood (not shown). These cells lie in the inner limiting membrane, a basement membrane composed of Muller glial cell endfeet and non-CNS cells (including blood vessels) that overlies the optic fibre layer.

PDGF-αR⁺ cells persist in the adult CNS

Fig. 10 shows a coronal section through the forebrain of an adult (approximately six-months-old) rat. PDGF-αR⁺ cells are visible in all regions of the section, including the optic nerves. Cell counts indicate that the number of cells expressing PDGF-αR in the thalamus declines from about 8% to about 4% of total cells between P10 and adulthood (Table 1). PDGF-αR⁺ cells are also present in the molecular and internal granule layers of the adult cerebellum (Fig. 6G,H); it is not possible to distinguish individual cells in the foliar white matter, because of the high scattering background from myelinated fibres.

O-2A lineage cells, but neither microglial cells nor cortical astrocytes, express PDGF-αR in vitro

The in situ hybridization data presented above, together with previous ¹²⁵I-PDGF binding studies on optic nerve cells in culture (Hart et al., 1989), suggested to us that PDGF-αR might be expressed solely by O-2A lineage cells in the CNS. We investigated this possibility further by attempting to bind ¹²⁵I-PDGF-AA to various non-neuronal rat brain cells in vitro, since PDGF-AA should only bind to cells that possess PDGF-αR
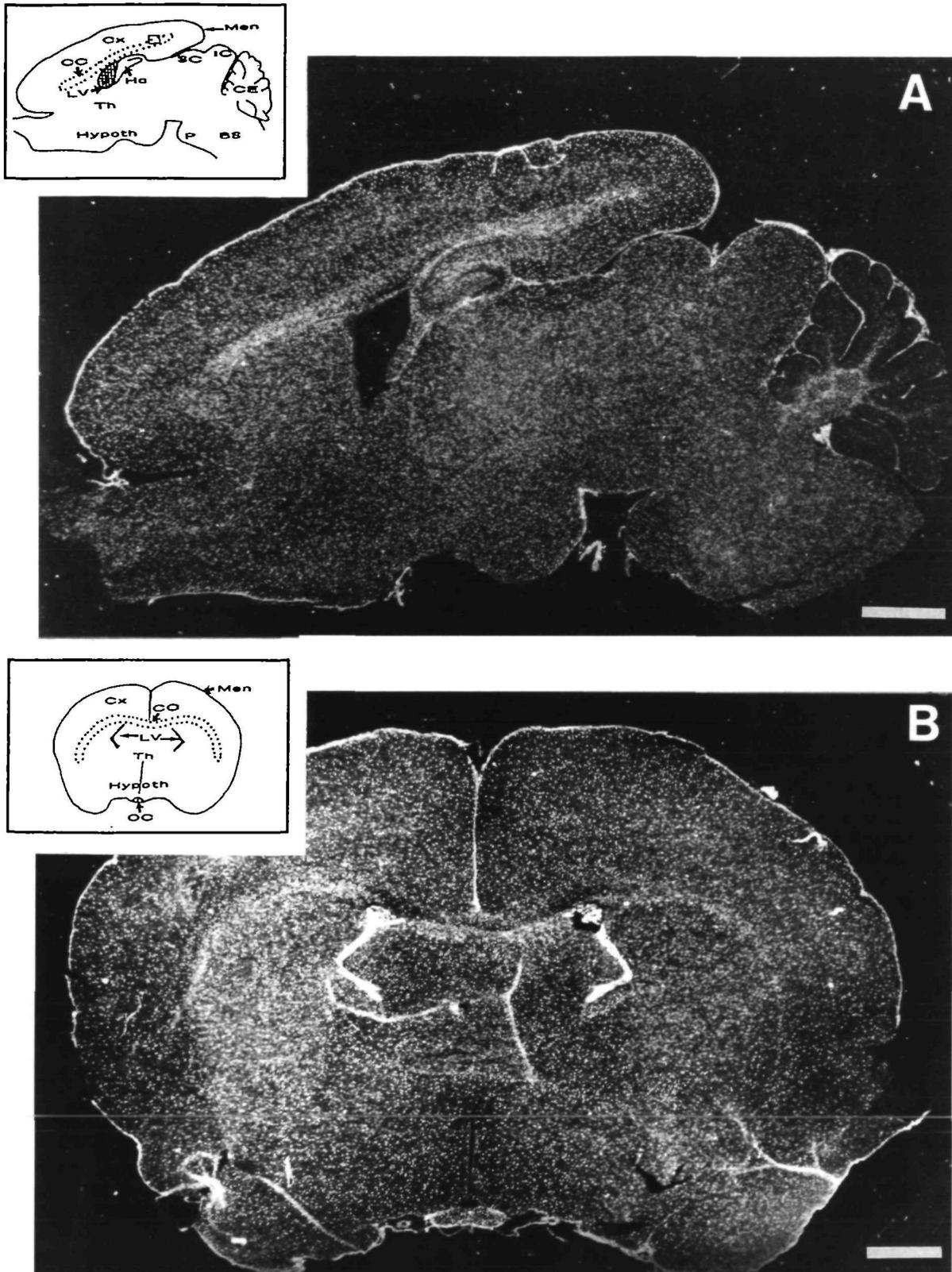
Fig. 7. Expression of PDGF-αR mRNA in the P10 rat brain. Panel A, sagittal section. Panel B, coronal section. PDGF-αR$^+$ cells are abundant and fairly evenly spaced throughout the brain, being somewhat more concentrated in the corpus callosum and the cerebellar white matter tracts. The choroid plexus within the lateral ventricles, and the meningeal membranes are strongly PDGF-αR$^+$ as before. Scale bar, 100 μm. Inset: Cx, cerebral cortex; CC, corpus callosum; Hc, hippocampus; Th, thalamus; Hypoth, hypothalamus; CE, cerebellum; BS, brainstem; P, pons; SC and IC, superior and inferior colliculi; LV, lateral ventricles; OC, optic chiasm.
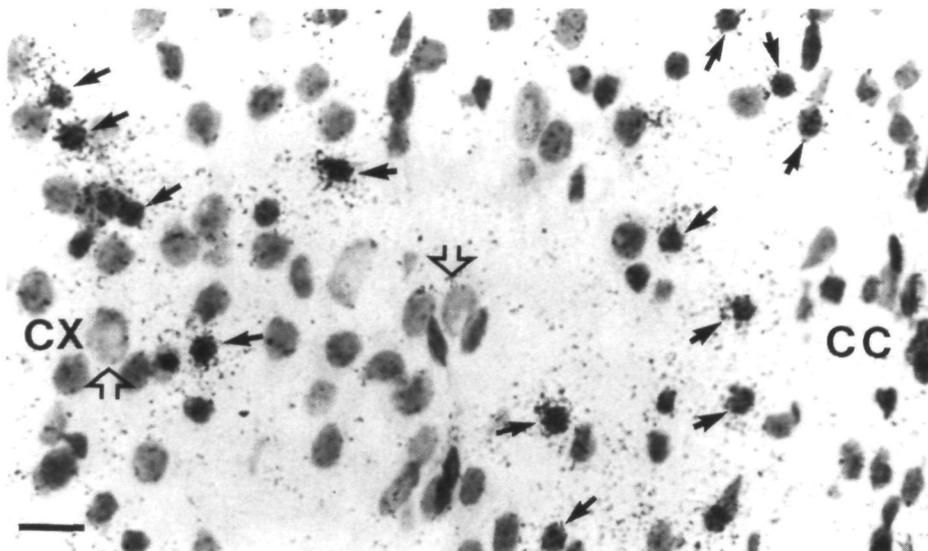
Fig. 8. Higher magnification bright field micrograph of the region within the frame of Fig. 7A, comprising part of the corpus callosum (CC) and the polymorphous cell layer of the cerebral cortex (CX). Exposed silver grains are associated with cells with small, densely-stained nuclei (small arrows). Neurons with large nuclei (large arrows) are negative for PDGF-αR. Scale bar, 10 μm.
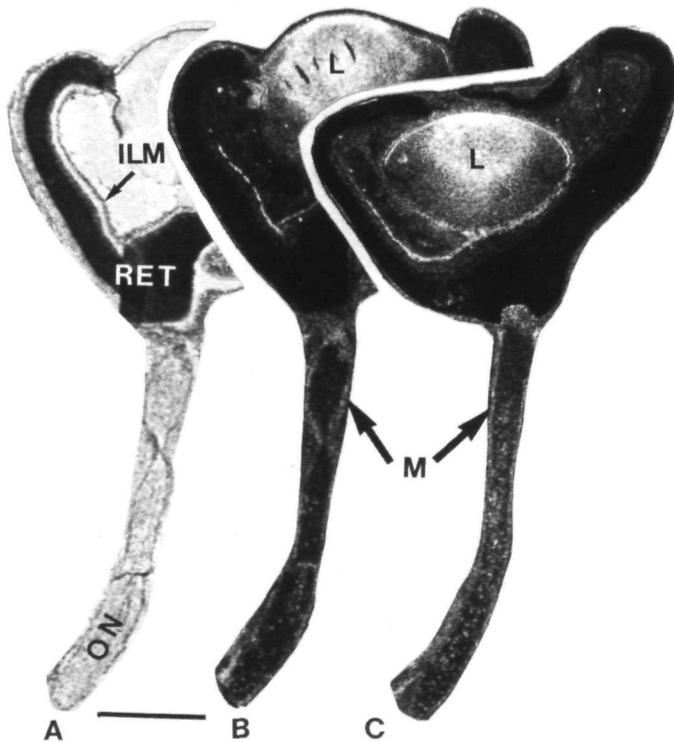
Fig. 9. Distribution of PDGF-αR mRNA in the perinatal rat optic nerve (ON) and retina (RET). The Fig. is a collage of three micrographs. Sections A and B, bright- and dark-field micrographs of a horizontal section through a P0 eye and optic nerve. A few PDGF-αR$^+$ cells are visible in the optic nerve, mostly towards the chiasmal end of the nerve (i.e. away from the eye). Section C, equivalent dark-field image of a P2 eye and optic nerve. At this age, there are many more PDGF-αR$^+$ cells in the nerve, and these are fairly evenly distributed apart from an exclusion zone next to the eye. At both P0 and P2 there is also intense labelling of the inner limiting membrane (ILM) of the retina, the anterior epithelium of the lens (L) and the meninges (M) around the optic nerve. Scale bar, 100 μm.

(Heldin et al., 1988; Gronwald et al., 1988). We established enriched cultures of rat O-2A progenitor cells, microglial cells (the macrophages of the CNS) and cortical (type-1-like) astrocytes (see Materials and methods for details). These different glial cell types were maintained for 24 hours in low-serum medium before incubating with ^{125}I-PDGF-AA in the presence or absence of a 100-fold excess of unlabelled PDGF-AA or PDGF-BB. NIH 3T3 cells, which are known to express PDGF-αR, were included as a control. Bound ^{125}I-PDGF was estimated by gamma counting of solubilized cells. Table 2 shows the results of these competitive binding experiments. NIH 3T3 cells bound ^{125}I-PDGF-AA specifically, as expected. O-2A progenitor cells also bound ^{125}I-PDGF-AA specifically, confirming our previous finding that O-2A lineage cells express PDGF-αR on their surface (Hart et al., 1989). Neither cortical astrocytes nor microglia bound ^{125}I-PDGF-AA specifically, indicating that neither of these cell types express PDGF-αR. In other experiments (not shown), cortical astrocytes were found to bind ^{125}I-PDGF-BB, suggesting that these cells express PDGF-βR in vitro (N. Pringle and W. Richardson, unpublished data).

Discussion

We have used in situ hybridization to visualize cells that express PDGF-αR mRNA in the rat CNS. From the spatial distribution of PDGF-αR$^+$ cells in the mature CNS, and the way these cells accumulate during development, we conclude that PDGF-αR is expressed by a subset of glial cells, but not neurons. We further conclude that PDGF-αR may be restricted to a single glial cell lineage in the CNS, the oligodendrocyte-type-2 astrocyte (O-2A) lineage. This conclusion is based on correlations between the distribution of PDGF-αR mRNA and histochemical markers of the O-2A lineage in situ (Reynolds and Wilkin, 1988; Hardy and Reynolds, 1991; LeVine and Goldman, 1988a,b), and in

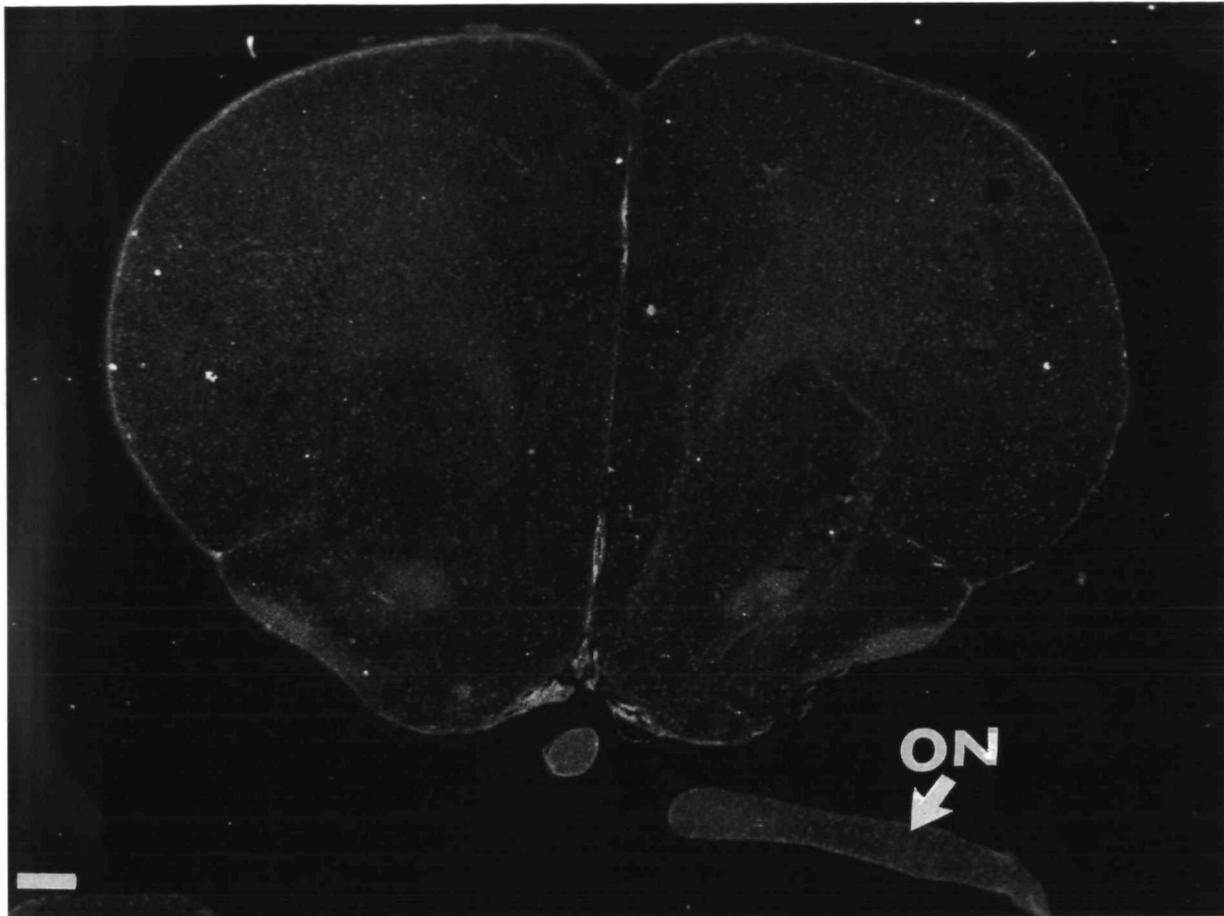**Fig. 10.** Expression of PDGF-αR mRNA in the adult rat forebrain and optic nerves (coronal section). Weakly PDGF-αR$^+$ cells are found in all areas of the adult forebrain and also in the optic nerves (ON). Scale bar, 100 μm.

Table 2. ^{125}*I-PDGF-AA binding to enriched cultures of glial cells from rat brain*

Competitor:-	None	PDGF-AA	PDGF-BB	Background (No cells)
O-2A Progenitors				
Expt 1	1523±35	421±40	231±9	483±120
Expt 2	294±20	85±11	ND	ND
Microglia				
Expt 3	473	984	1092	741
	598	1122	710	876
Expt 4	625	964	ND	717
	837	827	ND	776
Cortical astrocytes				
Expt 5	139±15	199±31	167±26	ND
Expt 6	209	250	167	ND
	293	203	116	ND
NIH 3T3				
Expt 7	3030±40	645±145	407±10	ND

Different populations of glial cells were isolated as described in Materials and methods, and grown in 24-well plates until semi-confluent (approximately 10^4 cells/well). NIH 3T3 fibroblasts were included as a positive control. ^{125}I-PDGF-AA binding in the presence or absence of competing unlabelled PDGF-AA or PDGF-BB was assessed as described in Materials and methods. Each experiment was performed in duplicate (both results tabulated) or triplicate (tabulated as mean ± standard deviation). Results are tabulated in counts per minute. NIH 3T3 cells and O-2A progenitors bound ^{125}I-PDGF-AA that could be competed with either PDGF-AA or PDGF-BB, as expected for cells that express PDGF-αR. Neither cultured cortical astrocytes nor microglial cells bound ^{125}I-PDGF-AA in a competable fashion, and therefore appear not to express PDGF-αR.

vitro studies that demonstrate PDGF-αR on O-2A lineage cells (Hart et al., 1989; McKinnon et al., 1990) but not other types of glial cells in culture (Hart et al., 1989 and this paper). PDGF-αR⁺ cells were found in most regions of the CNS, but occurred relatively infrequently in the subventricular germinal layer, suggesting that O-2A lineage cells may not start to express PDGF-αR until after (or just before) they migrate away from the subventricular zones towards their final destinations.

PDGF-αR is expressed by glial cells, not neurons

The spatial distribution of PDGF-αR⁺ cells in the postnatal rat CNS argues against these cells being neurons. Large numbers of PDGF-αR⁺ cells are present in developing white matter tracts such as the corpus callosum (Fig. 7), the foliar white matter of the cerebellum (Fig. 6) and the optic nerve (Fig. 9); these cells cannot all be neurons because there are few neuronal cell bodies in white matter generally, and none in the optic nerve. Even outside of white matter, the pattern of PDGF-αR⁺ cells is not what would be expected for neurons; PDGF-αR⁺ cells are distributed rather uniformly throughout the CNS, and are quite widely spaced, whereas the cell bodies of neurons are often closely packed in groups or sheets. For example, neurons in the postnatal cerebral cortex are arranged in discrete lamellae, whereas PDGF-αR⁺ cells show no sign of stratification, being distributed more-or-less evenly throughout the cortex (see Fig. 7, for example). Moreover, cells that are easily recognizable as neurons because of their location and/or distinctive morphology, such as cerebellar Purkinje cells (Fig. 6), retinal ganglion neurons (Fig. 9) or hippocampal neurons (Fig. 7), are invariably negative for PDGF-αR.

The time course on which PDGF-αR⁺ cells populate the developing CNS also argues for these cells being of glial, not neuronal lineage. Most neurons in the rat CNS become postmitotic before birth, whereas PDGF-αR⁺ cells continue to increase in number for some time after birth. In the cerebral cortex, for example, neurons are born in the subventricular zone between E11 and P0, with the peak time of production around E16/E17. As they are born, the neurons migrate away from the subventricular zone towards the pial surface, each wave of cells travelling further than the preceding one, so that those neurons that are born last end up closest to the pial surface (Jacobson, 1970). In contrast, PDGF-αR⁺ cells do not appear in the cortex until about E18, at which time they are confined to the lateral extremities of the intermediate zone (developing subcortical white matter) (Fig. 4). PDGF-αR⁺ cells subsequently spread throughout the cortex and continue to increase in numbers until at least P10, before declining again in the adult (Figs 4 to 6; Table 1). Thus, the spatial distribution of PDGF-αR⁺ cells in the developing and mature CNS, and the fact that they seem to proliferate postnatally, argue strongly that these cells correspond to glial cells, not neurons.

This conclusion is further supported by the appearance of the PDGF-αR⁺ cell nuclei in haematoxylin-

stained sections; invariably, the PDGF-αR hybridization signal is associated with small, round, densely stained nuclei that are characteristic of glial cells (Fig. 8). Blood vessels are readily apparent in these tissue sections, often being marked by rows of cells with elongated nuclei. These cells, which presumably represent endothelial cells and/or circulating blood cells, are always negative for PDGF-αR.

Is PDGF-αR restricted to the O-2A lineage?

O-2A lineage cells, but not other classes of glial cells, express PDGF-αR in vitro

What is known about PDGF receptors on glial cells? Hart et al. (1989) performed ¹²⁵I-PDGF binding experiments on cells freshly isolated from perinatal rat optic nerves, and the same cells grown for one day in vitro, and found that O-2A progenitor cells possess PDGF receptors with the binding characteristics of PDGF-αR, but no detectable PDGF-βR (Hart et al., 1989). This finding has since been corroborated by northern and western blot analysis of O-2A progenitor-enriched cultures prepared from perinatal rat cerebral cortex (McKinnon et al., 1990). Newly formed, postmitotic oligodendrocytes that develop in cultures of perinatal rat optic nerve cells initially retain PDGF-αR on their surface, but this is lost over the succeeding two or three days in vitro (Hart et al., 1989). Type-1 astrocytes, the other major macroglial cell type in the optic nerve, do not appear to express either PDGF-αR or PDGF-βR. Of the remaining cells in these cultures, which can constitute up to 60% of the total and consist mainly of microglial cells, endothelial cells, fibroblasts and leptomeningeal cells, less than 5% possess detectable PDGF-αR or PDGF-βR (Hart et al., 1989). We have performed ¹²⁵I-PDGF binding and northern blot analyses on cultured astrocytes from rat cerebral cortex, and find that these cells possess PDGF-βR, but not PDGF-αR (N. Pringle and W. Richardson, unpublished data; Table 2). Moreover, we can detect no specific binding of ¹²⁵I-PDGF-AA to microglial cells in vitro, indicating that microglia do not express PDGF-αR either (Table 2). Thus, apart from cells in the meningeal membranes and choroid plexus (Figs 4-6), the only cells in the CNS that are known to express PDGF-αR are O-2A progenitors and newly formed oligodendrocytes.

PDGF-αR⁺ cells, like O-2A progenitors, appear to migrate into the postnatal optic nerve from the optic chiasm

How do our data on the accumulation of PDGF-αR⁺ cells in the developing CNS compare with what is known about the development of the O-2A lineage in vivo? There is persuasive circumstantial evidence that O-2A progenitors are migratory cells in vivo, moving into developing white matter tracts from nearby germinal zones. For example, Small et al. (1987) found evidence consistent with the idea that O-2A progenitors migrate into the developing rat optic nerve from germinal zones in the brain via the optic chiasm. They cut optic nerves into thirds, dissociated the cells and

counted O-2A progenitors in each third separately in suspension. On the day of birth, there were relatively few O-2A progenitors in the nerve, and these were distributed in a gradient increasing towards the chiasmal end of the nerve. By P5 this gradient of cell number had largely disappeared, and the total number of cells had increased appreciably. These results are comparable with our data on the accumulation of PDGF-αR$^+$ cells in the nerve. At birth, there are relatively few PDGF-αR$^+$ cells in the nerve and these are concentrated towards the chiasmal end of the nerve (Fig. 9). By P2, the number of PDGF-αR$^+$ cells is several-fold higher (Table 1), and they are distributed fairly uniformly along the nerve except for a small exclusion zone next to the eye (Fig. 9). This exclusion zone includes the lamina cribrosa, a specialized structure that behaves as a physical barrier to prevent migration of O-2A progenitors into the retina (ffrench-Constant et al., 1988), which in most mammals remains unmyelinated throughout life.

The total number of PDGF-αR$^+$ cells in the rat optic nerve, extrapolated from numbers counted in sections of isolated nerves (Table 1), is in good agreement with the total number of O-2A progenitor cells estimated by other workers (Table 1), providing further evidence that PDGF-αR$^+$ cells correspond to O-2A progenitors. Since the numbers of PDGF-αR$^+$ cells are much less than the number of mature oligodendrocytes in the P15 and adult nerves (Vaughn, 1969), we conclude that oligodendrocytes stop expressing PDGF-αR soon after they differentiate in vivo, just as they do in vitro (Hart et al., 1989).

PDGF-αR$^+$ cells in the cerebral cortex: comparison with previous studies of O-2A lineage development in situ

At least two groups have studied the development of the O-2A lineage in the anterior forebrain by immunohistochemistry in situ. LeVine and Goldman (1988a,b) used a panel of antibodies including antibodies to ganglioside G$_{D3}$, which is expressed on the surface of immature neuroectodermal cells (Goldman et al., 1984), antibodies to carbonic anhydrase (CA), which is expressed mainly in oligodendrocytes (Cammer, 1984) but also in some astrocytes (Cammer and Tansey, 1988), antibodies against galactocerebroside (GC), a specific marker for oligodendrocytes in the CNS (Raff et al., 1978), and antibodies against myelin basic protein (MBP). From E16 into postnatal life, anti-G$_{D3}$ labelled small, round cells in the subventricular zones of the lateral ventricles. Anti-CA labelled cells with a variety of shapes from large, round cells that also labelled with anti-G$_{D3}$, to cells with the antigenic phenotype (G$_{D3}$$^-GC^+MBP^+$) and morphology of mature oligodendrocytes. The large, round CA$^+$ cells were first seen at E16 in the subventricular zones and in the intermediate zone (presumptive subcortical white matter); these were presumed to be the earliest cells committed to the oligodendrocyte lineage. At E18 the large, round cells were also found in the cingulum, and smaller G$_{D3}$$^+CA^+$ cells with a few thick processes

appeared in the subventricular zones at the lateral tips of the lateral ventricles, and in the more lateral aspects of the subcortical white matter. Between E18 and E20 G$_{D3}$$^+CA^+$ cells spread throughout the formative cortical white matter and overlying gray matter.

Hardy and Reynolds (1991) also studied oligodendrocyte development in the cerebral cortex by immunohistochemistry with anti-G$_{D3}$, anti-vimentin, anti-GC and anti-MBP, as well as antibodies to the myelin-specific cyclic nucleotide phosphodiesterase (CNP). These authors did not look at stages earlier than E19 but, at this age and above, their results were generally concordant with those of LeVine and Goldman (1988a,b). At E19-E20 they observed G$_{D3}$$^+$vimentin$^-$ cells that were either large and round, or smaller and bipolar, predominantly in the formative subcortical white matter, but also in the subventricular zones and the grey matter; some of these latter G$_{D3}$$^+$ cells may have been immature neurons. Between P0 and P2, many G$_{D3}$$^+$ cells were present in the subventricular zones and throughout the cortical grey and white matter; a few cells in the subventricular zone and inferior white matter coexpressed G$_{D3}$ and GC at this time. By P3-P4, the number of G$_{D3}$$^+$ cells had declined and these were found mainly in the cingulum and subcortical white matter adjacent to the subventricular zone. G$_{D3}$$^+$ cells were seen in decreasing numbers up to P12, while the numbers of cells expressing GC, CNP and MBP increased.

Is it possible to integrate these results with our own data on PDGF-αR$^+$ cells in the forebrain? At E16, we see a few PDGF-αR$^+$ cells inferior to the lateral ventricles outside of the subventricular zones, but none in the cerebral cortex (Fig. 2). We do not see PDGF-αR$^+$ cells in the cortex until E18, at which time they are confined to the lateral aspects of the presumptive subcortical white matter (Fig. 4). We also see some PDGF-αR$^+$ cells at the periphery of the subventricular zones at the lateral extremities of the lateral ventricles at E18 (Fig. 4), but none in the vicinity of the cingulum. At P0, PDGF-αR$^+$ cells are concentrated in the formative white matter, including the cingulum, and extend into the cortical grey matter up to the pial surface (Fig. 5). We believe that our data are compatible with those of LeVine and Goldman (1988a,b), if we assume that O-2A lineage cells do not start to express PDGF-αR until some time after they first become G$_{D3}$$^+CA^+$, i.e. after the "large, round" stage of their development. This might explain why we do not see any PDGF-αR$^+$ cells in the subventricular zone or intermediate zone at E16, or in the cingulum at E18. We propose that PDGF-αR expression commences in the G$_{D3}$$^+CA^+$ cells described by LeVine and Goldman (1988a) as having "slightly smaller cell bodies [with] one or more broad processes". Cells with this phenotype first appear at E18 in "the lateral aspects of the subventricular zone as well as in the overlying and more lateral white matter" (LeVine and Goldman, 1988b; compare our Fig. 4). We presume that in vivo, as in vitro, the O-2A lineage cells retain PDGF-αR until shortly after they start to express early markers of
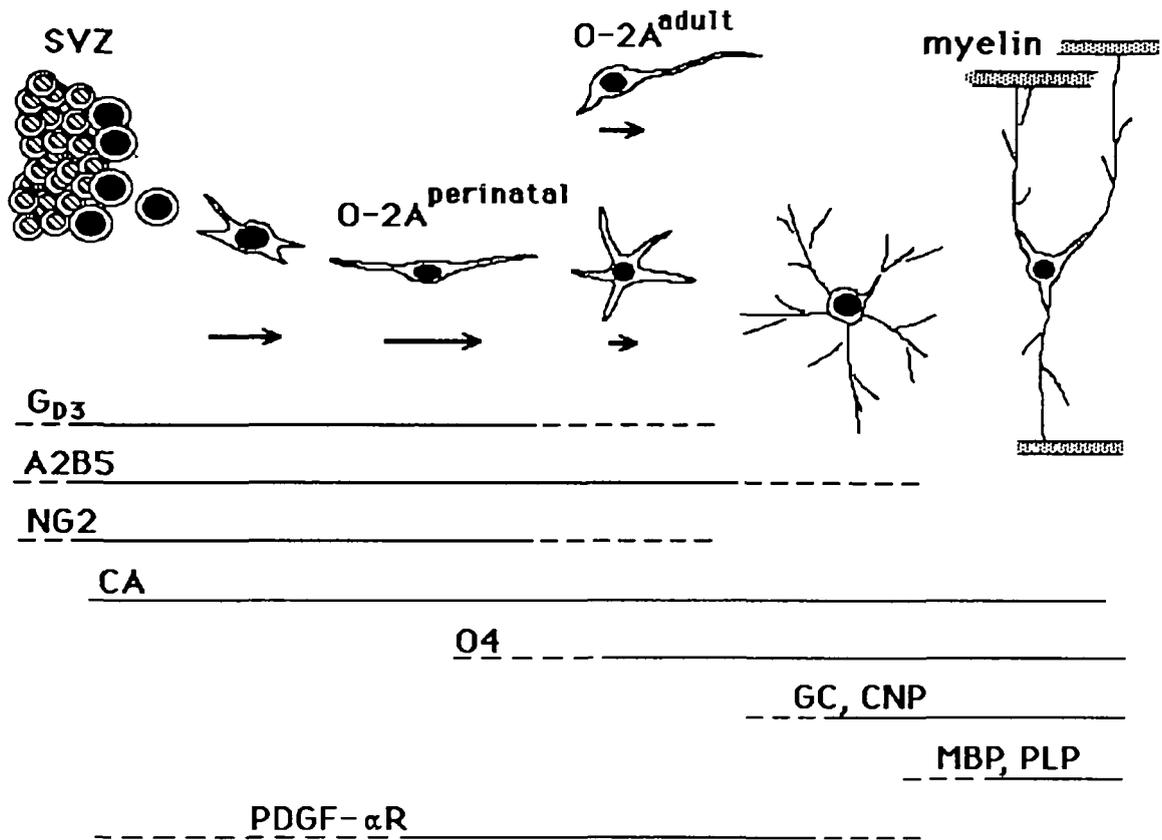
Fig. 11. A speculative view of the spatiotemporal development of the O-2A lineage in terms of the expression of PDGF-αR and various antigenic markers of the O-2A lineage. This diagram incorporates ideas and data from many studies, including our own. Antigenic markers not mentioned in the text are: O4, a monoclonal antibody raised against oligodendrocytes (Sommer and Schachner, 1980); PLP, proteolipid protein, a late structural protein of myelin. Dashed lines indicate uncertainty about the precise stage of gain or loss of the marker in question. Arrows indicate migratory cells; SVZ, subventricular zone.

oligodendrocyte differentiation such as GC (Hart et al., 1989) (see Fig. 11 for a schematic diagram).

It seems plausible, as suggested by LeVine and Goldman (1988a,b), that O-2A lineage cells migrate from subventricular germinal zones at or near the lateral tips of the lateral ventricles, first medially along the developing subcortical white matter tracts and then radially into the overlying cortex. This pattern of migration is also suggested by the way the distribution of PDGF-αR$^+$ cells evolves during cortical development (Figs 2 and 4).

PDGF-αR$^+$ cells in the developing cerebellum

LeVine and Goldman (1988a) observed G$_{D3}$$^+CA^+$ putative O-2A lineage cells with a gradation of morphologies in the P3 cerebellum, ranging from large, round cells without processes at the base of the cerebellum and in the presumptive germinal zones in the inferior colliculus (roof of the fourth ventricle), to more complex, process-bearing cells in the foliar white matter. Levine and co-workers studied a population of putative O-2A lineage cells in the developing rat cerebellum with antibodies to the NG2 chondroitin sulphate (Levine and Card, 1987; Levine and Stallcup,

1987). They found small numbers of NG2$^+$ cells in the cerebellar anlagen at E16; the number of these cells increased up to P5 and declined thereafter. In the adult, NG2$^+$ cells were especially prominent in the molecular layer. Reynolds and Wilkin (1988) found a few G$_{D3}$$^+$ cells in the developing inferior colliculus, the superior medullary vellum (the thin neck of tissue that connects the inferior colliculus to the base of the cerebellum) and the primitive folia of the P0 cerebellum. At P2, many G$_{D3}$$^+$ cells were present in the base of the cerebellum and throughout the folia, but not in the superior medullary vellum (SMV). From P2 to P5, the G$_{D3}$$^+$ cells became progressively localized to the developing white matter tracts, and GC$^+$CNP$^+$ cells began to accumulate. After P7, the number of G$_{D3}$$^+$ cells dwindled, until at P12 they were no longer detected. Large numbers of cells expressing one or more oligodendrocyte markers developed after the first postnatal week, first in the foliar white matter tracts and later in the internal granule cell layer, Purkinje cell layer and, after P15, in the molecular layer.

These studies suggested that oligodendrocyte precursors migrate after birth from germinal zones around the roof of the fourth ventricle, through the SMV and peduncles into the developing cerebellum. Our own

data on the accumulation of PDGF-αR$^+$ cells in the cerebellum (Fig. 6) are compatible with this idea if we once again make the assumption that the earliest stages of the O-2A lineage do not express PDGF-αR. Thus our hypothesis, that PDGF-αR$^+$ cells in the CNS represent predominantly (or exclusively) O-2A lineage cells, seems both internally consistent and compatible with previously published data. We presume that O-2A progenitors continue to express PDGF-αR until shortly after they differentiate into oligodendrocytes, as they do in vitro (Hart et al., 1989) (see Fig. 11).

PDGF-αR$^+$ cells in the adult CNS: O-2A^{adult} progenitors?

We observe PDGF-αR$^+$ cells in most regions of the adult (approximately 6 months old) rat CNS including the optic nerve, cerebral cortex and the molecular layer of the cerebellum (Figs 5 and 9), although they are less abundant and appear less intensely labelled than in young animals. It is known that O-2A progenitor cells persist in the adult CNS (ffrench-Constant and Raff, 1986; Wolswijk and Noble, 1989), where they presumably are required to replace oligodendrocytes that die naturally or as a result of injury. Alternatively, they might perform some other function unrelated to myelination in the adult. These O-2A^{adult} progenitors have somewhat different properties in vitro than their perinatal counterparts; they divide more slowly and take longer to differentiate in vitro, for example (Wolswijk and Noble, 1989). It has been reported (Chan et al., 1990) that O-2A^{adult} progenitors do not respond to PDGF in vitro, raising the possibility that they might no longer possess PDGF receptors. However, that study employed the PDGF-BB isoform, which has a significantly lower affinity than PDGF-AA for PDGF-αR (Heldin et al., 1988). It may be that O-2A^{adult} progenitors express reduced levels of PDGF-αR and for this reason, together with the lower affinity of PDGF-αR for PDGF-BB, they do not readily respond to PDGF-BB. Other workers found that O-2A^{adult} progenitors do divide in response to PDGF-AA in vitro (Wolswijk et al., 1991). Thus, it seems a reasonable supposition that PDGF-αR$^+$ cells in the adult CNS represent O-2A^{adult} progenitors and/or their newly differentiated progeny.

Conclusions

In summary, we have provided circumstantial evidence that argues in favour of the idea that PDGF-αR is expressed by O-2A lineage cells in the CNS, but not by neurons or other types of glial cells. Definitive proof of this hypothesis would require double-labelling studies with antibodies directed against specific subtypes of CNS cells. Nevertheless, the fact that PDGF-αR$^+$ cells occur mainly outside of the presumptive germinal zones in the subventricular layer suggests that PDGF does not act as a mitogen for O-2A lineage cells until just before, or after they migrate away from the subventricular layer towards their ultimate destinations. This raises the

question of what other polypeptide mitogen(s) might stimulate division of the earliest committed O-2A progenitors and their precursors in the subventricular zones. Members of the fibroblast growth factor (FGF) family might be involved at these early stages, because basic FGF is mitogenic for O-2A lineage cells in vitro (McKinnon et al., 1990; Bögler et al., 1990; Eccleston and Silberberg, 1985; Saneto and deVellis, 1985), and FGF receptor mRNA is concentrated in the subventricular cells of the developing chicken and rat CNS (Heuer et al., 1990; Wanaka et al., 1991). More needs to be learned about FGF receptors on O-2A lineage cells, and the expression of these receptors in vivo, before the relative roles of FGF and PDGF during O-2A lineage development can be fully explored.

References

Altman, J. (1966). Proliferation and migration of undifferentiated precursor cells in the rat during postnatal gliogenesis. *Exptl. Neurol.* 16, 263-278.

Ayer-Le Lievre, C., Stahlbom, P.-A. and Sara, V. R. (1991). Expression of IGF-I and -II mRNA in the brain and craniofacial region of the rat fetus. *Development* 111, 105-115.

Barres, B. A., Hart, I. K., Coles, H. C., Burne, J. F., Voyvodic, J. T., Richardson, W. D. and Raff, M. C. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* in press.

Bartlett, P. F., Noble, M. D., Pruss, R. M., Raff, M. C., Rattray, S. and Williams, C. A. (1981). Rat neural antigen (RAN-2): A cell surface antigen on astrocytes, ependymal cells, Muller cells and lepto-meninges defined by a monoclonal antibody. *Brain Res.* 204, 339-351.

Bartlett, W. P., Li, X.-S., Williams, M. and Benkovic, S. (1991). Localization of insulin-like growth factor-I mRNA in murine central nervous system during postnatal development. *Dev. Biol.* 147, 239-250.

Bögler, O., Wren, D., Barnett, S. C., Land, H. and Noble, M. (1990). Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of O-2A progenitor cells. *Proc. natn. Acad. Sci. U. S. A.* 87, 6368-6372.

Bunge, R. P. (1968). Glial cells and the central myelin sheath. *Physiol. Rev.* 48, 197-251.

Cammer, W. (1984). Carbonic anhydrase in oligodendrocytes and myelin in the central nervous system. *Annals N. Y. Acad. Sci.* 429, 494-497.

Cammer, W. and Tansey, F. A. (1988). Carbonic anhydrase immunostaining in astrocytes in the rat cerebral cortex. *J. Neurochem.* 50, 319-322.

Chan, C. L., Wigley, C. B. and Berry, M. (1990). Oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells from neonatal and adult rat optic nerve differ in their responsiveness to platelet-derived growth factor. *Dev. Brain Res.* 55, 275-282.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162, 156-159.

Chugani, D. C., Kedersha, N. L. and Rome, L. H. (1991). Vault immunofluorescence in the brain: new insights regarding the origin of microglia. *J. Neurosci.* 11, 256-268.

Eccleston, P. A. and Silberberg, D. H. (1985). Fibroblast growth factor is a mitogen for oligodendrocytes in vitro. *Develop. Brain Res.* 21, 315-318.

Eckenstein, F. P., Shipley, G. D. and Nishi, R. (1991). Acidic and basic fibroblast growth factors in the nervous system: distribution

and differential alteration of levels after injury of central versus peripheral nerve. *J. Neurosci.* **11**, 412-419.

Eisenbarth, G. S., Walsh, F. S. and Nirenburg, M. (1979). Monoclonal antibody to a plasma membrane antigen of neurons. *Proc. natn. Acad. Sci. U. S. A.* **76**, 4913-4917.

Ernfors, P., Wetmore, C., Olson, L. and Persson, H. (1990). Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* **5**, 511-526.

Feinberg, A. P. and Vogelstein, B. (1984). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **173**, 266-267.

ffrench-Constant, C., Miller, R. H., Burne, J. F. and Raff, M. C. (1988). Evidence that migratory oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells are kept out of the rat retina by a barrier at the eye end of the optic nerve. *J. Neurocytol.* **17**, 13-25.

ffrench-Constant, C. and Raff, M. C. (1986). Proliferating bipotential glial progenitor cells in adult rat optic nerve. *Nature* **319**, 499-502.

Fulton, B. P., Burne, J. F. and Raff, M. C. (1992). Visualization of O-2A progenitor cells in developing and adult rat optic nerve by quisqualate-stimulated cobalt uptake. *J. Neurosci.* in press.

Giulian, D. and Baker, T. J. (1986). Characterization of ameboid microglia isolated from developing mammalian brain. *J. Neurosci.* **6**, 2163-2178.

Goldman, J. E., Hirano, M., Yu, R. K. and Seyfried, T. N. (1984). G$_{D3}$ ganglioside is a glycolipid characteristic of immature neuroectodermal cells. *J. Neuroimmunol.* **7**, 179-192.

Gospodarowicz, D. (1984). Brain and pituitary growth factors. In *Hormonal Proteins and Peptides, Volume XII* (ed. C.H. Li.), pp. 205-230. New York: Academic Press.

Gronwald, R. G. K., Grant, F. J., Haldeman, B. A., Hart, C. E., O'Hara, P. J., Hagen, F. S., Ross, R., Bowen-Pope, D. F. and Murray, M. J. (1988). Cloning and expression of a cDNA coding for the human platelet-derived growth factor receptor: evidence for more than one receptor class. *Proc. natn. Acad. Sci. U. S. A.* **85**, 3435-3439.

Grothe, C., Zachmann, K. and Unsicker, K. (1991). Basic FGF-like immunoreactivity in the developing and adult rat brainstem. *J. comp. Neurol.* **305**, 328-336.

Hardy, R. and Reynolds, R. (1991). Proliferation and differentiation potential of rat forebrain oligodendroglial progenitors both *in vitro* and *in vivo*. *Development* **111**, 1061-1080.

Hart, I. K., Richardson, W. D., Heldin, C.-H., Westermark, B. and Raff, M. C. (1989). PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. *Development* **105**, 595-603.

Heldin, C.-H., Bäckström, G., Östman, A., Hammacher, A., Rönnstrand, L., Rubin, K., Nistér, M. and Westermark, B. (1988). Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. *EMBO J.* **7**, 1387-1393.

Heldin, C.-H. and Westermark, B. (1990). Platelet-derived growth factor: mechanism of action and possible *in vivo* function. *Cell Regul.* **8**, 555-566.

Heuer, F. G., Von Bartheld, C. S., Kinoshita, Y., Evers, P. C. and Bothwell, M. (1990). Alternating phases of FGF receptor and NGF receptor expression in the developing chicken nervous system. *Neuron* **5**, 283-296.

Jacobson, M. (1970). *Developmental Neurobiology,* New York: Holt, Rinehart and Winston, Inc.

Lawrence, J. B. and Singer, R. H. (1985). Quantitative analysis of *in situ* hybridization methods for the detection of actin gene expression. *Nucl. Acids Res.* **13**, 1777-1799.

Lee, K.-H., Bowen-Pope, D. F. and Reed, R. R. (1990). Isolation and characterization of the α platelet-derived growth factor receptor from olfactory epithelium. *Mol. Cell. Biol.* **10**, 2237-2246.

Levine, J. M. and Card, J. P. (1987). Light and electron microscopic localization of a cell surface antigen (NG2) in the rat cerebellum: association with smooth protoplasmic astrocytes. *J. Neurosci.* **7**, 2711-2720.

Levine, J. M. and Stallcup, W. B. (1987). Plasticity of developing cerebellar cells *in vitro* studied with antibodies against the NG2 antigen. *J. Neurosci.* **7**, 2721-2731.

LeVine, S. M. and Goldman, J. E. (1988a). Spatial and temporal

patterns of oligodendrocyte differentiation in rat cerebrum and cerebellum. *J. Comp. Neurol.* **277**, 441-455.

LeVine, S. M. and Goldman, J. E. (1988b). Embryonic divergence of oligodendrocyte and astrocyte lineages in developing rat cerebrum. *J. Neurosci.* **8**, 3992-4006.

Matsui, T., Heidaran, M., Miki, T., Popescu, N., La Rochelle, W., Kraus, M., Pierce, J. and Aaronson, S. (1989). Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. *Science* **243**, 800-804.

McCarthy, K. D. and De Vellis, J. (1980). Preparation of separate astroglial and oligodendroglial cultures from rat cerebral tissue. *J. Cell Biol.* **85**, 890-902.

McKinnon, R. D., Matsui, T., Dubois-Dalcq, M. and Aaronson, S. A. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5**, 603-614.

Millan, F. A., Denhez, F., Kondaiah, P. and Akhurst, R. J. (1991). Embryonic gene expression patterns of TGF β1, β2 and β3 suggest different developmental functions *in vivo*. *Development* **111**, 131-144.

Miller, R. H., David, S., Patel, R., Abney, E. R. and Raff, M. (1985). A quantitative immunohistochemical study of macroglial cell development in the rat optic nerve: *in vivo* evidence for two distinct astrocyte lineages. *Dev. Biol.* **111**, 35-41.

Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562.

Pringle, N., Collarini, E. J., Mosley, M. J., Heldin, C.-H., Westermark, B. and Richardson, W. D. (1989). PDGF A chain homodimers drive proliferation of bipotential (O-2A) glial progenitor cells in the developing rat optic nerve. *EMBO J.* **8**, 1049-1056.

Raff, M. C., Abney, E. R. and Fok-Seang, J. (1985). Reconstitution of a developmental clock in vitro: a critical role for astrocytes in the timing of oligodendrocyte differentiation. *Cell* **42**, 61-69.

Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F. and Noble, M. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* **333**, 562-565.

Raff, M. C., Miller, R. H. and Noble, M. (1983). A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature* **303**, 390-396.

Raff, M. C., Mirsky, R., Fields, K. L., Lisak, R. P., Dorfman, S. H., Silberberg, D. H., Gregson, N. A., Liebowitz, S. and Kennedy, M. (1978). Galactocerebroside: a specific cell surface antigenic marker for oligodendrocytes in culture. *Nature* **274**, 813-816.

Ranscht, B., Clapshaw, P. A., Price, J., Noble, M. and Seifert, W. (1982). Development of oligodendrocytes and Schwann cells studies with a monoclonal antibody against galactocerebroside. *Proc. Natn. Acad. Sci. U. S. A.* **79**, 2709-2713.

Reynolds, R. and Wilkin, G. P. (1988). Development of macroglial cells in rat cerebellum. II. An *in situ* immunohistochemical study of oligodendroglial lineage from precursor to mature myelinating cell. *Development* **102**, 409-425.

Richardson, W. D., Pringle, N., Mosley, M. J., Westermark, B. and Dubois-Dalcq, M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell* **53**, 309-319.

Richardson, W. D., Raff, M. and Noble, M. (1990). The oligodendrocyte-type-2 astrocyte lineage. *Semin. Neurosci.* **2**, 445-454.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning, a Laboratory Manual,* Cold Spring Harbor, New York: CSH Laboratory Press, Ed. 2.

Saneto, R. P. and De Vellis, J. (1985). Characterization of cultured rat oligodendrocytes proliferating in a serum-free, chemically defined medium. *Proc. Natn. Acad. Sci. U. S. A.* **82**, 3509-3513.

Sasahara, M., Fries, J. W. U., Raines, E. W., Gown, A. M., Westrum, L. E., Frosch, M. P., Bonthron, D. T., Ross, R. and Collins, T. (1991). PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* **64**, 217-227.

Small, R. K., Riddle, P. and Noble, M. (1987). Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature* **328**, 155-157.

Smits, A., Kato, M., Westermark, B., Nistér, M., Heldin, C.-H. and Funa, K. (1991). Neurotrophic activity of PDGF: rat neuronal cells possess functional PDGF β-receptors and respond to PDGF. *Proc. Natn. Acad. Sci. U. S. A.* **88**, 8159-8163.

Thoenen, H. (1991). The changing scene of neurotrophic factors. *Trends Neurosci.* **14**, 165-170.

Vaughn, J. E. (1969). An electron microscopic analysis of gliogenesis in rat optic nerves. *Z. Zellforsch.* **94**, 293-324.

Wanaka, A., Milbrandt, J. and Johnson, E. M., Jr (1991). Expression of FGF receptor gene in rat development. *Development* **111**, 455-468.

Wolswijk, G. and Noble, M. (1989). Identification of an adult-specific glial progenitor cell. *Development* **105**, 387-400.

Wolswijk, G., Riddle, P. and Noble, M. (1991). Platelet-derived growth factor is mitogenic for O-2A[adult] progenitors. *Glia* **4**, 495-503.

Yeh, H.-J., Ruit, K. G., Wang, Y.-X., Parks, W. C., Snider, W. D. and Deuel, T. F. (1991). PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell* **64**, 209-216.