Molecular Basis of Cell and Developmental Biology:
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Rescued by Deletion of the Neural Cell Adhesion Molecule

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Genetic Ablation of Polysialic Acid Causes Severe Neurodevelopmental Defects Rescued by Deletion of the Neural Cell Adhesion Molecule*

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Poly-α2,8-sialic acid (polySia) is a unique modification of the neural cell adhesion molecule, NCAM, tightly associated with neural development and plasticity. However, the vital role attributed to this carbohydrate polymer has been challenged by the mild phenotype of mice lacking polySia due to NCAM-deficiency. To dissect polySia and NCAM functions, we generated polySia-negative but NCAM-positive mice by simultaneous deletion of the two polysialyltransferase genes, St8sia-II and St8sia-IV. Beyond features shared with NCAM-null animals, a severe phenotype with specific brain wiring defects, progressive hydrocephalus, postnatal growth retardation, and precocious death was observed. These drastic defects were selectively rescued by additional deletion of NCAM, demonstrating that they originate from a gain of NCAM functions because of polySia deficiency. The data presented in this study reveal that the essential role of polySia resides in the control and coordination of NCAM interactions during mouse brain development. Moreover, this first demonstration in vivo that a highly specific glycan structure is more important than the glycoconjugate as a whole provides a novel view on the relevance of protein glycosylation for the complex process of building the vertebrate brain.

The cellular glycosylation machinery is a most impressive example of how cells enhance structural and functional complexity by use of only limited parts (<10%) of the genome. Glycans conjugated to lipids and proteins form the glycocalyx, the outer rim and prominent communication structure of animal cells (1, 2). Their paramount importance is emphasized by the growing group of congenital disorders of glycosylation, which manifest as severe multisystemic diseases including neuro-pathological symptoms (3).

Poly-α2,8-linked sialic acid (polySia)3 is a unique glycan added to the neural cell adhesion molecule, NCAM, and is known to exert an important influence on the development and function of the nervous system (4–6). The intriguing role assigned to polySia in promoting neurogenesis, migration, axon outgrowth, and synaptic plasticity has been explained predominantly by a negative regulation of cell-cell interactions due to the stereochemical properties of the large polyanion (shown schematically in Fig. 1A) (4, 7). Recent x-ray and neutron reflectivity data as well as direct force measurements confirm that an increased intermembrane repulsion in the presence of polySia overcomes homophilic NCAM binding and attenuates cadherin-dependent adhesion (8, 9). On the other hand, polySia is known to exert highly specific functions. For instance, NCAM trans-interactions with heparan sulfate proteoglycans involved in the formation and remodeling of hippocampal synapses depend on the presence of polySia (10, 11). Finally, competition experiments carried out with exogenously added polySia indicate that the carbohydrate polymer mediates autonomous, NCAM-independent functions. These concern the development of commissural axons in zebrafish (12), the strengthening of neuronal responses to brain-derived neurotrophic factor (BDNF) (13), and the functional modulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) subtype of glutamate receptors (14).

In marked contrast to the predicted central roles of NCAM and polySia in neurodevelopment and plasticity of the mature nervous system, mice lacking the NCAM gene (Ncam) (15, 16) and mice devoid of the NCAM-180 isoform (16) showed surprisingly mild phenotypes (for reviews, see Refs. 4 and 5). NCAM deficiency is accompanied by the loss of polySia (15, 16), and major defects observed in the Ncam knock-out models could be mimicked by enzymatic removal of polySia using endo-N-acetylenuraminidase (17). Combined, these findings led to the conclusion that polySia and not NCAM is the missing factor (18–22). Processes that can be disrupted by endo-N-acetylenuraminidase treatment are: (i) tangential migration of subventricular zone-derived neuroblasts along the rostral migratory stream to the olfactory bulb (18); (ii) lamination of the mossy fiber tract (22); (iii) long term potentiation and depression in the hippocampal CA1 region (19, 20); (iv) spatial learning (19); and (v) generation of the circadian locomotor rhythm in the suprachiasmatic nucleus (21).

The biosynthesis of polySia is the result of two polysialyltransferases, ST8Sia-II and ST8Sia-IV (23, 24). The two enzymes are highly conserved, but their time- and tissue-specific expression is independently regulated. ST8Sia-II is the dominating enzyme in the embryonic and early postnatal mouse, whereas ST8Sia-IV prevails in the adult (25, 26). The genetic ablation of St8sia-IV, therefore, generated mice in which the polySia synthesis was not significantly altered during development but almost completely absent in the adult (27). In contrast, depletion of St8sia-II generated animals with ongoing polySia synthesis in the adult brain (28). Both strains were born at Mendelian frequency, were fertile, and did not show gross anatomical abnormalities. Although distinct deficits have been identified in histological, electrophysiological, and
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behavioral analyses (27, 28), the data available today strongly suggest that each gene partially compensates for the absence of the other.

Information on the individual roles of NCAM and polySia during ontogeny is completely unavailable. Here we describe the phenotype of mice expressing normal NCAM levels in the absence of polySia, as obtained by simultaneous ablation of St8sia-II and St8sia-IV. The absence of polySia confers a lethal phenotype with specific defects of major brain fiber tracts. Phenotypic alterations that occurred in addition to those observed in NCAM-knock-out mice were completely reversed in triple knock-out animals lacking NCAM in addition to both polysialyltransferase genes. Collectively, our data demonstrate that polySia is essential in steering NCAM functions during development of the nervous system.

MATERIALS AND METHODS

Mice—All protocols for animal use were in compliance with German law and approved by the animal welfare committee of the Universität Hohenheim. St8sia-II and St8sia-IV single knock-out strains, which have been backcrossed with C57BL/6J mice for at least six generations, were intercrossed to obtain double heterozygous St8sia-II/+/St8sia-IV/+. To generate all different allelic combinations St8sia-II/+/St8sia-IV/− were backcrossed to St8sia-II/−/St8sia-IV/− or St8sia-II/−/St8sia-IV/+, respectively, and resulting St8sia-II/−/St8sia-IV/− mice were bred with St8sia-II/−/St8sia-IV/−. Wild-type mice were obtained from crosses between St8siaII/+/St8siaIV/± or between St8siaII/−/St8siaIV/++. Triple-knock-out mice deficient in NCAM and polysialyltransferase genes were obtained by intercrosses between St8sia-II/−/St8sia-IV/− and Ncam/−/− mice and subsequent backcrosses. All offspring were born in the expected Mendelian ratios. Genotyping was performed by PCR using the following primers: NCAM1 (5’-CGGAGAACCTCGGCTGAATCCATC-3’), NCAM2 (5’-CGGAGAACCTCGGCTGAATCCATC-3’), and NCAM3 (5’-TGGAGAACCGAGGCTCAACATGC-3’). Two-tailed t-test was applied. Chi-square tests were used to assess frequency distributions of the anterior commissure, the anterior and posterior limbs were followed on coronal serial sections, and diameters were measured perpendicular to their trajectory at the level of midline crossing (if applicable) to jointly assess both limbs, and directly rostral or caudal of the branching point for separate measurements of the anterior and posterior limbs, respectively. The rostrocaudal extent of the internal capsule was calculated as the product of the number of coronal sections on which the internal capsule was present multiplied by the thickness of the sections (50 μm). The cross-sectional areas of the corticospinal and mammillothalamic tracts were measured as illustrated in the respective figures (see “Results”), and for each animal the mean values of the left and right tracts were calculated.

DiI Tracing—DiI (Molecular Probes) was applied on fixed brains cut in coronal orientation from rostral up to the desired level. Crystals of DiI were placed unilaterally on the pars externa of the anterior olfactory nucleus to mark the anterior commissure or on the medullary pyramids to label the corticospinal tract of the hindbrain. After incubation in 4% paraformaldehyde for 2 weeks at 37 °C, 100-μm vibratome sections were cut in horizontal or coronal planes and examined with a fluorescence stereomicroscope (Leica MZ FLIII).

Statistics—Statistical analyses were performed using Graphpad Prism software. For comparisons of more than two groups, one-way ANOVA with Newman-Keuls multiple comparison test as a post-test (two-tailed) was applied. Chi-square tests were used to assess frequency distributions or to compare actual with expected frequencies.

RESULTS

Polysialyltransferase-deficient Mice Exhibit a Lethal Phenotype—Mice with single inactivation of the polysialyltransferase genes, St8sia-II (II−/−) (28) and St8sia-IV (IV−/−) (27), were cross-bred. Offspring from double-heterozygous animals (II−/−IV−/−; F6 backcross on C57BL/6J mice) were further intercrossed and yielded all expected genotypes including mice doubly deficient for the polysialyltransferases (II−/−IV−/−). Western blot analysis was used to display the expression of polySia (Fig. 1B, upper panel) and NCAM (Fig. 1B, lower panel) in brain homogenates of postnatal day 1 (P1) pups. Mice of all genotypes except II−/−IV−/− retained polySia-immunoreactivity. The complete absence of polySia in II−/−IV−/− animals excluded the existence of a third polysialyltransferase that could modify NCAM. Consistent with the high expression level of ST8Sia-II in perinatal animals (24–26), the absence of ST8Sia-II (Fig. 1B, II−/−IV−/− and II−/−IV−/−) lowers the polySia content more drastically than the absence of ST8Sia-IV (Fig. 1B, II−/−IV−/−+ and II−/−IV−/−). NCAM protein expression, in contrast, is not affected by the lack of polySia synthesis. The two major NCAM isoforms expressed at P1, NCAM-180 and −140 (29), are present at similar level in all genotypes (Fig. 1B, lower panel).

were embedded in 4% agar-agar (Roth) and cut into 50-μm serial sections using a vibrating microtome. Unstained free-floating vibratome sections were examined with a stereomicroscope (Zeiss SV 11) under modified dark field illumination. For standard histological examination, sections were air dried onto Superfrost Plus slides (Menzel Gläser), dehydrated and stained with cresyl violet/thionine (Nissl stain). Immunofluorescence staining of free-floating vibratome sections was performed with polyclonal rabbit anti-calbindin (Swant) and AlexaFluor 488-conjugated secondary antibodies (Molecular Probes) according to the manufacturers’ instructions. Micrographs were archived digitally using AxioVision version 3.1 software (Zeiss).

Morphometric Measurements—Area and length measurements were performed on digital images using NIH ImageJ. To address the dimensions of the anterior commissure, the anterior and posterior limbs were followed on coronal serial sections, and diameters were measured perpendicular to their trajectory at the level of midline crossing (if applicable) to jointly assess both limbs, and directly rostral or caudal of the branching point for separate measurements of the anterior and posterior limbs, respectively. The rostrocaudal extent of the internal capsule was calculated as the product of the number of coronal sections on which the internal capsule was present multiplied by the thickness of the sections (50 μm). The cross-sectional areas of the corticospinal and mammillothalamic tracts were measured as illustrated in the respective figures (see “Results”), and for each animal the mean values of the left and right tracts were calculated.

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stained tissue sections (heart, lung, stomach, pancreas, gut, liver, spleen, and kidney) did not show obvious abnormalities. In contrast to the dramatically affected development of polysialyltransferase-deficient mice, NCAM offsprings derived from N\textsuperscript{+}/N\textsuperscript{−}/N\textsuperscript{−} crossed at 9-12 weeks of age (n = 27, 54, 24 for N\textsuperscript{+}/N\textsuperscript{−}/N\textsuperscript{−} and N\textsuperscript{+}/N\textsuperscript{−}/N\textsuperscript{−}; p > 0.8, chi-square test).

Gain of NCAM Functions in polySia-negative Mice—Major defects in brain morphology as described for Ncam knock-out mice (N\textsuperscript{−}/N\textsuperscript{−}) (15, 16, 18, 22, 30, 31) were recapitulated in the polysialyltransferase-negative animals (I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−}), documenting the fact that they are caused by the absence of polySia and not by the lack of NCAM. Shared defects concern: (i) small olfactory bulbs (Fig. 2, see also Fig. 4B); (ii) a massive accumulation of cells in the proximal part of the rostral migratory stream (Fig. 2D); and (iii) the defasciculation and aberrant lamination of the mossy fiber tract (Fig. 3). Beyond that, I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} mice have unique defects including postnatal growth retardation and precocious death, and as will be demonstrated below (Figs. 4–6), they exhibit massive hydrocephalus and abnormalities of major brain fiber tracts.

This splitting of defects into two categories, (i) mild defects shared with N\textsuperscript{−}/N\textsuperscript{−} and (ii) severe defects that manifest exclusively in I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} mice, prompted us to hypothesize that the defects of the second category are due to a gain of NCAM function. To investigate this possibility, triple knock-out mice were generated by introducing N\textsuperscript{−} transgenes into I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} mice. The resulting N\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} animals were viable, and postnatal development was indistinguishable from wild-type and N\textsuperscript{−}/N\textsuperscript{−} animals (Fig. 1G). Like N\textsuperscript{−} mice, the triple knock-outs displayed small olfactory bulbs (Fig. 2F) and defasciculated mossy fiber tracts (Fig. 3, D and H).

A conspicuous malformation of mature I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} mice (3 weeks or older) was a domed cranium due to hydrocephalus formation. Compared with the normal brain morphology of double heterozygotes (I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−}; Fig. 4, A and C), sagittal and coronal brain sections of hydrocephalic I\textsuperscript{−}/I\textsuperscript{−}/V\textsuperscript{−}/V\textsuperscript{−} animals (Fig. 4, B and D) demonstrated a massive dilatation of the lateral and third ventricles in conjunction with thinning of the cerebral cortex, hypoplasia of the corpus callosum, and
Gain of NCAM Function Causes Lethality

This defect occurred in 100% of the II−/IV−/− mice displayed the dramatic phenotype as shown in Fig. 4, and II−/IV−/− mice (%), respectively). Ablation of NCAM in the triple knock-out mice fully restored the anterior commissure (Fig. 5, I), the pyramidal decussation (2, pyx), and the dorsal funiculus (3; contrast is selectively increased in the cortex (cc) and corpus callosum (cc), and the region of the anterior commissure. Note normal size of the corpus callosum (cc, arrowhead) and slight expansion of the lateral ventricles (lv, asterisk) of the II−/IV−/− specimen. In contrast, the fourth ventricle (4v), and the normal gross anatomy of the cerebellum (cb). Scale bars, 1 mm.

Anterograde tracing of the anterior limb of the anterior commissure with Dil highlights the defect (Fig. 5, A–D). In II−/IV−/− neonates this fiber tract is stunted and deviates early from its path (Fig. 5, B, D), whereas the tract is regularly shaped in double heterozygotes (Fig. 5, A, C, and E). Ablation of NCAM in the triple knock-out mice fully restored both limbs of the anterior commissure (Fig. 5, H and I, and TABLE ONE), demonstrating that loss of polySia resulted in a gain of NCAM function, which is responsible for the defects.

Moreover, the cross-sectional area of the corticospinal tract measured at the level of the medullary pyramids (1), the pyramidal decussation (2, pyx), and the dorsal funiculus (3; contrast is selectively increased in the insert) illustrates hypoplasia but complete crossing of the midline (dotted line) in II−/IV−/− mice. M and N, unstained coronal sections through the medulla showing the corticospinal tract (red-framed) of mature control (M) and II−/IV−/− mice (N) at the level of the seventh cranial nerve (VII, black arrow). O, statistical evaluation of the cross-sectional corticospinal tract area. Bars present means ± S.D. of n = 6 (control and II−/IV−/−) or n = 3 (N−/−; N−/− II−/IV−/−) mice. p < 0.0001, one-way ANOVA; a, b, and c, significant differences between groups, p < 0.001). Scale bars, 500 μm.

Displacement and deformation of the hippocampal formation and the fimbria. In contrast, the fourth ventricle (exemplarily shown in Fig. 4B) is not significantly enlarged and the gross anatomy of the cerebellum appears normal. Of 16 II−/IV−/− mice (derived from 11 different litters) that survived for more than 3 weeks, seven animals (derived from six different litters) displayed the dramatic phenotype as shown in Fig. 4, B and D, and six animals exhibited a moderate ventriculomegaly (see Fig. 2D for an example). Only three double-null mutants but all double heterozygotes (II−/IV−/Ⅰ−/Ⅰ−; eight in total) had normal-sized ventricles (p < 0.001, chi-square test). Newborn II−/IV−/− mice mostly displayed a mild dilatation of the lateral ventricles (see Fig. 5G for an example), whereas severe ventricular dilatation was rare (3 of 14 neonates examined). Among mature II−/IV−/− animals the drastic reduction in body weight was observed irrespective of the different ventricular conditions (mean weights ± S.D. of animals with marked (n = 7), moderate (n = 6), and no ventricular dilatation (n = 3) were 7.2 ± 2.8, 7.8 ± 3.3, and 6.6 ± 2.2 g, respectively). Together, these observations suggest that hydrocephalus in polySia-negative mice develops progressively and is not linked to growth retardation and lethality. In accordance with reported data (15, 32), none of the six N−/− or N−/− II−/IV−/− animals additionally analyzed in the present study developed signs of hydrocephalus, but all displayed a slight enlargement of the lateral ventricles (Figs. 4E and 5F). Thus, compared with NCAM-deficient mice, the II−/IV−/− animals have a significantly increased incidence of hydrocephalus (p < 0.05, chi-square test).

A feature observed exclusively in the polySia-negative II−/IV−/− mice was the absence of the anterior commissure (Fig. 5, A–I), which connects the olfactory structures and lateral parts of the temporal lobe. This defect occurred in 100% of the II−/IV−/− animals, even in the rare cases in which ventricles were not dilated, as in the example shown in Fig. 5I (p < 0.001, chi-square test of II−/IV−/− (n = 18) versus control (n = 12), N−/− (n = 6), or N−/− II−/IV−/− (n = 5), respectively).

FIGURE 3. Aberrant lamination of hippocampal mossy fibers in II−/IV−/− and Ncam-deficient mice. A–D, calbindin immunofluorescence (green) and nuclear counterstaining with 4′,6-diamidino-2-phenylindole (blue) of the hippocampal formation in 4–6-week-old wild-type (wt, A, N−/− (B), II−/IV−/− (C), and II−/IV−/−/N−/− (D), CA3, CA3 subfield of the hippocampus; dg, dentate gyrus. E–H, higher magnification of the calbindin-stained mossy fiber bundles corresponding to the boxed areas in A–D, showing the normal fasciculation and separation of the suprapyramidal (sp) and infrapyramidal (ip) mossy fiber bundles in the wild type and their altered appearance in the other genotypes. Scale bars, 100 μm.

FIGURE 4. Formation of hydrocephalus in II−/IV−/− mice. A–E, montage of cresyl violet-stained sagittal sections (A and B) and unstained coronal sections (C–E) under modified dark field illumination through brains from mature II−/IV−/− (A, C), II−/IV−/− (B, D), and N−/− II−/IV−/− animals (E). B and D represent cases with severe dilatation of the lateral (lv) and third ventricles (3v), thinning of the cortex (cc) and corpus callosum (cc), and distortion of the hippocampus (hp) and fimbria (f). In B, note the deformation and reduced size of the olfactory bulb (ob), the normal size of the fourth ventricle (4v), and the normal gross anatomy of the cerebellum (cb). Scale bars, 1 mm.

FIGURE 5. Malformations of anterior commissure and corticospinal tract in II−/IV−/− mice. A and B, Dil tracing of the anterior branch of the anterior commissure (ac) in P2 control and II−/IV−/−, respectively. Assembly of horizontal sections with selective contrast enhancement reveals normal midline (dotted line) crossing in controls (A) and aberrant guidance and fasciculation in II−/IV−/− littermates (B, arrows). C and D, cresyl violet-stained sections corresponding to A and B. E–H, cresyl violet-stained coronal sections of P1 brains at the level of the anterior commissure (ac) in II−/IV−/− (E), N−/− (F), II−/IV−/− (G), and N−/− II−/IV−/− (H) mice. The arrow in G indicates a rudiment of the posterior branch of the anterior commissure. Note normal size of the corpus callosum (cc, arrowhead) and slight expansion of the lateral ventricles (lv, asterisk) of the II−/IV−/− specimen. In contrast, the fourth ventricle (4v), and the normal gross anatomy of the cerebellum (cb). Scale bars, 100 μm.
reversed by additional deletion of NCAM (N+/− II+/− IV+/−; Fig. 6, B (triple) and G).

In contrast, fiber tracts such as the corpus callosum, fimbria, and hippocampal commissure, found to be seriously damaged in hydrocephalic II−/− IV−/− adults (Fig. 4), were perfectly developed in II−/− IV−/− neonates and in mature II−/− IV−/− mice not showing severe hydrocephalus (examples are shown for corpus callosum in Fig. 5, G (for neonatal) and I (for mature brain)). In the few mice with almost regularly shaped ventricles, the width of the cerebral cortex from the pial surface to the white matter was indistinguishable from wild-type, double-heterozygous, or N+/− II+/− IV+/− animals (see Figs. 5, I and J, and 6B). Therefore, defects of these brain structures, as well as the distortion of the hippocampus (Fig. 4, B and D) seem to be secondary to ventricular dilatation.

Important in this context is the marked expansion of the hippocampus that was observed in all II−/− IV−/− mice without hydrocephalus (see Fig. 6B). Although further studies will be required to evaluate the basis of this effect, we hypothesize that spatial alterations due to the reduction of the internal capsule enable a passive extension of adjacent tissue to fill the available space. Conversely, an increase of hydrostatic pressure of the cerebrospinal fluid, the most likely reason for the development of hydrocephalus, would counteract this expansion. Fiber tracts, which were regularly shaped and properly positioned in all II−/− IV−/− mice, are the ipsilaterally projecting lateral olfactory tract (Fig. 5J) and fasciculus retroflexus (Fig. 6, E and F), the optic nerve and chiasm (data not shown), and the optic tract (Fig. 6B) and posterior commissure (Fig. 6F).

**DISCUSSION**

The animal models described in this article allow the dissection between NCAM and polySia functions during mouse neurodevelopment. By simultaneous deletion of St8sia-II and St8sia-IV, a complete elimination of polySia was achieved, but in contrast to findings in Ncam knock-out mice, expression of NCAM was retained. As summarized in TABLE TWO, the severe phenotype of II−/− IV−/− mice combines two types of defects: (i) defects that establish independent of the presence of NCAM and are shared by polysialyltransferase- (II−/− IV−/−) and NCAM-depleted mice (N+/− or N+/− II+/− IV−/−); and (ii) defects caused by a gain of NCAM functions. These alterations manifest exclusively in II−/− IV−/− mice and are fully reversed by the additional deletion of the NCAM gene in N+/− II+/− IV−/− mice.

The gain of NCAM functions in II−/− IV−/− mice causes anomalies of specific brain fiber tracts, progressive hydrocephalus, and impaired postnatal growth and viability. The complete rescue of these fatal developmental defects by deletion of NCAM demonstrates unequivocally that the major function of polySia is to mask NCAM and thus guarantee that NCAM contacts take place in a highly organized, time- and sitespecific manner. Invertebrates, which do not synthesize polySia-like structures, control the cell surface presentation of NCAM orthologues (FasII and apCAM) by internalization and proteolytic degradation (34, 35). It is likely that concealing NCAM by polysialylation provides an additional regulatory level required in the complex vertebrate organism.

As in the present study, deleterious NCAM effects have been described in two other systems with ectopic NCAM presentation. Dominant embryonic lethality was observed in mice mis-expressing NCAM.

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**TABLE ONE**

**Size comparison of the anterior commissure of polysialyltransferase and Ncam-deficient mice.**

<table>
<thead>
<tr>
<th>Anterior commissure</th>
<th>Thicknessa</th>
<th>N+/−</th>
<th>II−/− IV−/−</th>
<th>N+/− II+/− IV−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midline</td>
<td>325 ± 49</td>
<td>325 ± 36</td>
<td>0 ± 0</td>
<td>312 ± 25</td>
</tr>
<tr>
<td>Anterior limbb</td>
<td>227 ± 22</td>
<td>207 ± 21</td>
<td>12 ± 28d</td>
<td>234 ± 26</td>
</tr>
<tr>
<td>Posterior limbc</td>
<td>164 ± 11</td>
<td>149 ± 24</td>
<td>35 ± 30d</td>
<td>151 ± 10</td>
</tr>
</tbody>
</table>

a Defined under “Materials and Methods.” Only animals with complete series of coronal sections were evaluated.

b Mean values of left and right limbs per mouse.

c In four mice rudiments of the anterior limb were detected in one hemisphere.

d In four mice rudiments of the posterior limb were detected on both sides or in one hemisphere, respectively (n = 2, each).

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**FIGURE 6.** Size reduction of the internal capsule and mammillothalamic tract in II−/− IV−/− mice. A, montage of cresyl violet-stained horizontal sections showing the rostral caudal extent of the internal capsule (ic, arrows) and the fornix (asterisks) in P1 II−/− IV−/− (left) and II−/− IV−/− (right) littermates. B, unstained coronal sections of the posterior (dorsal) thalamus showing aberrant fiber projections (arrow) and absence of the fornix in II−/− IV−/− mice (for neonatal) and I (for mature brain)). In the few mice with almost regularly shaped ventricles, the width of the cerebral cortex from the pial surface to the white matter was indistinguishable from wild-type, double-heterozygous, or N+/− II+/− IV+/− animals (see Figs. 5, I and J, and 6B). Therefore, defects of these brain structures, as well as the distortion of the hippocampus (Fig. 4, B and D) seem to be secondary to ventricular dilatation.

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**TABLE TWO**

**Anterior commissure thickness comparison in control and NCAM deficient mice.**

<table>
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<td>Anterior limbb</td>
<td>227 ± 22</td>
<td>207 ± 21</td>
<td>12 ± 28d</td>
<td>234 ± 26</td>
</tr>
<tr>
<td>Posterior limbc</td>
<td>164 ± 11</td>
<td>149 ± 24</td>
<td>35 ± 30d</td>
<td>151 ± 10</td>
</tr>
</tbody>
</table>

a Defined under “Materials and Methods.” Only animals with complete series of coronal sections were evaluated.

b Mean values of left and right limbs per mouse.

c In four mice rudiments of the anterior limb were detected in one hemisphere.

d In four mice rudiments of the posterior limb were detected on both sides or in one hemisphere, respectively (n = 2, each).
Gain of NCAM Function Causes Lethality

TABLE TWO

<table>
<thead>
<tr>
<th>NCAM-independent defects (polySia-autonomous; (H^{-}/IV^{-/-} \rightarrow N^{-}/N^{-}/H^{-}/IV^{-/-} ))</th>
<th>NCAM-dependent defects (gain of NCAM function; (H^{-}/IV^{-/-} \rightarrow N^{-}/N^{-}/H^{-}/IV^{-/-} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small olfactory bulbs and rostral migratory stream expansion</td>
<td>Complete agenesis of anterior commissure</td>
</tr>
<tr>
<td>Delamination of mossy fibers</td>
<td>Severe hypoplasia of corticospinal tract</td>
</tr>
<tr>
<td></td>
<td>Size reduction of internal capsule</td>
</tr>
<tr>
<td></td>
<td>Hypoplasia of mammillothalamic tract</td>
</tr>
<tr>
<td></td>
<td>High incidence of hydrocephalus</td>
</tr>
<tr>
<td></td>
<td>Growth retardation and precocious death</td>
</tr>
</tbody>
</table>

as a soluble protein (36), and most recently, alterations reminiscent to schizophrenia were described in a conditional mouse model over-expressing soluble NCAM in late stages of neural development and in the adult (37). Including the current study, the animal models engineered to allow ectopic NCAM interactions prove impressively the importance of a strictly controlled NCAM homeostasis in the living system.

Although polySia-NCAM is abundantly expressed throughout brain development (38), gain of NCAM function because of polySia-deficiency affects a well defined and highly diverse set of commissural and non-commissural fiber tracts. This diversity argues against a common cellular mechanism underlying the defects. To deduce which step of axonal development or maintenance is actually impaired by the untimely NCAM interactions, future studies must concentrate on investigating the dysgenesis of each individual fiber tract. These studies must also address the questions of whether fatal NCAM contacts in \(H^{-}/IV^{-/-} \) mice are due to premature interactions in cis (i.e. in the plane of the membrane) or in trans (i.e. between apposing cells or cells and extracellular matrix) and whether homophilic or heterophilic NCAM interactions are affected (for review, see Ref. 39).

Remarkably, depletion of L1, like depletion of polysialyltransferases, induces a complex phenotype including hydrocephalus and defective brain fiber tracts (40–43). Recent studies separated the phenotype of L1-deficient mice into defects caused by the loss of homo- and heterophilic L1-interactions (44). Although the hydrocephalus develops because of the lack of homophilic L1 interactions, malformations such as hypoplasia of the corpus callosum and corticospinal tract can be clearly attributed to failures of heterophilic L1 binding (44). Because L1 is a lateral binding partner of NCAM (45), we are currently investigating the possibility that NCAM/L1 interactions are enhanced in the absence of polySia leading to sequestration and functional inactivation of L1. In contrast, signaling via the NCAM-GFRα1 complex should not be influenced by polySia depletion, because both GFRα1 and GDNF (glial cell line-derived neurotrophic factor) bind to polysialylated and non-polysialylated forms of NCAM (46).

Defects of the olfactory bulbs, expansion of the rostral migratory stream, and delamination of hippocampal mossy fibers observed in \(H^{-}/IV^{-/-} \) mice are shared with all other polySia-negative models independently of the presence of NCAM (15, 16, 18, 22, 30, 31). Consequently, the function of polySia in these areas is not the masking of NCAM but may be the modulation of other cell surface interactions by stereocellular means. As discussed extensively in earlier studies, polySia prevents inappropriate contacts of cells with their environment, permitting tangential migration of precursors in the rostral migratory stream to the olfactory bulbs (18, 31) and supporting correct fasciculation of mossy fibers (22, 30).

In conclusion, the lethal phenotype of polysialyltransferase-deficient mice and its rescue by additional ablation of NCAM reveals that the unique and highly specific posttranslational modification with polysialic acid plays a vital role in steering NCAM interactions in vivo.

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REFERENCES

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