Korean J Biol Sci 3: 259-267, 1999

Differential Expression of NCAM-180 in the Olfactory System and Retina of the Rat

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Key Words:
NCAM-180
Olfactory epithelium
Olfactory bulb
Retina
Optic nerve
Regenerative capacity

The expression of the neural cell adhesion molecule-180 (NCAM-180), which accumulates at contact sites between cells and may be responsible for the stabilization of cell contacts, was studied in the olfactory system and retina of developing and adult rats. From embryonic day 12 onwards, which was the earliest stage examined, the NCAM-180 pathway directing to the presumptive olfactory bulb was observed. In later stages, olfactory neurons and fasciculating axons in the olfactory epithelium and nerve fiber layer and glomeruli of the olfactory bulb expressed NCAM-180. From postnatal day 0, immunolabelling pattern of the olfactory epithelium and olfactory bulb were the same as that during later stages. NCAM-180 immunoreactivity was present on differentiating retinal cells and persisted on those cells throughout adulthood. However, contrary to the olfactory nerve which remained detectable in the adult, the optic nerve was only transiently expressed with NCAM-180 and was no longer detectable in the adult. The presence of NCAM-180 in olfactory tissues suggests their possible role in pathfinding, differentiation, fasciculation and synaptic plasticity. The continued presence of NCAM-180 in the olfactory system examined may underlie its continuous cell turnover and regenerative capacity. The continuous expression of NCAM-180 in ganglion cells, bipolar cells and photoreceptor cells, also suggests potential regenerating capability and some plastic functions for these cells in the adult. Since the expression of NCAM-180 by the optic nerve was restricted to the period of special histogenetic events, for example, during axonal growth and synaptogenesis, it is possible that the lack of NCAM-180 in the adult optic nerve might cause a nonpermissive environment for the regeneration and result in regenerative failure of this system.

The proper functioning of the nervous system depends on the precise location of cells and specific connections that they make with each other. Forming these connections involves cell migration, axonal outgrowth and selective synaptogenesis. Key roles in this targeting process are known to be played by a variety of adhesion molecules present in the neuronal cell membrane. One of these is NCAM (neural cell adhesion molecule). NCAM is the most prevalent Ca^{2+} independent cell adhesion molecule in vertebrate tissues and regulates cell-cell adhesion via homophilic interactions. Following NCAM binding, transmembrane signalling is believed to be activated, resulting in increased intracellular calcium. By mediating cell adhesion to other cells and to the extracellular matrix and by activating intracellular signalling pathways, NCAM influences cell migration, neurite extension, fasciculation and possibly formation of synapses in the brain (Rothbard et al., 1982; Allan and Greer, 1998; Ronn et al., 1998). There are at least 20-30 distinct isoforms of NCAM that are generated as a result of alternative splicing of the product of a single gene (Murray et al., 1986; Owens et al., 1987). The isoforms can be grouped into three main size classes that differ in their mode of attachment to the membrane or the size of their intracellular domains. NCAM-180 and NCAM-140 are integral membrane proteins, whereas NCAM-120 is attached to membranes by a phosphatidylinositol linkage (He et al., 1986) and can be released spontaneously from membranes (Edelman., 1986). NCAM-180, the component with the largest cytoplasmic domain, appears developmentally later than the two other components (Pershon and Schachner, 1987). It specifically associates with brain spectrin, shows restricted motility and has been suggested to play a role in stabilization of cell contacts (Pollerberg et al., 1986; Pollerberg et al., 1987). The expression of NCAM-180 appears to accompany neuronal differentiation, and are abundant in differentiating

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neurons, but absent from cells in NCAM positive proliferative zones (Pollerberg et al., 1985).

The olfactory system represents a unique system in which to study differentiation and regeneration of neurons. This system shows continuous neuronal turnover persisting in the adult animal (Graziadei and Monti Graziadei, 1978a, b; Miragall and Monti Graziadei, 1982). Newly differentiated neurons continuously project their axons into the olfactory bulb and establish functional synapses in the CNS (Pinching and Powell, 1971). An experimental animal, when provoked by ablation of the olfactory nerve, showed massive degeneration of olfactory neurons followed by subsequent replacement by a new neuronal generations originating from the epithelium (Monti Graziadei and Graziadei, 1979; Monti Graziadei, 1992). The ectopically transplanted epithelium also consistently differentiates into a normal olfactory organ (Magrassi and Graziadei, 1996). Moreover, the olfactory axons developed from the ectopically located epithelium and connected with unconventional targets (Magrassi and Graziadei, 1985; Koo and Graziadei, 1995b). In contrast, the optic system is a typical example of an adult mammalian central nervous system (CNS) that do not regenerate beyond the site of injury (Aguayo et al., 1990; Oorschot and Jones, 1990). It is also known to be highly specific, and a point to point match up between retinas and the brain is essential for normal function. In experimental conditions, retinal axons always make consistent projections to its proper target even though they enter brain locations which are distinctly off the optic tract (Fujisawa et al., 1984; Harris, 1986; Koo and Graziadei, 1995a).

Since the olfactory epithelium is continuously undergoing turnover and show plasticity in sysnaptic connections, the mechanisms of axonal guidance may be alternatively organized from that seen in other systems such as the optic system which shows extreme specificity of axonal connection and extreme limit of regeneration. The present study asks whether these two systems will show any difference in the expression of NCAM-180 which is mediating special histogenetic events during later stages of development. Immunocytochemistry was used in determining the differential expression of NCAM-180 in both the developing and adult rat olfactory and optic systems, especially the retina and optic nerve.

Materials and Methods

Sprague-Dawley rat fetuses were obtained from timed pregnant females. The day on which the vaginal plug was found was assigned as embryonic day 0 (E0). Pregnant females were anesthesized with ketamine (Yuhan, 20 mg/weight kg) and the fetuses were dissected out. Embryos were taken at 12, 14, 15, 17, 18 and 20th day of gestation. Neonatal, 7, 34 days old and adult animals (P0, P7, P34 and adult, respectively) were also used. Fetuses and neonatal animals were fixed by immersion in 4% paraformaldehyde overnight at 4℃. Older animals were fixed with cold 4% paraformaldehyde by transcardinal perfusion and left overnight in 2% paraformaldehyde at 4℃. Following fixation, the specimens were dehydrated through a series of cold ethanols and toluene, and finally embedded in Paraplast. Sections of 10 micron thickness were cut on a regular microtome and mounted on gelatin subbed slides.

For conventional histology, sections were stained with Gill's hematoxylin and Bodian silver impregnation method. For immunocytochemistry, sections were dewaxed through a series of ethanol, water and phosphate buffered saline (PBS) washes and incubated with 3% normal goat serum. With rat monoclonal antibody against NCAM-180 (Sigma), sections were incubated in a humidified petri dish overnight at 4℃. The primary antibodies were diluted 1:500 in PBS containing gelatin and merthiolate. Following incubation with primary antibody, sections were rinsed and incubated with goat anti-rat IgG at 1:100 for 30 minutes at room temperature. Sections were then treated with PAP (Peroxidase Anti-Peroxidase) for 30 minutes and reacted with 0.05% 3,3-diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer containing 0.01% H_2O_2. Sections were mounted in 90% glycerol.

Results

The goal of this study was to examine differences in the expression of surface NCAM-180 on neural cells of the olfactory and optic systems in relation to their regenerative capability. Experiments were mainly performed in the olfactory epithelium, olfactory bulb and retina.

Differential expression of NCAM-180 in the olfactory epithelium and olfactory bulb

Using immunocytochemical methods, the spatial and temporal pattern of NCAM-180 expression in the embryonic, postnatal and adult rat olfactory system was studied. The study was initiated at E12 when a few pioneer olfactory fibers are present and associated migrating cells course through the nasal mesenchyme. From E12, which was the earliest stage studied, to the adult, NCAM-180 immunoreactivity was observed along the basal surface of the olfactory epithelium and around the developing telencephalon.

As early as E12, staining with the anti-NCAM-180 antibody marked a distinct pathway originating in the olfactory epithelium and extending all the way to the presumptive olfactory bulb (Fig. 1A). A similar result was also observed at later stages through E16 (Fig. 1B). From E14, immunoreactivity of NCAM-180 was also expressed in differentiating the olfactory epithelium, specially olfactory neurons which are situated in the middle layer (Fig. 2). The antibodies labeled the

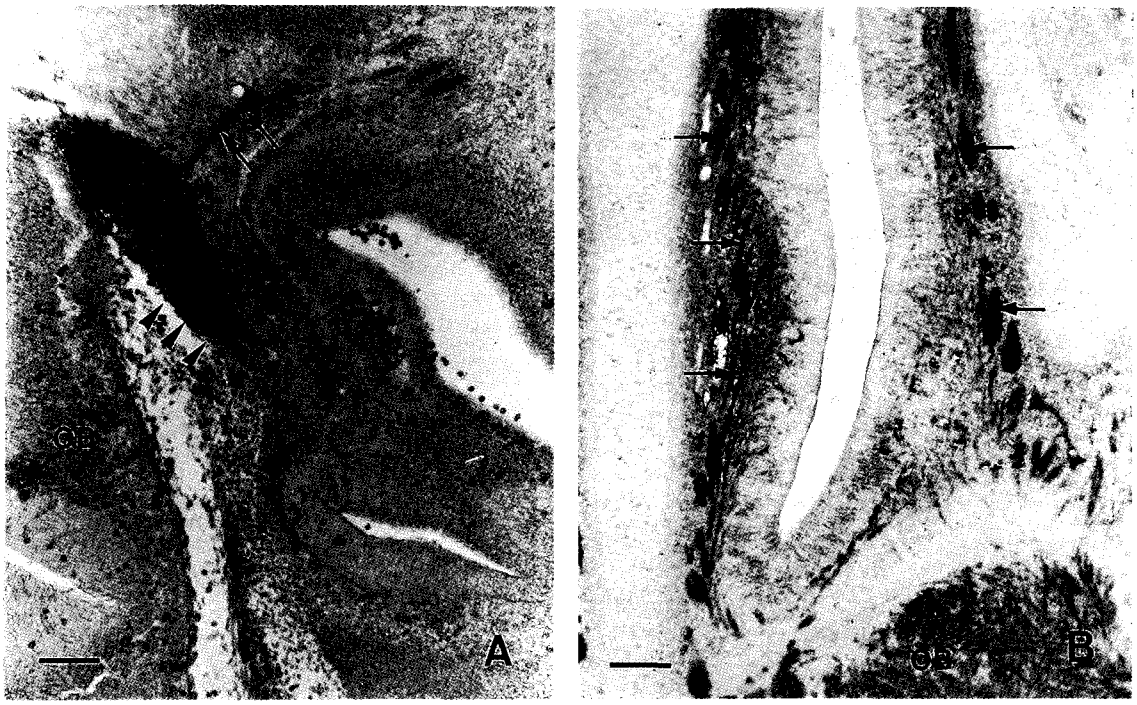

Fig. 1. Horizontal section of a E12 embryo. A, This immunohistochemical photomicrograph shows the NCAM-180 pathway (arrows) from the olfactory epithelium to the olfactory bulb (OB). The presumptive olfactory nerve fiber layer at the outer edge of the bulb is also stained (arrowheads). B, Immunocytochemistry microscopic localization of NCAM-180 of E15 embryo. Arrows indicate the NCAM-180 immunoreactive pathway running to the developing olfactory bulb (OB). Scale bar=27 μm.

cell bodies, dentrites and axons of olfactory neurons. However, no supporting cells or basal cells expressed detectable levels of NCAM-180. In later stages, the fasciculating axons of the olfactory nerve were stained with anti-NCAM-180 (Fig. 3). Axon bundles found in the *lamina propria at birth and in later stages were* also strongly stained. From P0, this immunolabelling pattern of NCAM-180 was the same as that during later stages of development and in adults (Fig. 4). In the olfactory bulb, the nerve fiber layer stained intensely with anti-NCAM-180. At P0, no clear glomerular structures were yet present in the olfactory bulb, but intense staining was apparent in the nerve fiber layer and the developing glomerular layer (Fig. 5). From P7 glomeruli were readily distinguished and they showed strong immunoreaction. However, there was a difference in the intensity of the stain between glomeruli with some of them staining more intensely than others. There was also a difference in the intensity of the stain between

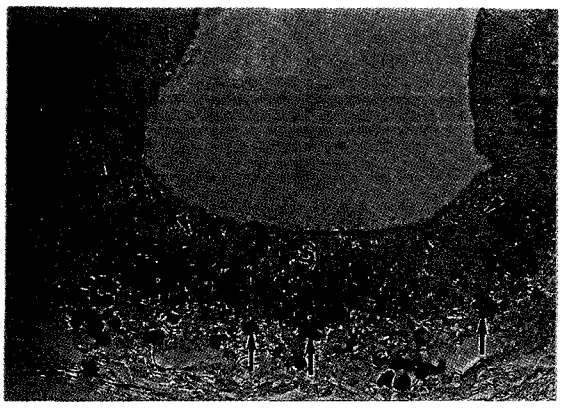

Fig. 2. The olfactory epithelium of the E17 rat. This horizontal section shows olfactory neurons stained with NCAM-180 in the epithelium. Most of the stained cells are situated in the middle layer of the epithelium (ON) whereas a few cells are stained in the basal layer (arrows). The arrowheads point to the dendrites of olfactory neurons stained with NCAM-180. Almost all the olfactory neurons are stained with NCAM. Supporting cells (S) are negative. Scale bar=6 μm.

Fig. 3. The olfactory epithelium of the E18 rat. The fasciculating axon bundles (arrows) are stained with NCAM-180. The nerve fiber layer of the olfactory bulb (OB) is also immunoreactive. Scale bar=27 μm.

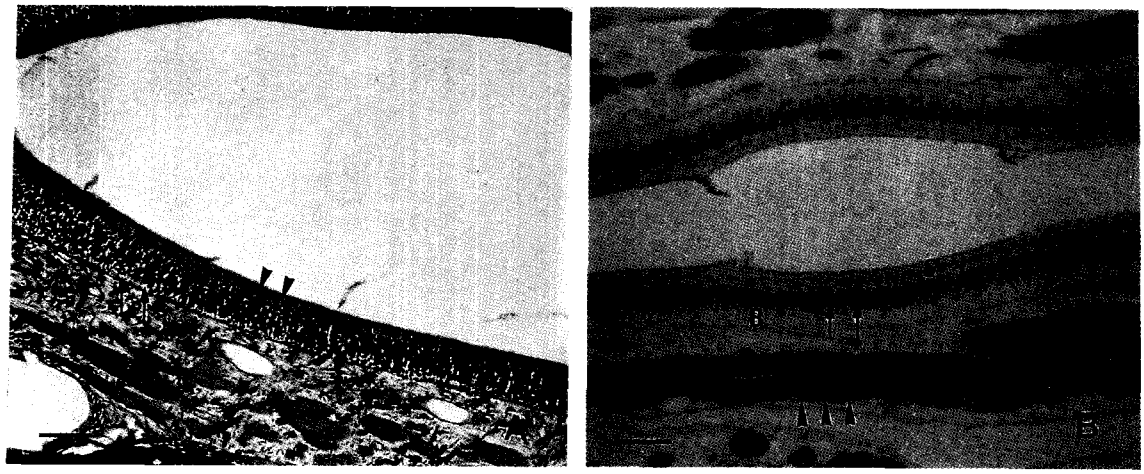

Fig. 4. Horizontal section of an adult rat. A, Silver stained photograph of the adult rat olfactory epithelium. Cell bodies of basal cells situated in the basal layer (arrows), olfactory neurons in the middle layer and supporting cells in the laminal parts of the epithelium (arrowheads). OL, olfactory nerves. B, Olfactory epithelium of an adult. Almost all olfactory neurons (arrows) are stained with NCAM-180. Supporting cells (S) and basal cells (B) are negative. Axon bundles are also labeled (arrowheads). Scale bars=23 μm (B) and 27 μm (A).

nerve fibers and glomeruli, with the later stains less intense compared to the nerves (Fig. 6). Immunolabelling for NCAM in the external plexiform layer was negative. This general pattern of staining did not change throughout adult life.

Differential expression of NCAM-180 in the retina

Retinas and optic nerves from rats were examined on E12 through stages of the adult. NCAM-180 was found in a different retinal position with developmental age. At the onset of photoreceptor birth (E12), NCAM-180 immunoreactivity was present in cells of the retinal pigment epithelium (RPE) and on the inner surface of

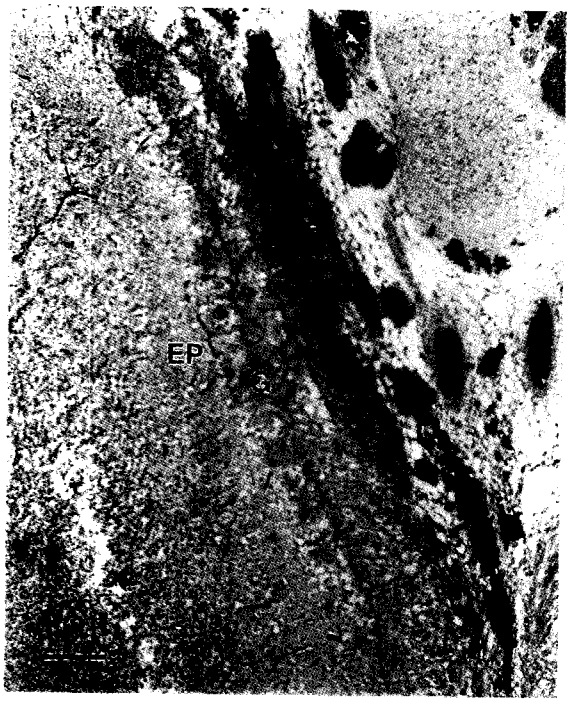

Fig. 5. Localization of NCAM-180 in the olfactory bulb of the P0 rat. No clear glomerular structures are evident yet, but intense staining is apparent in the nerve fiber layer and the developing glomerular layer (G). In contrast, labeling for NCAM-180 in the external plexiform layer (EP) is negative. Scale bar=27 μm.

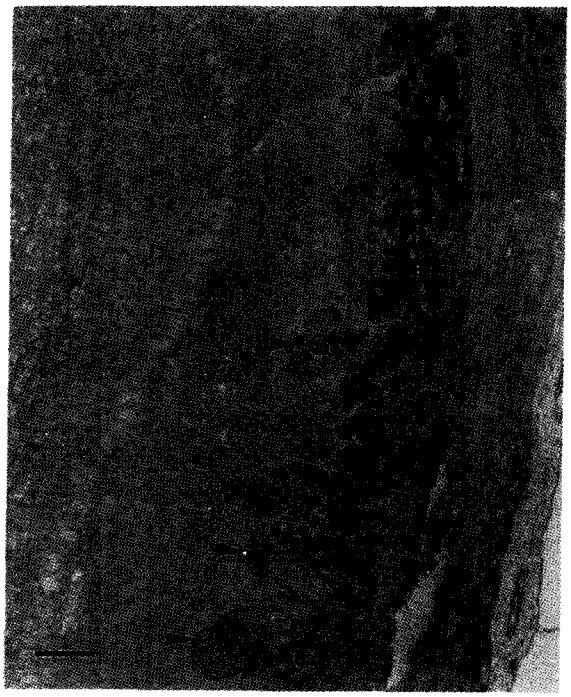

Fig. 6. The olfactory bulb of the P34 rat. The nerve fiber layer (NF) and the glomerular layer (G) are stained with NCAM-180. The glomeruli are stained less intensely than the nerve fiber bundles. Some glomeruli are stained more intensely (arrowheads) than others. Scale bar=6 μm.

Fig. 7. Transverse section of an eyeball of a E12 rat. Antibodies to NCAM-180 label the retinal pigment epithelial cells (arrows). Immunolabeled cells are also observed in the inner layer of the retinal epithelium (arrowheads). Scale bar=27 μm.

the retinal epithelium (Fig. 7) and persisted in these areas throughout development. NCAM-180 was not found in the neural layer of the retina at early stages, but by stage E17, labelling appeared in differentiating bipolar and ganglion cell layers (Fig. 8). In addition to the bipolar and ganglion cell layers, immunoreactivity also became positive in the photoreceptor cell body layer from P0 (Fig. 9). The inner plexiform layer, outer plexiform layer and outer segment of the photoreceptor layer (rod and cone cell layer) were also immunoreactive from P7. However, both plexiform layers and the outer segment of photoreceptor layer were more weakly labeled than the cell body layers. From P7 onwards,

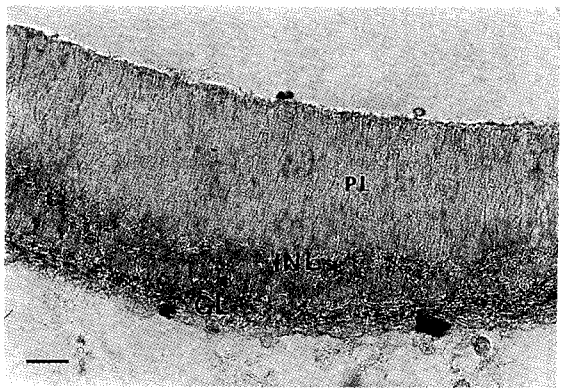

Fig. 8. Localization of NCAM-180 in the developing retina of the E17 rat. NCAM-180 is stained in the inner nuclear layer (bipolar cell layer, INL) and ganglion cell layer (GL), but not detectable in the photoreceptor cell body layer (outer nuclear layer, PL). Scale bar=6 μm.

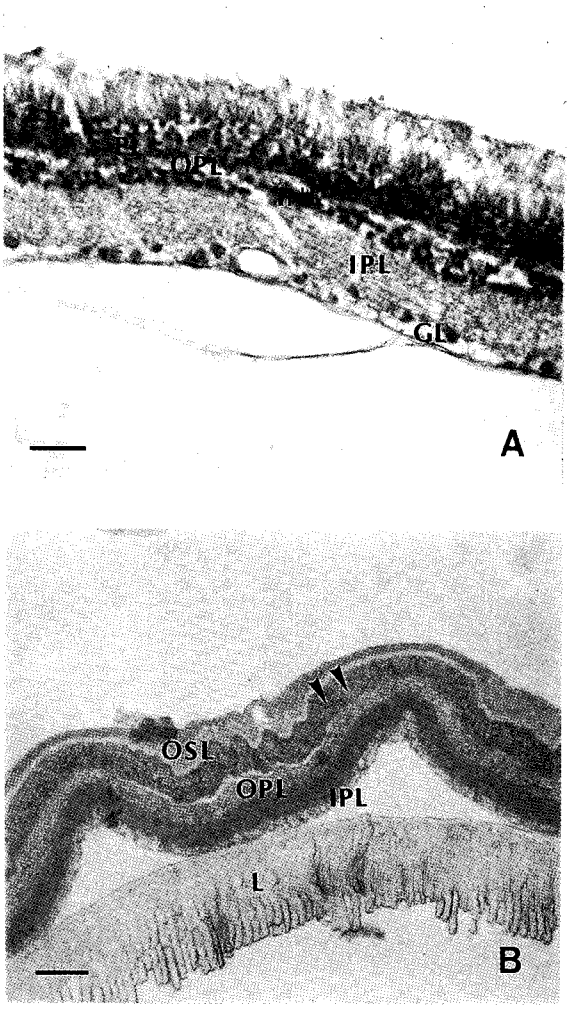

Fig. 9. Horizontal section of an adult rat. A, Hematoxylin stained horizontal section of the adult rat retina. Photograph shows six layers of the retina. PL, photoreceptor cell body layer (outer nuclear layer); OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GL, ganglion cell layer. B, Immunocytochemical staining for NCAM-180 in a P34 rat. Photoreceptor cell body layer is NCAM-180 positive (arrowheads) in addition to other layers seen in Fig. 8. The inner plexiform layer (IPL), outer plexiform layer (OPL) and the outer segment of photoreceptor layer (rod and cone of the photoreceptor layer, OSL) are also stained. Both plexiform layers and outer segment of photoreceptor layer are more weakly stained compared to the cell body layers. L, lens. Scale bar=27 μm.

the general immunolabelling pattern of NCAM-180 in the retina, was the same as that during later developmental stages and adult. At E18, the optic nerve and bundled axons of ganglion cells, was immunoreactive for NCAM-180 (Fig. 10). However, the expression of NCAM-180 decreased in the optic nerve near P7 and disappeared in P34.

Discussion

How axons reach their destination is a fundamental issue for neurobiologists. It is important to understand how axons connect with their final target because this connection underlies the basic functioning of the nervous

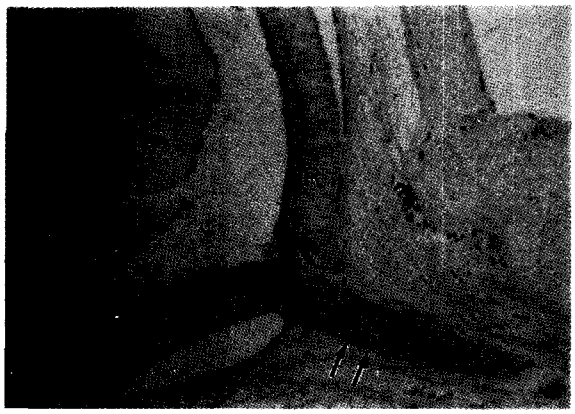

Fig. 10. Distribution of NCAM in E19 rat. Arrows show that the optic nerve is NCAM-180 positive. L, lens; R, retina. Scale bar=27 μm.

system. The olfactory system shows continuous neuronal turnover and regenerative capability throughout adulthood. However, the capacity of adult axons to regenerate is very limited within CNS including optic nerve fibers. For successful regeneration, axons must have proper cell adhesion molecules which interact with the environment and supports axonal growth in addition to the intrinsic capacity of the axon itself. Adult nerve fibers express Thy-1 which has been implicated in the inhibition of neurite outgrowth (Tiveron et al., 1992). Conversely, other important molecules promoting axonal growth in the embryo does not appear to be available in regenerating adult optic fibers (Bates et al., 1999). Thus, the most available CAM that might be able to mediate growth of adult nerve fibers is NCAM. NCAM is an immunoglobulin superfamily molecule that is widely expressed on neurons and glia and mediates cell adhesion (Rutishauser et al., 1988), attachment, and neurite growth throughout the nervous system (Fredett et al., 1993; Lars et al., 1995; Joosten et al., 1996; Ronn et al., 1998).

In this study, expressions of NCAM-180 were studied in the olfactory epithleium, olfactory bulb and retina of pre- and post-natal rats using a monoclonal anti-NCAM antibody which recognized NCAM-180.

NCAM-180 expression in the olfactory epithelium and olfactory bulb

Several previous studies support the idea that NCAM plays an important role in normal development of olfactory tissues. NCAM mutant or knock out mice studies have shown a defect in cell migration in the olfactory bulb (Huaiyu et al., 1996) and the formation of the olfactory bulb (Ronn et al., 1998).

This study provides an exception to previously published results (Paz et al., 1995; Minana et al., 1998), in which NCAM-180 is expressed at a pathway as early as or even prior to the arrival of the pioneer axon and then remains in the olfactory nerve at a high level even in the adult. This demonstration of a preformed substrate pathway provides evidence that this molecule is involved in axonal guidance. In later stages, the olfactory neurons in the epithelium and nerve fiber layer and glomerular layer of the olfactory bulb were also strongly lableled with anti-NCAM-180 in both embryonic and adult animals. This is not unexpected since the olfactory system has the ability of continuous neuronal turnover into adult life. Continuous expression of NCAM-180 may reflect the persistent neural plasticity of this tissue. The differentiating and mature olfactory neurons were the only cells labeled with antibody in the olfactory epithelium. This is in agreement with previous data, in which NCAM-180 is associated with nonproliferating, postmitotic, and differentiating neurons. According to several authors, NCAM-180 of three major forms of NCAM was first expressed on postmitotic and postmigratory cells (Pollerberg et al., 1986), relatively poor at promoting neurite outgrowth (Doherty et al., 1990) and accumulated at sites of cell contact (Pollerberg et al., 1987). In this respect the results of this study differ from that of Minana et al. (1998). In their experiments with the primary culture of cortical astrocytes, cells expressed NCAM-180 during proliferation. Then, during cell differentiation this isoform progressively disappeared (Minana et al., 1998). Since the olfactory system shows notable exception to other nervous system with its continually regenerating ability, this difference may represent that parameter. However, down-regulation of NCAM-180 also has been reported even in the olfactory system. In toads, expression of antibody decreased near the end of larval development and was absent in post-metamorphic and adult animals although total NCAM continued to be expressed in differentiating olfactory epithelium, olfactory fibers, and in the olfactory bulb of post-metamorphic and adult animals (Paz et al., 1995). Since the study was done on toads, the results can not be directly compared to mammals. Furthermore, recently it was reported that there are two NCAM-180 specific monoclonal antibodies, 481 and D3, and they showed different patterns of immunoactivity in the adult mouse (Kramer et al., 1997). The D3 antibody showed an age dependent decrease of activity in CNS coincident with the establishment of stable cell-cell contacts, while the 481-specific epitope remained positive throughout development and adulthood. It remains unclear if these two possess different roles necessary for normal development or regeneration within CNS and the olfactory system. Further work is required on possible isoforms of NCAM-180 including these two in order to come to any definite conclusions regarding roles of NCAM-180 in various nervous tissues.

NCAM-180 expression in the retina

The author previously hypothesized that NCAM plays a role in the developing optic pathway. In vitro studies have suggested that NCAM plays important roles in

orienting the axon toward the optic tectum (Neugebauer et al., 1988) and is also critical for axon extension in the optic pathway (Stier and Schlosshauer, 1995) and fasciculation (Thanos et al., 1984). Antibody blocking studies using chick optic fibers also demonstrated partial inhibition of neurite growth on astrocytes, implying that NCAM participates in this growth. However, the relative importance of NCAM seems to vary with developmental age. Anti-NCAM had no detectable effects on neurite outgrowth of the ganglion cell by E7 in rat, but E11 retinal neurons were strongly dependent on NCAM function (Neugebauer et al., 1988).

NCAM has been demonstrated on several types of retinal cells (Doherty et al., 1990; Rutishauser et al., 1988; Zhang et al., 1992) including marginal endfeet of neuroepithelial cells (Balak et al., 1987), lens epithelial cells (katar et al., 1993) and on fasciculating axons and growth cones at all stages of development (Bartsch et al., 1989).

This study demonstrates that the distribution of NCAM-180 in the retina varies with developmental stages. At stage E12, immunoreactivity of NCAM-180 was first seen on the pigment of epithelial cells and retinal epithelial cells. Ganglion cells were stained first in neural retinal layers and followed by bipolar cells and photoreceptor cells. This immunoreactive pattern seems to be consistent with cell differentiation. At late embryonic (from E18) and early postnatal ages (to P7), the optic nerve expressed NCAM- 180. However, beginning with the second postnatal week, immunoreactivity of NCAM-180 decreased from the optic nerve and finally disappeared at P34. This transient appearance of NCAM-180 at those stages in the optic nerve, must be related to their role for specific histogenetic events such as axonal growth and synaptogenesis. From the P7 outer plexiform layer, the inner plexiform layer and outer segment of the photoreceptor layer were also weakly stained. Immunoreactive staining at P7 was similar to that observed in the adult.

According to the study of Fliesler et al. (1990), adult isoforms of total NCAM related its presence in the plexiform layer, and nerve fiber layer. Minor reactivity was also detected in nuclear layers, but not in the outer segment of photoreceptor layer and pigment epithelium suggesting that NCAM is not a likely participant in the process of neural retina-pigment epithelium adhesion in the adult eye. This different expression in total NCAM and NCAM-180 might suggest different roles of three major isoforms of NCAM in different histogenetic events during development. Moreover, in the salamander, both NCAM total and NCAM-180 were continuously expressed in the optic nerve as well as the retina and tectum of developing and adult animals (Becker et al., 1993). In the crushed adult optic nerve, regenerating fibers were also NCAM-180 positive. The sustained expression of NCAM-180 in adult salamanders might be due to paedomorphosis.

Studies demonstrating that NCAM can mediate neuronal outgrowth of optic fibers have been done mainly on chicks, thus, it is not clear these generalize to mammals. Even studies on rats illustrated that while embryonic cortical neurons could grow on substrates of NCAM, retinal neurons could not (Hankin and Lagenaur, 1994). Thus definite roles of NCAM including NCAM-180 during development will need to be determined in future research.

Taken together, this observation concludes that NCAM-180 expressions in the olfactory systems show features in common with the optic system and that the differences in NCAM-180 expression of nerves may be related in regenerative failure of the optic system. NCAM-180 was stained only on mature olfactory neurons, not other types of cells in the olfactory epithelium, while it changed in distribution on three types of retinal cells with developmental stages. This is in agreement with the previous data, in which NCAM-180 is associated with nonproliferating, postmitotic, differentiating neurons. However, the expression of NCAM-180 persisted in olfactory neurons and olfactory nerves as well as the nerve fiber layer and the glomeruli of the olfactory bulb. Moreover, even cells in the retina and plexiform layers demonstrated continuous expression of this molecule. This opposes the general pattern of NCAM-180 expression known to be down-regulated during development. The functional implications of the expression of this form are most likely linked to its function in the stabilization of cell contacts. In contrast, the optic nerve showed down-regulated expression. The difference between the olfactory nerve and optic nerve may reflect the regenerative capability, continuous cell turnover, and extreme plasticity in an abnormal environment documented for the olfactory tissue. This might suggest that NCAM-180 or different isoforms of NCAM-180 play different roles in different tissues. The results also suggest that regeneration capability of the optic nerves may not depend on the ganglion cell itself, but on the extension of the optic nerve. However, other factors that contribute to the establishment of their projection need to be considered. In conclusion, NCAM-180 may accompany several histogenetic events, for example, the differentiation of neuron, fasciculation, pathfindig, axonal growth and synaptogenesis. Results also lead to the hypothesis that adult optic axon are deficient in NCAM-180 critical for successful regeneration. NCAM-180 is developmentally down-regulated in the optic nerve and not expressed in mature neurons. Regenerative failure may, therefore, be attributable to changes within the nerve as well as any inhibitory environment.

References

Aguayo AJ, Carter DA, Zwimpfer JF, Vidal-Sanz M, and Bray GM (1990) Axonal regeneration and synapse formation in the injured CNS adult mammals. In: Bjorklund A and Aguayo A (eds), Brain Repair, Stockton Press, New York, p 251-270.

Allan DW, and Greer JJ (1998) Polysialylated NCAM expression during motor axon outgrowth and myogenesis in the fetal rat. *J Comp Neurol* 391: 275-292.

Balak K, Jacobson M, Sunshine J, and Rutishauser U (1987) Neural cell adhesion molecule expression in *Xenopus* embryos. *Dev Biol* 119: 540-550.

Bartsch U, Kirckhoff F, and Schachner M (1989) Immunohistological localization of the adhesion molecules L1, N-CAM, and MAG in the developing and adult optic nerve of mice. *J Comp Neurol* 284: 451-462.

Bates CA, Becker CG, Miotke JA, and Meyer RL (1999) Expression pf polysialylated NCAM but not L1 or N-cadherin by regenerating adult mouse optic fibers in vitro. *Exp Neurol* 155: 128-139.

Becker T, Becker CG, Niemann U, Naujoks-Manteuffel C, Gerardy-Schahn R, and Roth G (1993) Amphibian-specific regulation of polysialic acid and the neural cell adhesion molecule in development and regeneration of the retinotectal system of the salamander *Pleurodeles waltl. J Comp Neurol* 336: 532- 544.

Doherty P, Cohen J, and Walsh FS (1990) Neurite outgrowth in response to transfected N-CAM changes during development and is modulated by polysialic acid. *Neuron* 5: 209-219.

Edelman GM (1986) Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annu Rev Cell Biol* 2: 81-116.

Fliesler SJ, Cole GJ, Adler AJ (1990) Neural cell adhesion molecule (NCAM) in adult vertebrate retinas:tissue localization and evidence against its role in retina-pigment epithelium adhesion. *Exp Eye Res* 50: 474-482.

Fredette B, Rutishauser U, and Landmesser L (1993) Regeneration and activity dependence of N-cadherin, NCAM isoforms and polysialic acid on chick myotubes during development. *J Cell Biol* 123: 1867-1888.

Fujisawa H (1984) Pathways of retinotectal projections in developing *Xenopus* tadpoles revealed by selective labelling of retinal axons with horse radish peroxidase. *Dev Growth Diff* 26: 656-664.

Graziadei PPC and Monti Graziadei GA (1978a) Continuous nerve cell renewal in the olfactory system. In: Jacobson M (ed), Book of Sensory Physiology, Springer-Verlag, New York, pp 55-82.

Graziadei PPC, and Monti Graziadei GA (1978b) Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *J Neurocytol* 8: 1-18.

Hankin MH and Lagenaur CF (1994) Cell adhesion molecules in the early developing mouse retina:retinal neurons show preferential outgrowth in vitro on L1 but not N-CAM. J *Neurobiol* 25: 472-487.

Harris WA (1986) Homing behavior of axons in the embryonic vertebrate brain. *Nature* 320: 266-269.

He HT, Barbet J, Chaix JC, and Gordis, C (1986) Phosphatidylinositol is involved in the membrane attachment of N-CAM-120, the smallest component of the neural cell adhesion molecule. *EMBO J* 5: 2489-2494.

Huaiyu H, Tomasiewicz H, Magnuson T, and Rutishauser U (1996) The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. *Neuron* 16: 735-743.

Joosten EAJ, Reshilov LN, Gispen WH, and Bar PR (1996) Embryonic form of NCAM and development of the rat corticospinal tract: immuno-electron microscopical localization during spinal white matter ingrowth. *Dev Brain Res* 94: 99-105.

Katar M, Alcala J, and Maisel H (1993) NCAM of the mammalian lens. *Curr Eye Res* 12: 191-196.

Koo H and Graziadei PPC (1995a) Eye primordium transplantation in *Xenopus* embryo. *Anat Embryol* 191: 155-170.

Koo H and Graziadei PPC (1995b) Cell migration from the transplanted olfactory placode in *Xenopus. Anat Embryol* 191: 171-181.

Kramer I, Hall H, Bleistein U, and Schachner M (1997) Developmenally regulated masking of an intracellular epitope of the 180 kDa isoform of the neural cell adhesion molecule NCAM. *J Neurosci Res* 49: 161-175.

Lars C, Ronn B, Bock E, Linnemann D, and Jahnsen H (1995) NCAM antibodies modulate induction of long term potentiation in rat hippocampal CA1. *Brain Res* 677: 145-151.

Magrassi L and Graziadei PPC (1985) Interaction of the transplanted olfactory placode with the optic stalk and the diencephalon in *Xenopus laevis* embryos. *Neuroscince* 15: 903-921.

Magrassi L and Graziadei PPC (1996) Lineage specification of olfactory neural precursor cells depends on continuous cell interactions. *Dev Brain Res* 96: 11-27.

Minana R, Sancho-Tello M, Climent E, Segui JM, Renau-Piqueras J, and Guerri C (1998) Intracellular location, temporal expression, and polysialylation of neural cell adhesion molecule in astrocytes in primary culture. *Glia* 24: 415-427.

Miragall F and Monti Graziadei GA (1982) Experimental studies on the olfactory marker protein. II. Appearance of the olfactory marker protein during differentiation of the olfactory sensory neurons of mouse: an immunohistochemical and autoradiographic study. *Brain Res* 239: 245-250.

Miragall F, Kadmon G, and Schachner M (1989) Expression of L1 and N-CAM cell adhesion molecules during development of the mouse olfactory system. *Dev Biol* 135: 272-286.

Monti Graziadei GA and Graziadei PPC (1979) Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *J Neurocytol* 8: 197-213.

Monti Graziadei GA (1992) Cell migration from the olfactory neuroepithelium of neonatal and adult rodents. *Dev Brain Res* 70: 65-74.

Murray BA, Hemperly JJ, Edelman GM, and Cunningham BA (1986) Alternatively spliced mRNAs code for different polypeptide chains of the chicken neural cell adhesion molecule (NCAM). *J Cell Biol* 102: 189-194.

Neugebauer KM, Tomaselli KJ, Lilien J, and Reichardt LF (1988) N-cadherin, NCAM, and integrins promote retinal neurite outgrowth on astrocytes *in vitro. J Cell Biol* 107: 1177-1187.

Oorschot DE and Jones DG (1990) Axonal regeneration in the mammalian central nervous system: a critique of hypothesis. *Adv Anat Embryol Cell Biol* 119: 1-118.

Owens GC, Edelman GM, and Cunningham BA (1987) Orientation of the neural cell adhesion molecule (NCAM) gene: alternative exon usage as the basis for different membrane associated domains. *Pro Natl Acad Sci USA* 84: 294-298.

Paz DA, Alonso DG, Pisano A, Casco VH, Knudsen KA, and Peralta Soler A (1995) Expression of isoforms of the neural cell adhesion molecule (NCAM) and polysialic acid during the development of the Bufo arenarum olfactory system. *Int J Dev Biol* 39: 1005-1013.

Pershon E and Schachner M (1987) Immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM during postnatal development of the mouse cerebellum. *J Cell Biol* 105: 569-576.

Pinching AJ and Powell TPS (1971) The neuron types of the glomerular layer of the olfactory bulb. *J Cell Sci* 9: 305-345.

Pollerberg EG, Sadoul R, Gordis C, and Schachner M (1985) Selective expression of the 180-kD component of the neural cell adhesion molecule NCAM during development. *J Cell Biol* 101: 1921-1929.

Polellerberg GE, Schachner M, and Davoust J (1986) Differentiation state-dependent surface mobilities of two forms of the neural cell adhesion molecule. *Nature* 324: 462-465.

Pollerberg GE, Burridge K, Krebs K, Goodman S, and Schachner M (1987) The 180 kD component of the neural cell adhesion molecule NCAM is involved in cell-cell contacts and cytoskeleton membrane interactions. *Cell Tissue Res* 250: 227-236.

Ronn LC, Hartz BP, and Bock E (1998) The neural cell adhesion molecule (NCAM) in development and plasticity of the

nervous system. *Exp Gerontol* 33: 853-864.
Rothbard JB, Brackenbury R, Cunningham BA, and Edelman GM (1982) Difference in the carbohydrates structures of neural adhesion molecules from adult and embryonic chicken brains. *J Biol Chem* 257: 11064-11069.
Rutishauser U, Acheson A, Hall K, Mann DK, and Sunshine J (1988) The neural cell adhesion molecule(NCAM) as a regulator of cell-cell interactions. *Science* 240: 53-57.
Stier G, and Schlosshauer A (1995) Axonal guidance in the chicken retina. *Development* 121:1443-1454
Thanos S, Bonhoeffer F, and Rutishauser U (1984) Fiber-fiber interaction and tectal cues influence the development of the chicken retinotectal projection. *Proc Natl Acad Sci USA* 81: 1906-1910.
Tiveron MC, Barboni E, Rivero FBP, Gormley AM, Seeley PJ, Grosveld F, and Morris R (1992) Selective inhibition of neurite outgrowth on mature astrocytes by Thy-1 glycoprotein. *Nature* 355: 745-748.
Zhang H, Miller RH, and Rutishauser U (1992) Polysialic acid is required for optimal growth of axons on a neuronal substrate. *J Neurosci* 12: 3107-3114.

[Received June 4, 1999; accepted July 10, 1999]