Published in final edited form as:

Neuroscience. 2007 October 26; 149(2): 328–337.

Down-regulation of the axonal PSA-NCAM expression coincides with the onset of myelination in the human fetal forebrain

Igor Jakovcevski^{*}, Zhicheng Mo, and Nada Zecevic[#]

University of Connecticut Health Center, Farmington, CT, USA

Abstract

The polysialic acid (PSA) modification of neural cell adhesion molecule, which reduces NCAM-mediated cell adhesion, is involved in several developmental processes, such as cell migration, axonal growth, pathfinding, and synaptic plasticity. It has been suggested that PSA-NCAM expression may inhibit myelination. To clarify the relationship between myelination and the expression of PSA-NCAM we systematically investigated its expression in the human forebrain from embryonic stage to midgestation (19–24 gestation weeks, gw). Immunofluorescence on cryosections showed that PSA-NCAM is expressed at the earliest stage studied (5.5 gw) in the primordial plexiform layer of the telencephalon, which mainly consists of neuronal processes. At midgestation, cortical axonal tracts in the emerging white matter were PSA-NCAM+, but they were not yet myelinated, based on the lack of myelin basic protein (MBP) immunoreaction. To follow the progression of myelination we developed organotypic slice cultures that included the subventricular and intermediate zones of the fetal forebrain. In freshly prepared slices, similar to cryosections, axonal tracts were PSA-NCAM+ but did not express MBP. After 5 days in culture there was a dramatic increase in MBP expression around the axons of the intermediate zone, which suggested the onset of myelination. Simultaneously with MBP up-regulation PSA-NCAM expression in axons was completely lost, as demonstrated both with immunofluorescence and Western blot analysis. These results support the idea that in the human fetal forebrain axonal PSA-NCAM expression is inversely related to primary myelination.

Keywords

development; immunohistochemistry; midgestation; myelination; organotypic slice culture; subventricular zone

INTRODUCTION

Neuron Cell Adhesion Molecule (NCAM) was the first cell adhesion molecule identified in the nervous system. Its ubiquitous and early expression in nervous system is well documented across species (Moore et al., 1998). It is expressed by nearly all postmitotic neurons, Schwann cells, oligodendrocytes and astrocytes (Seilheimer and Schachner, 1988). There are three major isoforms of NCAM at molecular weight of 180, 140 and 120 kD, produced by alternative splicing of primary transcript (Santoni et al., 1989). Early during development NCAM is undergoing post-translational modification by attachment of polysialic polymer to its fifth Ig-like domain (Ong et al., 1998). While all isoforms of NCAM may be sialylated, 180kD form

[#] Corresponding author: Nada Zecevic, M.D., Ph.D., Neuroscience Department, University of Connecticut Health Center, 263 Farmington Ave. Farmington, CT 06030-3401, Tel: 1-860-679-1768; fax: 1-860-679-8766, E-mail: nzecevic@neuron.uchc.edu.

^{*} Present address – Center for Molecular Neurobiology, Hamburg, Germany

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is the main carrier of polysialic acid (PSA; Franceschini et al., 2001). The PSA modification of NCAM is believed to reduce NCAM-mediated cell adhesion (Kiss et al., 2001), and to be involved in dynamic cellular processes, such as cell migration, axonal growth, pathfinding and synaptic plasticity (Rutishauser and Landmesser, 1996; Decker et al., 2000; Bruses and Rutishauser, 2001; Kleene and Schachner, 2004). After establishment of axonal projections and synaptic contacts, the amount of PSA-NCAM in the brain decreases (Rutishauser and Landmesser, 1996).

In the human brain expression of PSA-NCAM was previously described in the fetal ganglionic eminence (Ulfig and Chan, 2004) and in the adult cerebral cortex (Arellano et al., 2002) and hippocampus (Ni Dhuill et al., 1999; Arellano et al., 2004). Presence of PSA-NCAM, as an embryonic form of NCAM, in the adult human brain was related to brain pathology, such as schizophrenia (Barbeau et al., 1995) and pituitary tumors (Trouillas et al., 2003). Of special interest is that PSA-NCAM has been implicated as an inhibitor of primary myelination in mice (Charles et al., 2000). Moreover, this molecule is re-expressed by demyelinated axons in human multiple sclerosis lesions (Charles et al., 2002).

In this study we investigate the relationship between primary myelination and the expression of PSA-NCAM in the human fetal forebrain. The level of myelination was estimated by the expression of myelin basic protein (MBP), a marker of terminally differentiated oligodendrocytes (Pfeiffer et al., 1993; Jakovcevski and Zecevic, 2005a). In organotypic slice culture of human fetal forebrain, the increased MBP expression was demonstrated after 3–5 days in vitro (Jakovcevski and Zecevic, 2005a). We now show that, simultaneously with up-regulation of MBP expression, there is a down-regulation of PSA-NCAM immunoreactivity in the same cultures. Our results suggest that in the human fetal forebrain the onset of myelination is inversely related to the amount of PSA-NCAM expressed by axons.

EXPERIMENTAL PROCEDURES

Tissue

Human fetuses (5–24 gw; $n = 15$, see table 1) were obtained after legal abortions performed at the Obstetrics and Gynecology Clinic, University of Belgrade (Serbia), and from the Brain Bank, The Albert Einstein College of Medicine (Bronx, NY). The Institutional Ethics Committees of the University of Belgrade, the Albert Einstein College of Medicine, and the University of Connecticut approved the tissue collection, and informed consent was obtained from the parents. The handling of tissue was performed in accordance with regulations set forth by the Institutional Ethics Committees and the Helsinki Convention. No evidence of disease or developmental abnormalities was discovered after ultrasonic and neuropathological examination. The time between the abortion and the fixation of the collected tissue was on average 15 min. The ages of the embryos and fetuses were estimated on the basis of weeks after ovulation, crown-rump length (Olivier and Pineau, 1962), and anatomical landmarks (O’Rahilly et al., 1987). Tissue blocks were cut frontally through the level of basal ganglia, the thalamus, and the internal capsule. More detailed description of the midgestational forebrain tissue used in this study with schematic drawings is available in our previous publication (Jakovcevski and Zecevic, 2005b, Fig 4A).

Tissue was fixed in 4% paraformaldehyde in 0.1M phosphate buffer, cryoprotected by immersion in 30% sucrose, and frozen in isopentane cooled to -70°C . Frozen tissue was cut into 15- μm -thick sections for immunohistochemistry.

Organotypic slice culture

Human fetal brains ($n = 4$; age 19–23 g.w.) were kept in the ice-cold Hank's balanced salt solution (HBSS, Sigma, St. Louis, MO) with 0.75% glucose and were dissected within 2 h after extraction. The pia was stripped and frontal and occipital poles were excluded from the slice dissection; therefore, slices were cut from the rostral end of the lateral ventricle through the middle part of the fetal brain. Pieces of tissue were kept on ice-cold HBSS until cut. Dimensions of each slice, containing the neocortical SVZ and the adjacent white matter, were approximately $1 \times 1 \times 0.5$ cm. Tissue was embedded in 3% low-melted agarose and cut frontally into 400- μ m-thick slices with a Vibroslice (model VSLM1, World Precision Instruments, Sarasota, FL). Slices were transferred to 30-mm membrane inserts, placed in six-well plates containing Dulbecco's modified Eagle's medium (DMEM, Hepes modification; Sigma) with 120 mM D-glucose, 2 mM L-glutamine, N2 (Gibco, Gaithersburg, MD), 5% fetal bovine serum (FBS, Hyclone, Logan, UT), and antibiotic-antimycotic (Gibco). Slices were incubated for 2 days in culture (DIC) or 5 DIC at 37°C with 5% CO₂ and then fixed in 4% paraformaldehyde, cryoprotected with 30% sucrose and mounted on glass slides. Slices were re-sectioned using a cryostat into 15- μ m-thick sections and processed for immunohistochemistry.

Dissociated cell culture

Cell cultures were prepared from the VZ/SVZ of the fetal forebrain (16 gw), dissected from the frontally cut hemispheres as a tissue band approximately 2000 μ m high from the VZ surface (Zecevic et al., 2005). Tissue was dissociated with 0.05% trypsin-0.02% EDTA (Invitrogen, Carlsbad, CA) and triturated through a fire-polished pipette. Cells were resuspended in DMEM/F12 (Invitrogen) containing 10% FBS (supplemented with B27 (Invitrogen), and seeded into poly-L-lysine (Sigma) coated eight-well chamber slides (BD Falcon, San Jose, CA) at a concentration of 4×10^4 cells/well. Cell cultures were incubated at 37°C with 5% CO₂ and 95% O₂ for seven days, fixed in 4% paraformaldehyde for 10 minutes, and processed for immunostaining with primary and secondary antibodies as described below.

Antibodies

PSA-NCAM was detected with the rat monoclonal IgM antibody (1:100; Becton-Dickinson, San Jose, CA; Walsh and Doherty, 1993). Oligodendrocyte progenitor cells were labeled with antibody against platelet-derived growth factor receptor (PDGFR- α , mouse, 1:25; Pharmingen, San Diego, CA; Lu et al., 2000) and chondroitin sulfate proteoglycan (NG2, rabbit, 1:100, Chemicon, Temecula, CA). Oligodendrocytes were labeled with antibody to MBP (mouse, SMI-99, 1:100; Sternberger Monoclonals, Lutherville, MD; Weidenheim et al., 1992). Rabbit antibody against the transcription factor Olig2 was gift from Dr. C. D. Stiles, Harvard Medical School, Boston, MA (1:5000; Jakovceviski and Zecevic, 2005b). Pan Dlx antibody, rabbit anti-DLL antibody (1:40, Rakic and Zecevic, 2003) was a gift Dr. Y. Kohtz. Neurons were labeled with monoclonal (mouse) antibodies to microtubule-associated protein 2 (MAP2) (1:200; Sigma), and β -III-tubulin (1:200; Sigma). Radial glia and astrocytes were labeled with anti-glial fibrillary acidic protein (GFAP; rabbit, 1:50; Dako Carpinteria, CA). A short (1–2 min.) incubation in bisbenzamide (Sigma) was used to reveal the cell nuclei.

Controls were done by omitting the primary antibody, which resulted in a lack of immunofluorescence.

Immunofluorescence and image analysis

Immunohistochemistry was performed as described before (Jakovceviski and Zecevic, 2005a). Various primary antibodies and corresponding fluorescein- and rhodamine-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used (see above). Sections were viewed with a confocal laser-scanning microscope (Carl Zeiss, LSM 410) and

Zeiss Axioplan fluorescent microscope. Image processing was done in Adobe Photoshop 6.0 software (Adobe Systems Inc, San Jose, CA)

Western blot analysis

Brain slices from 19 and 20 gw fetuses were dissected through the parietal lobe, and tissue samples were homogenized in a glass homogenizer in radioimmunoprecipitation assay buffer with protease inhibitors and sonicated for 1 min. Homogenates were run on 7% (for PSA-NCAM) or 15% (for MBP and β -actin) SDS-PAGE, and transferred to a nitrocellulose membrane (Hybond P; Amersham Biosciences, Piscataway, NJ). Membranes were blocked with 5% nonfat milk in TBST buffer (Tris 10mM, NaCl 150mM, Tween-20 0.05%, pH 7.4), and incubated overnight at 4°C with primary antibodies (MBP, 1:500; PSA-NCAM, 1:500), followed by incubation for 1 hour at room temperature with the horseradish peroxidase conjugated secondary antibodies. The signals were detected by enhanced chemiluminescence (Amersham Biosciences). Densitometric analysis of bands was done with Image J program (NIH).

RESULTS

Expression pattern of PSA-NCAM during the first half of intrauterine development

In order to identify PSA-NCAM-positive (+) cells we performed single and double immunohistochemical analysis of fetal brains age 5.5 to 24 weeks (table 1) with PSA-NCAM antibody and cell-type specific antibodies. At embryonic age (5.5 gw, n=2) PSA-NCAM is strongly expressed in the human forebrain (Fig. 1A–E). The immune reaction is localized in the preplate or primordial plexiform layer (Marin-Padilla, 1983), above the ventricular zone, in the neuronal processes co-labeled with neuronal marker β -III-tubulin (Fig. 1A–E). At 10 gw, the expression of PSA-NCAM is mainly localized in two parallel strata, above and below cortical plate, in the layer I and pre-subplate, respectively (Fig. 1F). These two fetal layers contain afferent fibers. PSA-NCAM is also abundant in the lateral ganglionic eminence and cells streaming from this ventral region towards dorsolateral cerebral cortex (Fig. 1G, H). At these early stages of development, dispersed cells in cortical ventricular/subventricular zone (VZ/SVZ) express MBP-like proteins (Golli), but this expression is not related to myelination (Zecevic et al., 1998, Tosic et al., 2002).

At midgestation (19–24 gw), the next stage available for this study, PSA-NCAM is highly expressed on axonal tracts in the intermediate zone, the future white matter. In addition, PSA-NCAM is expressed by numerous progenitor cells around lateral ventricles, in the VZ and SVZ zones (Fig. 2A–F). To clarify the identity of PSA-NCAM+ cells in the SVZ we co-labeled them with various glial and neuronal cell markers. Early oligodendrocyte progenitors in the SVZ are labeled with PDGFR α and NG2 antibodies, but none of these cells seem to express PSA-NCAM (Fig. 2C). Another commonly used oligodendrocyte progenitor marker is Olig2 transcription factor, which also labels some neuronal progenitors in the human forebrain (Jakovcevski and Zecevic, 2005b). Although majority of Olig2+ cells did not express PSA-NCAM (Fig. 2D), a small subpopulation of the Olig2+/PSA-NCAM+ cells was present in the VZ/SVZ (Fig. 2D, inset).

Radial glia cells, labeled with GFAP, extend long radial fibers from VZ/SVZ to the cortical plate (Zecevic, 2004). Neither of those fibers, nor the cell bodies of radial glia cells in the VZ are co-labeled with PSA-NCAM (Fig. 2E, F).

On the other hand, a subpopulation of neuronal progenitors in the SVZ, labeled with MAP2, or pan-Dlx antibody, co-express PSA-NCAM (Fig. 3A, B). To further clarify cell of PSA-NCAM+ progenitor cells we immunolabeled the dissociated mixed cell cultures obtained from

fetal SVZ (16 gw). In these cultures, we confirmed co-localization of PSA-NCAM with neuronal (β -III-tubulin), but not with astroglial marker, GFAP (Fig. 3C, D).

Cell bodies of more mature neurons, which either migrate through the intermediate zone (MAP2+) or are already in the cortical plate (NeuN+, MAP2+), do not express PSA-NCAM (not shown). These double labeling experiments demonstrate that PSA-NCAM is present on a subpopulation of neuronal progenitor cells (MAP2, β -III-tubulin), whereas majority of radial glia fibers (GFAP+), or oligodendrocyte progenitor cells (NG2, PDGFR α) and cell bodies of mature neurons (NeuN), are not labeled with PSA-NCAM. However, axonal tracts in the intermediate zone express PSA-NCAM at midgestation.

Expression pattern of PSA-NCAM on axonal tracts at midgestation

PSA-NCAM is highly expressed by axonal tracts in the emerging forebrain white matter (the intermediate zone, Fig. 2C–E; 4C, D). During midgestation, the first MBP+ pre-myelinating oligodendrocytes appear in the telencephalic white matter (Jakovcevski and Zecevic, 2005a). In all forebrain regions studied (corpus callosum, intermediate zone, and internal capsule), MBP+ myelinating oligodendrocytes are present only in areas with low level of PSA-NCAM expression on axons. Furthermore, in the subcortical tracts where myelination has started at midgestation, only fibers that are PSA-NCAM negative show MBP+ immunoreaction (Fig. 4A–F). We conclude that at midgestation myelination progresses through regions in which PSA-NCAM expression is down-regulated.

PSA-NCAM expression in organotypic slice cultures

In order to test whether PSA-NCAM expression changes during progression of myelination, we analyzed slice cultures from human fetal subventricular and intermediate zones at midgestation (Jakovcevski and Zecevic, 2005a). Initially in slice cultures PSA-NCAM is abundant in the intermediate zone (Fig. 5A, B), but after five days *in vitro*, becomes virtually undetectable by immunofluorescence (Fig. 5C, D). Conversely, numerous MBP reactive oligodendrocytes are present in five days old slices (Fig. 5E), but not in the brain sections from the same gestational age and regions (Jakovcevski and Zecevic, 2005a). Most notably, MBP+ processes on slices appear to follow the axons, suggesting myelination (Jakovcevski and Zecevic, 2005a). Immunoblot confirmed this result, showing significantly smaller amounts of axonal, 200 kD PSA-NCAM isoform at 5DIC (Fig. 6A). A 35 % decrease of PSA-NCAM was statistically significant ($p < 0.05$, ANOVA with Dunnett *post-hoc* test; $n = 3$, in 2 independent experiments). On the same brain slices of the 19 gw fetal brain, the expression of MBP is significantly increased by 72 % after 5DIC compared to the initial values (0 DIC) (Fig. 6B). We conclude that with the progression of primary myelination, axonal expression of PSA-NCAM is downregulated.

DISCUSSION

Presented results suggest a relation between the onset of myelination and the down-regulation of PSA-NCAM expression by axons in the developing white matter of the human fetal telencephalon. This relation seems to be well conserved in mammals, since studies in mice have shown that the onset of myelination depends on down-regulation of PSA-NCAM on axonal surface, but the removal of PSA-NCAM alone was not sufficient to promote myelination *in vitro* (Charles et al., 2000). Accordingly, PSA-NCAM in rodents is expressed at first on all growing neuronal tracts, but myelination occurs only on axons that down-regulate PSA-NCAM (Bartsch et al., 1990; Oumesmar et al., 1995). This is also supported by present findings in the human fetal brain.

Various signals were proposed to be essential for the onset of myelination. For example, although mouse oligodendrocytes *in vitro* have the capacity to form myelin-like membranes in the absence of neurons (Temple and Raff, 1986), co-culture with neurons increases MBP gene expression (Macklin et al., 1986), suggesting that axons provide a necessary signal for the onset of myelination. This notion is supported by *in vivo* experiments in rat, which demonstrated that oligodendrocyte progenitor cell proliferation and subsequent myelination depend on the electrical activity in axons (Barres and Raff, 1993; Demerens et al., 1996; Fields and Stevens-Graham, 2002). Other reports stress the importance of axon diameter for the myelination over either progression of oligodendrocyte differentiation or electrical activity of axons (Colello et al., 1995). In our human slice culture model, a prominent increase in myelination coincided with PSA-NCAM down-regulation, which argues for a possible role of PSA-NCAM signaling mechanism as one of the regulators of primary myelination in the human fetal brain. This is further supported by the finding that in multiple sclerosis axons, which fail to regenerate myelin sheathes, also re-express PSA-NCAM (Charles et al., 2002). However, when Schwann cells engineered to express PSA-NCAM were transplanted into a spinal cord injury site they promoted myelination (Papastefanaki et al., 2007). This indicates that a negative connection between PSA-NCAM and myelination observed in our study may be cell-type specific.

In rodents, the strongest PSA-NCAM staining was detected on growing axon bundles (Daston et al., 1996; Seki and Arai, 1999), in agreement with our findings in the human fetal brain. Expression of PSA-NCAM by various progenitor cell populations in the central nervous system was reported (Gascon et al., 2007; Vitry et al., 2001; Allan and Greer, 1998; Oumesmar et al., 1995), including *in vivo* expression by glial restricted progenitors in the perinatal mouse subventricular zone (Marshall and Goldman, 2002). *In vitro* isolated PSA-NCAM+ cells considered “oligodendrocyte pre-progenitors” (Ben-Hur et al., 1998; Decker et al., 2000) were used to give rise to both glia and neurons upon transplantation, depending on their insertion site in the developing mouse brain (Vitry et al., 2001). We, however, could not confirm the expression of PSA-NCAM by either early oligodendrocyte progenitor cells or astroglial cells. The population of PSA-NCAM+ cells in the human fetal subventricular zone represents neuronal progenitor cells, positive for several neuronal markers (β -III-tubulin, MAP2, pan Dlx) and negative for oligodendrocyte precursor markers studied here (PDGFR α , NG2), as well as for astroglial lineage markers, vimentin and GFAP. The small proportion of Olig2+/PSA-NCAM+ cells probably represents neuronal progenitors, although it is possible that a subpopulation of oligodendrocyte progenitors during very early stages of development expresses PSA-NCAM. This would be consistent with recent reports describing various oligodendrocyte progenitors subpopulations in rodents (Spassky et al., 2000; Kessaris et al., 2006; Parras et al., 2007), and possibly in humans, as we discussed previously (Jakovcevski and Zecevic, 2005b). The fact that at midgestation glia cell populations in the human brain do not express PSA-NCAM, as has been shown in rodents, might be due to either inter-species differences or difference in studied stages of development. In addition, astroglial expression of PSA-NCAM reported earlier was related to the reactive astrocytes (Oumesmar et al., 1995; Charles et al., 2002; Camand et al., 2004). It is not unusual that cell adhesion molecules show different patterns of expression during development and in regeneration. Another cell adhesion molecule, close homolog of L1, present only on axons during development, is highly expressed by the reactive astrocytes after spinal cord injury (Jakovcevski et al., 2007).

In conclusion, our results on PSA-NCAM expression in the human fetal forebrain show similarities and some differences comparing to studies in other mammals. In contrast to rodents, PSA-NCAM expression in human fetal subventricular zone was confined to neuronal and not glia progenitors. However, similar to reports in rodents, the PSA-NCAM expression on premyelinating axons, and its down regulation at the onset of myelination, suggests a

phylogenetically conserved mechanism related to myelination. To elucidate if the connection between PSA-NCAM expression and myelination is causative awaits further studies.

Acknowledgements

This study was supported by grant RG-3083-B3/1 from the National Society for Multiple Sclerosis (to N.Z.). Human fetal tissue was obtained from Tissue Repository, The Albert Einstein College of Medicine, Bronx, NY. Authors wish to thank C. D. Stiles, D. H. Rowitch, and Y. Kohtz for generous gifts of antibodies, and A. Irintchev for critical reading of the manuscript. Parts of this work were presented as an abstract on 36th Annual Meeting of the Society for Neuroscience, 2006.

Supported by grant RG-3083-B3/1 from the National Society for Multiple Sclerosis

References

- Allan DW, Greer JJ. Polysialylated NCAM expression during motor axon outgrowth and myogenesis in the fetal rat. *J Comp Neurol* 1998;391:275–292. [PubMed: 9492201]
- Arellano JI, DeFelipe J, Munoz A. PSA-NCAM immunoreactivity in chandelier cell axon terminals of the human temporal cortex. *Cereb Cortex* 2002;12:617–624. [PubMed: 12003861]
- Arellano JI, Munoz A, Ballesteros-Yanez I, Sola RG, DeFelipe J. Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain* 2004;127:45–64. [PubMed: 14534159]
- Barbeau D, Liang JJ, Robitaille Y, Quirion R, Srivastava LK. Decreased expression of the embryonic form of the neural cell adhesion molecule in schizophrenic brains. *Proc Natl Acad Sci U S A* 1995;92:2785–2789. [PubMed: 7708724]
- Barres BA, Raff MC. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 1993;361:258–260. [PubMed: 8093806]
- Bartsch U, Kirchhoff F, Schachner M. Highly sialylated N-CAM is expressed in adult mouse optic nerve and retina. *J Neurocytol* 1990;19:550–565. [PubMed: 2243247]
- Ben-Hur T, Rogister B, Murray K, Rougon G, Dubois-Dalcq M. Growth and fate of PSA-NCAM+ precursors of the postnatal brain. *J Neurosci* 1998;18:5777–5788. [PubMed: 9671666]
- Bruses JL, Rutishauser U. Roles, regulation, and mechanism of polysialic acid function during neural development. *Biochimie* 2001;83:635–643. [PubMed: 11522392]
- Camand E, Morel MP, Faissner A, Sotelo C, Dusart I. Long-term changes in the molecular composition of the glial scar and progressive increase of serotonergic fibre sprouting after hemisection of the mouse spinal cord. *Eur J Neurosci* 2004;20:1161–1176. [PubMed: 15341588]
- Charles P, Hernandez MP, Stankoff B, Aigrot MS, Colin C, Rougon G, Zalc B, Lubetzki C. Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proc Natl Acad Sci U S A* 2000;97:7585–7590. [PubMed: 10840047]
- Charles P, Reynolds R, Seilhean D, Rougon G, Aigrot MS, Niezgoda A, Zalc B, Lubetzki C. Re-expression of PSA-NCAM by demyelinated axons: an inhibitor of remyelination in multiple sclerosis? *Brain* 2002;125:1972–1979. [PubMed: 12183343]
- Colello RJ, Devey LR, Imperato E, Pott U. The chronology of oligodendrocyte differentiation in the rat optic nerve: evidence for a signaling step initiating myelination in the CNS. *J Neurosci* 1995;15:7665–7672. [PubMed: 7472517]
- Daston MM, Bastmeyer M, Rutishauser U, O'Leary DD. Spatially restricted increase in polysialic acid enhances corticospinal axon branching related to target recognition and innervation. *J Neurosci* 1996;16:5488–5497. [PubMed: 8757261]
- Decker L, Avellana-Adalid V, Nait-Oumesmar B, Durbec P, Baron-Van Evercooren A. Oligodendrocyte precursor migration and differentiation: combined effects of PSA residues, growth factors, and substrates. *Mol Cell Neurosci* 2000;16:422–439. [PubMed: 11085879]
- Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. Induction of myelination in the central nervous system by electrical activity. *Proc Natl Acad Sci USA* 1996;93:9887–9892. [PubMed: 8790426]
- Fields RD, Stevens-Graham B. New insights into neuroglia communication. *Science* 2002;298:556–562. [PubMed: 12386325]

- Franceschini I, Angata K, Ong E, Hong A, Doherty P, Fukuda M. Polysialyltransferase ST8Sia II (STX) polysialylates all of the major isoforms of NCAM and facilitates neurite outgrowth. *Glycobiology* 2001;11:231–239. [PubMed: 11320061]
- Gascon E, Vutskits L, Jenny B, Durbec P, Kiss JZ. PSA-NCAM in postnatally generated immature neurons of the olfactory bulb: a crucial role in regulating p75 expression and cell survival. *Development* 2007;134:1181–1190. [PubMed: 17301083]
- Jakovcevski I, Wu J, Karl N, Leshchyn'ska I, Sytnyk V, Chen J, Irintchev A, Schachner M. Glial scar expression of CHL1, the close homolog of the adhesion molecule L1, limits recovery after spinal cord injury. *J Neurosci* 2007;27:7222–7233. [PubMed: 17611275]
- Jakovcevski I, Zecevic N. Sequence of oligodendrocyte development in the human fetal telencephalon. *Glia* 2005a;49:480–491. [PubMed: 15578660]
- Jakovcevski I, Zecevic N. Olig transcription factors are expressed in oligodendrocyte and neuronal cells in human fetal CNS. *J Neurosci* 2005b;25:10064–10073. [PubMed: 16267213]
- Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci* 2006;9:173–179. [PubMed: 16388308]
- Kiss JZ, Troncoso E, Djebbara Z, Vutskits L, Muller D. The role of neural cell adhesion molecules in plasticity and repair. *Brain Res Brain Res Rev* 2001;36:175–184. [PubMed: 11690614]
- Kleene R, Schachner M. Glycans and neural cell interactions. *Nat Rev Neurosci* 2004;5:195–208. [PubMed: 14976519]
- Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. *Neuron* 2000;25:317–329. [PubMed: 10719888]
- Macklin WB, Weill CL, Deininger PL. Expression of myelin proteolipid and basic protein mRNAs in cultured cells. *J Neurosci Res* 1986;16:203–217. [PubMed: 2427736]
- Marin-Padilla M. Structural organization of the human cerebral cortex prior the appearance of the cortical plate. *Anat Embryol* 1983;168:21–40. [PubMed: 6650855]
- Marshall CA, Goldman JE. Subpallial Dlx2-expressing cells give rise to astrocytes and oligodendrocytes in the cerebral cortex and white matter. *J Neurosci* 2002;22:9821–9830. [PubMed: 12427838]
- Moore SS, Hale P, Byrne K. NCAM: a polymorphic microsatellite locus conserved across eutherian mammal species. *Anim Genet* 1998;29:33–36. [PubMed: 9682447]
- Ni Dhuill CM, Fox GB, Pittock SJ, O'Connell AW, Murphy KJ, Regan CM. Polysialylated neural cell adhesion molecule expression in the dentate gyrus of the human hippocampal formation from infancy to old age. *J Neurosci Res* 1999;55:99–106. [PubMed: 9890438]
- Oumesmar BN, Vignais L, Duhamel-Clerin E, Avellana-Adalid V, Rougon G, Baron-Van Evercooren A. Expression of the highly polysialylated neural cell adhesion molecule during postnatal myelination and following chemically induced demyelination of the adult mouse spinal cord. *Eur J Neurosci* 1995;7:480–491. [PubMed: 7773445]
- Olivier G, Pineau H. Horizons de Streeter et age embryonnaire. *Bull Ass Anat* 1962;47e:573–576.
- O'Rahilly R, Muller F, Hutchins GM, Moore GW. Computer ranking of the sequence of appearance of 73 features of the brain and related structures in staged human embryos during the sixth week of development. *Am J Anat* 1987;180:69–86. [PubMed: 3661464]
- Ong E, Nakayama J, Angata K, Reyes L, Katsuyama T, Arai Y, Fukuda M. Developmental regulation of polysialic acid synthesis in mouse directed by two polysialyltransferases, PST and STX. *Glycobiology* 1998;8:415–424. [PubMed: 9499389]
- Papastefanaki F, Chen J, Lavdas AA, Thomaidou D, Schachner M, Matsas R. Grafts of Schwann cells engineered to express PSA-NCAM promote functional recovery after spinal cord injury. *Brain*. 2007 [Epub ahead of print]
- Parras CM, Hunt C, Sugimori M, Nakafuku M, Rowitch D, Guillemot F. The proneural gene *Mash1* specifies an early population of telencephalic oligodendrocytes. *J Neurosci* 2007;27:4233–4242. [PubMed: 17442807]
- Peretto P, Giachino C, Aimar P, Fasolo A, Bonfanti L. Chain formation and glial tube assembly in the shift from neonatal to adult subventricular zone of the rodent forebrain. *J Comp Neurol* 2005;487:407–427. [PubMed: 15906315]

- Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. *Trends Cell Biol* 1993;3:191–197. [PubMed: 14731493]
- Rakic S, Zecevic N. Emerging complexity of layer I in human cerebral cortex. *Cereb Cortex* 2003;13:1072–1083. [PubMed: 12967924]
- Rutishauser U, Landmesser L. Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. *Trends Neurosci* 1996;19:422–427. [PubMed: 8888519]
- Santoni MJ, Barthels D, Vopper G, Boned A, Goridis C, Wille W. Differential exon usage involving an unusual splicing mechanism generates at least eight types of NCAM cDNA in mouse brain. *EMBO J* 1989;8:385–392. [PubMed: 2721486]
- Seilheimer B, Schachner M. Studies of adhesion molecules mediating interactions between cells of peripheral nervous system indicate a major role for L1 in mediating sensory neuron growth on Schwann cells in culture. *J Cell Biol* 1988;107:341–351. [PubMed: 3292543]
- Seki T, Arai Y. Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. *Anat Embryol (Berl)* 1991;184:395–401. [PubMed: 1952111]
- Seki T, Arai Y. Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *J Neurosci* 1993;13:2351–2358. [PubMed: 7684771]
- Seki T, Arai Y. Different polysialic acid-neural cell adhesion molecule expression patterns in distinct types of mossy fiber boutons in the adult hippocampus. *J Comp Neurol* 1999;410:115–125. [PubMed: 10397399]
- Spassky N, Olivier C, Perez-Villegas E, Goujet-Zalc C, Martinez S, Thomas J, Zalc B. Single or multiple oligodendroglial lineages: a controversy. *Glia* 2000;29:143–148. [PubMed: 10625332]
- Temple S, Raff MC. Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. *Cell* 1986;44:773–779. [PubMed: 3948247]
- Tosic M, Rakic S, Matthieu J-M, Zecevic N. Identification of Golli and Myelin Basic Proteins in human brain during early development. *Glia* 2002;37:219–228. [PubMed: 11857680]
- Trouillas J, Daniel L, Guigard MP, Tong S, Gouvernet J, Jouanneau E, Jan M, Perrin G, Fischer G, Tabarin A, Rougon G, Figarella-Branger D. Polysialylated neural cell adhesion molecules expressed in human pituitary tumors and related to extrasellar invasion. *J Neurosurg* 2003;98:1084–1093. [PubMed: 12744370]
- Ulfing N, Chan WY. Expression patterns of PSA-NCAM in the human ganglionic eminence and its vicinity: role of PSA-NCAM in neuronal migration and axonal growth? *Cells Tissues Organs* 2004;177:229–236. [PubMed: 15459479]
- Vitry S, Avellana-Adalid V, Lachapelle F, Evercooren AB. Migration and multipotentiality of PSA-NCAM+ neural precursors transplanted in the developing brain. *Mol Cell Neurosci* 2001;17:983–1000. [PubMed: 11414788]
- Walsh FS, Doherty P. Factors regulating the expression and function of calcium-independent cell adhesion molecules. *Curr Opin Cell Biol* 1993;5:791–796. [PubMed: 8240822]
- Weidenheim KM, Kress Y, Epshteyn I, Rashbaum WK, Lyman WD. Early myelination in the human fetal lumbosacral spinal cord: characterization by light and electron microscopy. *J Neuropathol Exp Neurol* 1992;51:142–149. [PubMed: 1538238]
- Zecevic N, Andjelkovic A, Matthieu J-M, Tosic M. Myelin basic protein immunoreactivity in the human embryonic CNS. *Dev Brain Res* 1998;105:97–108.
- Zecevic N. Specific characteristics of radial glia in the human fetal telencephalon. *Glia* 2004;48:27–35. [PubMed: 15326612]
- Zecevic N, Chen Y, Filipovic R. Contributions of cortical subventricular zone to the development of the human cerebral cortex. *J Comp Neurol* 2005;491:109–122. [PubMed: 16127688]

List of abbreviations

DIC

days in culture

DMEM

	Dulbecco's modified Eagle's medium
FBS	fetal bovine serum
HBSS	Hank's balanced salt solution
GFAP	glial fibrillary acidic protein
gw	gestation weeks
MAP2	microtubule associated protein 2
MBP	myelin basic protein
NCAM	neural cell adhesion molecule
NG2	chondroitin sulfate proteoglycan
PBS	phosphate buffered saline
PDGFRα	platelet derived growth factor receptor alpha
PSA	polysialic acid
SVZ	subventricular zone
VZ	ventricular zone

Figure 1.

PSA-NCAM expression in early developmental stages. (A–E) In the embryonic (5.5 gw) forebrain the PSA-NCAM is expressed in the primordial plexiform layer (PPL), above the ventricular zone (VZ). (A, B) Low magnification of the frontal section through the cerebral vesicle immunostained with (A) PSA-NCAM (red) or (B) β-III-tubulin (green). (C–E) Higher magnification of the cerebral vesicle (boxed area in A). Double-immunofluorescence demonstrates PSA-NCAM (red) in neuronal processes labeled with β-III-tubulin (green) (D) Arrow points to a double-labeled cell in the VZ. (E) Overlay. (F–H) At 10 gw, (F) strong PSA-NCAM (red) expression in the cerebral cortex is bilaminar, in layer I and in the pre-subplate (SP), whereas it is faint in the cortical plate (CP). (G) At the same age, PSA-NCAM is expressed

in the lateral ganglionic eminence (LGE) and in (H) a “stream” of cells connecting LGE and the lateral cortex (asterisk). LV - lateral ventricle; Cx – cerebral cortex. Scale bars: (F) 20µm (A–E, G) 100µm, (H) 50µm.

Figure 2.

PSA-NCAM expression at midgestation (19–24 gw). (A, B) Immunofluorescence for PSA-NCAM (red) in the ventricular zone (VZ). PSA-NCAM is expressed by the neural progenitor cells (arrows). Some of the subventricular zone (SVZ) progenitors are also PSA-NCAM+. On panel A, nuclei are counter-stained with bis-benzamide (blue). (C) Double-immunofluorescence for PDGFR α (green), marker for early oligodendrocyte precursor cells, and PSA-NCAM (red). (D) Double immunofluorescence for Olig2 (green) and PSA-NCAM (red) in the VZ/SVZ. Inset-higher magnification of a cell co-labeled with both markers. (E) Double immunofluorescence for GFAP (green) and PSA-NCAM (red) in the SVZ of the medial cortex of the frontal lobe. Axons in the IZ, the emerging white matter, are strongly PSA-NCAM+ (red). (F) Enlarged detail from the box in E showing VZ/SVZ expression of GFAP (green)

and PSA-NCAM (red). LV - the lateral ventricle. Scale bars: 25 μm (A–C), 100 μm (D, E), 20 μm (F).

Figure 3.

PSA-NCAM is expressed in neuronal progenitors in vivo and vitro. (A, B) Neuronal progenitors in the 19 gw old cortical subventricular zone (SVZ) labeled with (A) MAP2 (green) and (B) Dll (pan Dlx antibody, green) are co-labeled with PSA-NCAM (red). Nuclei are counter-stained with bis-benzamide (blue). (C, D) In dissociated mixed cell culture at 16 gw (C) GFAP (green) and PSA-NCAM (red) are not co-localized in astroglia, whereas (D) β -III-tubulin+ cells (green, arrows) are co-labeled with PSA-NCAM (red), co-localization seen in yellow. Scale bar (A, B) 10 μ m, (C, D) 25 μ m.

Figure 4.

Axonal expression of *PSA-NCAM* in the thalamus and forebrain at midgestation. (A–F) Double-immunofluorescence for MBP (A, B, green) and PSA-NCAM (C, D, red) of the thalamus (Th), (A, C, E) and the internal capsule (IC), (B, D, F) at 22 gw Panels E, F show overlapped images. Scale bar: 25 μ m.

Figure 5.

PSA-NCAM and MBP immunoreactivity in slice cultures. (A, B) Immunofluorescence for PSA-NCAM (red) on slices from human midgestational forebrains fixed immediately (0 DIC). Blue on panels B, D is nuclear counterstaining with bis-benzamide (bb). (C, D) On 5 DIC slice cultures there is virtually no PSA-NCAM expression (a lack of red staining). (E) On the same slices (5 DIC) MBP+ oligodendrocytes (arrows, red) and fibers are frequently demonstrated. Shown are representative images from 4 independent experiments. Scale bars: 25 μm.

Figure 6. Western blot analysis of PSA-NCAM and MBP expression in the slice cultures of the human fetal brain at 19 gw. Panel A shows representative blots at 0 DIC and 5DIC; panel B shows mean values + standard error of mean (SEM). Actin is used as a loading control. In panel B, initial (0 DIC) values for PSA-NCAM and MBP are normalized to 100%. An asterisk indicates significant difference from 0 day ($p < 0.05$). Protein loading: 20 μ g/well.

Table 1

Human fetal brains used in this study

Case number	Gender	Gestation weeks	Sections	Slice culture
1	?	5 (16)	+	
2	?	5.5. (17)	+	
3	?	10	+	
4	F	19	+ WB	
5	M	19	+WB	
6	M	19	+	+
7	F	20	+	
8	M	22	+ WB	+
9	F	22	+	
10	F	22	+	+
11	F	22	+	
12	M	23	+	
13	M	23	+	
14	M	23	+	+
15	F	24	+	

Carnegie stages are in parentheses. F, Female; M, male; WB, Western blot.