Top Curr Chem (2015) 366: 55–96
DOI: 10.1007/128_2013_446

Published online: 30 May 2013

Polysialic Acid in Brain Development and Synaptic Plasticity

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Abstract Polymers of sialic acid can be produced by pro- and eukaryotic cells. In vertebrates polysialic acid consists of α2,8-linked *N*-acetylneuraminic acid and is most prominent during nervous system development. Polysialic acid is produced by two complementary sialyltransferases, ST8SiaII and ST8SiaIV. The major, but not the only, carrier of polysialic acid is the neural cell adhesion molecule (NCAM). In this review we highlight how polySia dictates the interactions of various cell types during development and plasticity of the vertebrate central nervous system on different molecular levels. Recent progress in generating mouse models with differential ablation of the polysialyltransferases or NCAM revealed the dramatic impact of polysialic acid-negative NCAM on brain development and elaborate electrophysiological studies allowed for new insights into the role of polysialic acid in regulating synaptic plasticity and learning. The implications of dysregulated polysialylation for brain disease and neuropsychiatric disorders are discussed.

Keywords Axon development · Brain disease · Cell surface glycosylation · Neural cell adhesion molecule (NCAM) · Synaptogenesis

This work has been supported by grants from the Deutsche Forschungsgemeinschaft, Neuroscience Program of the Compagnia di San Paolo, and BMBF grant 01EW1106/ NeuConnect in the frame of ERA-NET NEURON.

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Contents

1 Introduction

Polysialic acid (polySia or PSA[1]) occurs as a capsular polysaccharide of neuroinvasive bacterial pathogens (see Jakobsson et al. [1]) and as a unique glycan structure of a small set of eukaryotic cell surface proteins [2, 3]. In mammals, polySia consists of linear chains of α2,8-glycosidically linked *N*-acetylneuraminic acid residues (Fig. 1a) with a variable degree of polymerization ranging from 8 up to approximately 90 sugar units and comprises approximately 10% of the total protein-bound neuraminic acid in the developing brain [4, 5]. Early physicochemical investigations predict that at least parts of the polySia chain exhibit an extended helical conformation with a basal unit of approximately nine sialic acids [6–8]. Due to the negative charge of the nine-carbon monosaccharide, polySia forms a hydration shell, which increases the hydrodynamic radius of the polySia carrier and enlarges the space between adjacent cells (Fig. 1b) [9–12].

The most prominent protein modified by polySia is the neural cell adhesion molecule (NCAM), the prototypic member of the immunoglobulin family of adhesion molecules. Discovered as a synaptic glycoprotein more than 35 years

[1] The most commonly used abbreviation for polysialic acid in neuroscience is PSA but in tumor biology, PSA stands for prostate specific antigen. To avoid confusion we prefer to use polySia to abbreviate polysialic acid.

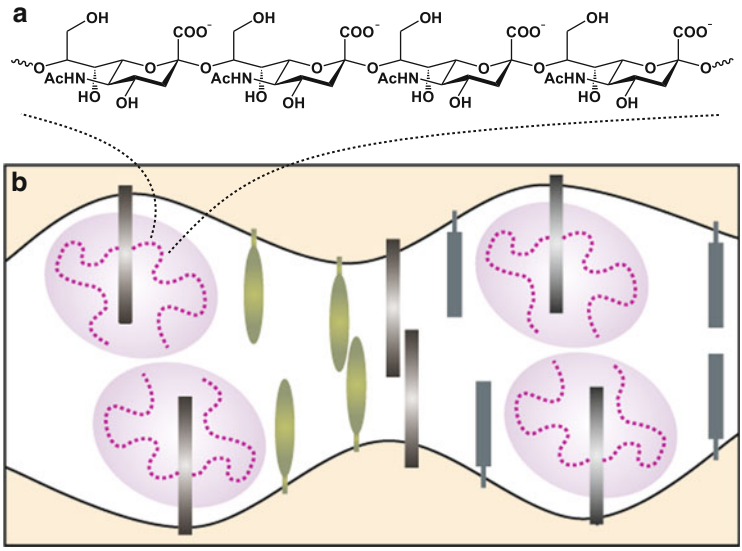

Fig. 1 Eukaryotic polySia structure. (**a**) α2,8-glycosidically linked *N*-acetylneuraminic acid residues. (**b**) PolySia increases the hydrodynamic radius, shields of interactions of its carrier protein and increases intermembrane space affecting interactions of various other cell surface proteins

ago [13], some of the first analyses already indicated striking differences in sialic acid content and biochemical properties of NCAM isolated from either embryonal or adult nervous tissue [14, 15]. At about the same time polySia was identified as a major source of sialic acid in the glycoprotein fraction of embryonic rat brain and hence could be assigned to NCAM [4, 16]. Since then, numerous studies in vitro and in cell-based approaches have shown that polysialylation decreases NCAM-mediated homophilic adhesion [11, 12, 17–20] as well as NCAM signaling functions induced by homophilic or heterophilic NCAM interactions [21–23].

However, NCAM is not the only carrier of polySia. A limited number of other polysialylated proteins have been described including the scavenger receptor CD36 in human milk [24], neuropilin-2 on human dendritic cells [25–27], and the polysialic acid synthesizing enzymes themselves, which can polysialylate their own *N*-glycans in a process termed autopolysialylation [28–31]. In the nervous system, occurrence of polySia on sodium channel alpha subunits of adult rat brain synaptosomal fractions has been reported [32] and most recently a subfraction of the synaptic cell adhesion molecule SynCAM 1 has been identified as a target for polysialylation in the early postnatal mouse brain [33]. The latter study also established that polysialylation attenuates homophilic adhesion of SynCAM 1 in a bead aggregation assay, implying that polySia serves as a potent regulator of Syn-CAM 1 interactions in vivo, as is known for NCAM (Fig. 1b).

2 Polysialic Acid Biosynthesis

In 1995 two different polysialyltransferase genes were independently characterized in four groups [34–37]. In these studies, each of the two enzymes was shown to be capable of producing polySia in vitro. Initially named STX [38] and PST-1 [34], the enzymes were designated ST8SiaII and ST8SiaIV according to a systematic nomenclature of sialyltransferases introduced in 1996 by Tsuji, Datta, and Paulson [39]. ST8SiaII and ST8SiaIV show a high sequence homology and are typical members of the mammalian sialyltransferase family with a type II *trans*-membrane topology, a short *N*-terminal cytoplasmic tail, a stem region, and a large catalytic domain facing the Golgi lumen (Fig. 2a) [40, 41]. The catalytic domain includes the sialylmotifs L, S, and VS, three conserved sequences that are found in all mammalian sialyltransferases and are involved in substrate binding [41–43]. The polysialyltransferases contain two additional structurally unique polybasic motifs, termed polysialyltransferase domain [44] and polybasic region [45, 46], respectively (PD and PBR; Fig. 2a). While the polysialyltransferase domain is part of the catalytic domain, the polybasic region is located in the stem region and seems to be involved in acceptor substrate recognition. Replacement of basic amino acids identified arginine residues within both motifs that are essential for polysialylation [45, 46]. In addition, interference with *N*-glycosylation of the polysialyltransferases and in particular the prevention of autopolysialylation leads to the formation of inactive enzymes [28, 47, 48].

Using cytidine 5′-monophosphate (CMP) – activated sialic acid as donor (see [49]), ST8SiaII and ST8SiaIV catalyze the transfer of multiple α2,8-linked sialic acid residues to, in the case of NCAM, a highly variable, di-, tri-, or tetraantennary *N*-linked core glycan [50–54] (Fig. 2b). As determined in vitro, terminally α2,3- or α2,6-sialylated galactose residues bound in α1,4-linkage to *N*-acetyl glucosamine can be used as acceptor sites for polysialylation [55, 56]. Although NCAM carries six *N*-glycosylation sites, the addition of polySia in vivo is restricted to sites 5 and 6, located in the fifth Ig-like domain (Ig5; Fig 2b) [52, 53, 57]. Mutational analyses identified an acidic patch in the first fibronectin type III repeat (FN1) that is critical for polysialylation [58] and hence might interact with the polybasic region of the polysialyltransferases [45]. Further deletion and replacement studies revealed the role of an alpha helix in Fn1 and the region linking FN1 and Ig5 in positioning of the Ig5 *N*-glycans for polysialylation with some but limited flexibility [58–60]. The latter studies suggest that not only protein–protein interaction but also proper spacing between the membrane and a particular *N*-glycosylation site are key determinants for site-specific polysialylation of only selected protein acceptors, such as NCAM and SynCAM 1 (Fig. 2b) [33, 58].

In the absence of specific enzymes that could degrade polySia at the cell surface, polySia expression in vertebrates seems to be regulated mainly by the balance between the synthesis of polysialylated structures and their internalization from the cell surface, which in the case of NCAM leads to either lysosomal degradation or recycling [61–63]. During mouse brain development, the expression of the two

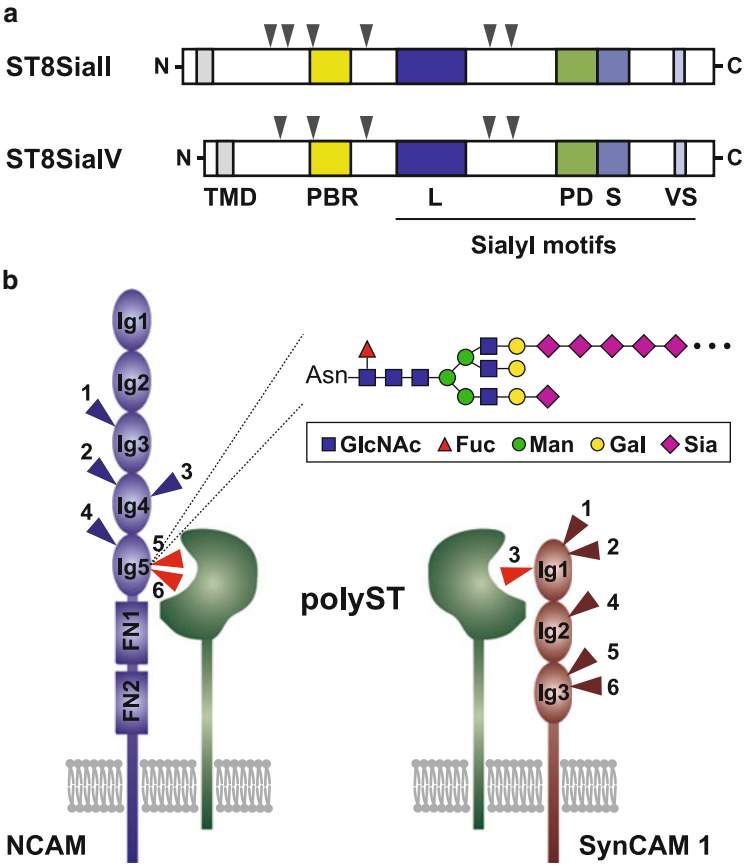

Fig. 2 Biosynthesis of polySia on NCAM and SynCAM 1. (**a**) Domain structure of the polysialyltransferases ST8SiaII and ST8SiaIV with the polybasic region (PBR), the polysialyltransferase domain (PD), and the sialylmotifs large (L), small (S), and very small (VS) of the catalytic domain. The relative positions of the *N*-glycans are indicated by *arrowheads*. *TMD* transmembrane domain. (**b**) Structure of the type I transmembrane proteins NCAM and SynCAM 1, example for a complex, here triantennary, core glycan with terminal sialic acid(s), and model of NCAM and SynCAM 1 in complex with a Golgi-resident polysialyltransferase (polyST) for site-specific polysialylation. *Ig* Ig domains, *Fn* fibronectin type III repeats, *triangles N*-glycosylation sites. Parts of panel **b** are modified from [33]

polysialyltransferases and the level of NCAM peaks during the third trimester [64] and in the perinatal phase, the entire pool of NCAM is polysialylated [65, 66]. Analyses of polysialyltransferase-deficient mice in this time window reveal that a loss of ST8SiaIV is completely compensated by the remaining activity of ST8SiaII. Conversely, in the absence of ST8SiaII more than 50% of the available NCAM are still fully polysialylated [65, 66]. Thus, biosynthesis of polySia under these conditions is limited by the availability of NCAM as the major acceptor. As detailed below (Sect. 4.2), an untimely appearance of polySia-negative NCAM causes severe defects

in brain development. The overly high enzyme levels in the embryonic and early postnatal period, therefore, might be crucial to guarantee that all NCAM is fully polysialylated during critical developmental periods. Possibly this overcapacity explains that a small fraction of SynCAM 1 is used as an alternative acceptor for polysialylation just during this phase [33].

3 Patterns of Polysialic Acid Expression

3.1 Developmental PolySia Patterns

PolySia is most prevalent during nervous system development. In mice the expression of both polysialyltransferases starts with neural tube closure at embryonic day (E) 8.5 and polySia is detected from E9 onwards [67, 68]. In general, polySia is widespread during embryonic and early postnatal brain development and most if not all neurons seem to be positive for polySia at some stage of their differentiation [69]. Staining has been detected on radial glia of the developing cortex and mesencephalon [64, 70] as well as on Bergmann and Müller glia, i.e., the radial glia of cerebellum and retina [71–73]. The most prominent polySia expression is found on interneuron precursors that migrate tangentially from the subventricular zone of the lateral ventricle to the olfactory bulb [74, 75]. This neurogenic niche is derived from the embryonic lateral ganglionic eminence and persists into adulthood. In the course of brain development polySia is also found on migrating precursors of cortical interneurons [76, 77] and cerebellar granule cells [71]. As shown for, e.g., Cajal–Retzius cells, the first neuron population growing out axons in the cortical primordium, or for fiber tracts like the optic nerve, the corticospinal tract, and thalamocortical fibers, developing axons of the rodent brain display strong polySia-immunoreactivity as well [72, 76, 78–80]. Finally, polySia is present during synapse formation of hippocampal neurons [81, 82] and plays a decisive role in the functional maturation of GABAergic inhibition which determines the time window of the so-called critical period of plasticity in the visual cortex [83] (see Sect. 5.2). Together, these findings point towards multiple functions of poly-Sia at all stages of neurogenesis. Correspondingly, the polysialyltransferases have broad and overlapping expression patterns in these developmental stages [68, 84].

As studied on the whole brain level and during development of the mesencephalic dopaminergic system in mice, mRNA expression of both polysialyltransferases increases dramatically after E10.5 and reaches plateau levels between E13.5 and E14.5, which are maintained until birth [64]. The time-course of ST8SiaII and ST8SiaIV upregulation is almost identical and precisely parallels a steep increase in the NCAM transcript level. In the course of postnatal brain development both polysialyltransferases are downregulated. In contrast to the moderate reduction of ST8SiaIV, a sharp drop of ST8SiaII mRNA occurs in a rather narrow time window between postnatal day (P) 5 and P11 followed by declining polySia [66]. At these

lower transcript levels a close correlation of polysialyltransferase expression and polySia formation becomes evident. Because the amount of NCAM remains almost constant, reduced polysialylation causes a gradual appearance of polySia-negative NCAM [66]. Highly consistent with these studies on the whole brain level, a decline of polySia has been observed in the prefrontal and visual cortex during the second and third week of postnatal development, which in the visual cortex is preceded by declining mRNA levels of ST8SiaIV and a particularly pronounced drop of ST8SiaII between P9 and P12 [83, 85, 86]. Moreover, postnatal downregulation of polySia but constant levels of NCAM were detected in the human prefrontal cortex [87].

Among the three major splice variants of NCAM only the two transmembrane isoforms NCAM-140 and NCAM-180 occur in their polysialylated form in brain lysates of embryonic and postnatal mouse brain [66, 67, 76]. In contrast, the glycolipid anchored isoform NCAM-120 is barely detectable at birth but massively upregulated during postnatal development without being polysialylated [66]. These findings contrast with in vitro data showing that all three NCAM isoforms can serve as polySia acceptors due to their identical extracellular domains [88]. Consistent with NCAM-120 being the characteristic isoform of mature oligodendrocytes and myelin sheaths, the increasing levels of NCAM-120 parallel the course of myelination during postnatal brain development [89, 90]. The lack of polysialylated NCAM-120 in postnatal mouse brain, therefore, may be explained by differential expression patterns of polysialyltransferases and NCAM-120. In contrast, oligodendrocyte precursors are positive for polySia in development and also during lesion-induced recruitment in the adult brain [91–96]. However, for both neuronal and oligodendrocyte precursors the NCAM isoform patterns remain to be determined.

3.2 PolySia Patterns of the Mature Brain

Under healthy conditions, polySia vanishes almost completely within the first 3 weeks of postnatal development, coinciding with the completion of major morpho-genetic events. There are, however, various hotspots of polySia expression in the mature brain. Most prominent, migrating neuroblasts arising from the neurogenic niches of the anterior subventricular zone [74, 75, 97, 98] and early postmitotic granular cell precursors in the subgranular layer of the hippocampal dentate gyrus [99–104] are characterized by their high polySia content and have been observed in all mammals including man [105–107]. Other major sources of polySia in the adult brain comprise widely spread subsets of interneurons and a population of immature neurons in layer II of the paleocortex. PolySia-positive interneurons were observed in different cortical areas, including prefrontal cortex [108, 109], piriform cortex [110], and hippocampus [111], as well as in the amygdala [112, 113]. Although polySia is best known by its intense expression in immature precursor stages, the polySia-positive interneurons of the cortex are mature neurons as evidenced by the presence of NeuN as an indicator of differentiated neurons, together with interneuron markers, mainly GAD67 and either calbindin, somatostatin, or parvalbumin

depending on the cortical area under consideration [114]. Compared to polySia-negative interneurons, these cells receive less synaptic input and have reduced dendritic arborization and spine numbers, suggesting that polySia is a negative regulator of interneuron connectivity and possibly allows for plasticity of inhibitory cortical networks [114]. As discussed in detail elsewhere [69, 115], the identity of the population of immature neurons in the paleocortex is enigmatic. Besides being polySia-positive, the immature cells display further features of neuronal precursors, like expression of doublecortin and the lack of NeuN [110, 116]. Despite a conflicting report [117], studies in rodents and cats provide substantial evidence that these cells are generated prenatally and maintain their immature phenotype into adulthood [118, 119]. Together with a comparative analysis of various mammalian and non-mammalian species [120], the data indicate that some immature polySia- and doublecortin-positive cells are also present in layers II and III of the mammalian neocortex. Most recently, the first evidence has been obtained that at least some of these immature neurons have the potential for maturation. After massive interference with olfactory processing by bulbectomy, the numbers of polySia- and doublecortin-positive cells in the piriform cortex layer II of adult rats were reduced in favor of increased numbers of differentiated, NeuN-positive neurons [121].

In addition to these examples of polySia immunoreactivity comprising the surface of neuronal cell somata and processes, some differentiated neurons of the mature brain are characterized by a polySia-negative soma while displaying polySia on their neurites. Most notably, most, if not all, hippocampal mossy fibers show intense polySia staining, although their somata in the granule cell layer are polySia-negative [77]. A similar situation was observed for pyramidal cells of the hippocampal CA1 region. Although the cell layer itself is polySia-negative [122], polySia immunoreactivity is detected on axons and dendrites of the CA1 pyramidal cells [123, 124]. Furthermore, as reviewed in great detail elsewhere [69, 125], wide areas of the adult brain retain a diffuse pattern of polySia staining. In thalamic and striatal regions this staining cannot yet be assigned to defined cell populations. In contrast, the more prominent diffuse polySia immunoreactivity of the adult hypothalamo-neurohypophysial system has been studied comprehensively [126–131]. Pronounced changes of polySia patterns occur during the glial and synaptic remodeling that accompany the physiological regulation of neuro-hormone release. While some of this polySia could be assigned to neurons of hypothalamic magnocellular nuclei, astrocytes and in particular their fine perineuronal processes are a major source of polySia in the hypothalamus. Strikingly, enzymatic removal of polySia by endosialidase injection prevents the rearrangement of synapses and astrocytic processes, indicating that polySia is a prerequisite for these changes [128, 130, 131]. Besides these hypothalamic astrocytes, polySia is found on other astrocytic cells of the adult brain, like the pituicytes of the neurohypophysis [132] and radial glia-like tanycytes in the ependymal layer of the third ventricular wall sending processes into the mediobasal hypothalamus [133]. Furthermore, polySia is also formed by reactive astrocytes, activated in response to various insults [92, 134–136].

3.3 Polysialyltransferase Activity in the Mature Brain

In general, polySia immunoreactivity and the combined mRNA expression of polysialyltransferases are well correlated [68, 84, 137–140]. Despite considerable overlap there are marked differences in tissue- and time-specific mRNA expression patterns suggesting an independent regulation of ST8SiaII and ST8SiaIV at the transcriptional level. Most notably, ST8SiaII is predominant during embryonic development, while ST8SiaIV is the major polysialyltransferase of the adult brain [66, 68, 84, 137]. Accordingly, polySia is drastically reduced in the brain of adult ST8SiaIV-negative mice as detected by Western blot analysis or immunohistochemistry [66, 141, 142]. However, polySia expression is retained on newborn neurons in the neurogenic niches of the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricle [141]. Loss of ST8SiaII has less effect on the polySia level but Western blot analysis of different brain regions indicates clear reductions in some parts of the brain [143]. First immunohistochemical data demonstrated a loss of polySia in the subgranular zone of the dentate gyrus, but normal levels of immunoreactivity were detected in the subventricular zone of the lateral ventricle and the descending stream of rostrally migrating-neuroblasts destined to become olfactory bulb interneurons [143]. Thus, both polysialyltransferases jointly produce polySia during subventricular zone neurogenesis and the loss of one enzyme can be largely compensated by the other. In contrast, ST8SiaII seems to be solely responsible for polySia synthesis in newborn granule cells of the adult dentate gyrus. However, the prominent polySia staining on the mossy fibers of the mature dentate granule cells is retained in the absence of ST8SiaII but completely abolished by the loss of ST8SiaIV [141, 143]. These findings match perfectly the ST8SiaII and ST8SiaIV mRNA expression patterns [84]. During the early stage of their life the newborn granule cell precursors in the subgranular layer express high levels of ST8SiaII, whereas only ST8SiaIV has been detected over the entire depth of the granular cell layer and consequently is associated with mature granule cells [84]. Interestingly, therefore, the expression patterns of the two polysialyltransferases during neurogenesis of dentate granule cells recapitulate the developmental profiles on the cellular level.

A direct comparison of polySia immunoreactivity in the cortex of young adult ST8SiaII- and ST8SiaIV-deficient mice corroborated the differential contribution of the two enzymes in the hippocampal dentate gyrus but also indicated a small overlap [142]. Minor populations of immature polySia-positive neurons remain in the ST8SiaIV-negative subgranular zone and some isolated polySia-positive fibers are still present throughout the granular cell layer of ST8SiaII-deficient mice. Moreover, this study clearly demonstrates that ST8SiaIV is solely responsible for polySia expression in mature cortical interneurons, whereas ST8SiaII is the major polysialyltransferase of the immature neurons in the paleocortex [142]. Remarkably, ST8SiaIV activity may drive maturation of these immature neurons, because ST8SiaIV deficiency leads to increased numbers of polySia- and doublecortin-positive immature neurons in the paleocortex layer II. In contrast, many of the

immature granule neurons displayed aberrant locations and morphology in ST8SiaII-deficient animals, suggesting a role for ST8SiaII in their terminal differentiation [142].

4 Role of Polysialic Acid in Brain Development

4.1 NCAM and PolySia are Implicated in Neural Tube Closure

A function of the earliest expression of polySia during neural tube closure is inferred from the premature polysialylation of NCAM observed in the splotch mutant mouse, a model of Waardenburg syndrome type I caused by pax3 mutations [144]. Indeed, pax3 mutations may affect the balanced expression of polysialylated NCAM, since NCAM and ST8SiaII are downstream targets of this transcription factor [145, 146]. The vital importance of tightly controlled NCAM interactions during these early stages of development was unequivocally demonstrated by the dominant embryonic lethality of mice in which all membrane-associated forms of NCAM were replaced by a soluble, secreted form of its extracellular domain [147]. Analysis of chimeric embryos revealed severe defects by E8.5–E9.5. The embryos were truncated with reduced numbers of poorly formed somites and neural tube defects. Embryos derived almost entirely from homozygous mutant ES cells exhibited the same lethal phenotype, indicating that the secreted NCAM is producing this phenotype through heterophilic rather than homophilic interactions [147].

Although not addressed in this study, the drastic effects of uncontrolled, overshooting NCAM interactions imply that the onset of polysialylation at E9 is used to limit NCAM interactions during neural tube closure. Noteworthy in this context are the pronounced effects of valproic acid and retinoic acid on the polySia-NCAM system. Both are potent teratogens in humans and cause defects of neural tube closure with different periods of sensitivity in mice [148]. Valproic acid increases the polySia to NCAM ratio, while retinoic acid accelerates polysialylation of NCAM, at least in cell culture experiments, by augmenting ST8SiaIV but decreasing ST8SiaII mRNA levels [149–151].

4.2 PolySia-Deficient Mouse Models Reveal Distinct Modes of PolySia Engagement in Neuronal Migration and Axon Tract Development

Analyzing mice with partial or complete ablation of polySia disclosed the crucial role of NCAM polysialylation for mammalian brain development [141, 143, 152–155]. The first models with an extensive loss of polySia were mice with

genetic ablation of all NCAM isoforms [151] or with a deletion of an exon specific for the 180 kD isoform of NCAM [152]. Surprisingly, these NCAM-deficient animals turned out to be viable and fertile and showed a grossly normal brain development. Both NCAM mutant mice, however, display two prominent neuroanatomical defects (for comprehensive review, see [156, 157]). First was a size reduction of the olfactory bulbs caused by a migration deficit of subventricular zone-derived olfactory interneuron precursors, the major polySia-positive cell type in the wild-type brain (see Sect. 3.2) [152, 158–160]. Second was a defective lamination of mossy fibers projecting from the dentate gyrus to the CA3 subfield of Ammon's horn [161, 162]. Both phenotypic traits must be explained by the loss of polySia and not NCAM because they could be copied by enzymatic removal of the sugar polymer leaving the NCAM protein backbone unaltered [158, 162].

Consistent with the potential of the polysialyltransferase ST8SiaII to compensate almost entirely for a loss of ST8SiaIV during the developmental phase (see Sect. 2), no defects of brain morphology were detected in the ST8SiaIV-negative mice [141]. Conversely, the partial reduction of polySia levels in the developing brain explains the malformation of the hippocampal mossy fiber tract observed in ST8SiaII-deficient mice, which is reminiscent of the respective phenotype of the *Ncam*-knockouts [143]. Since mice with genetic ablation of NCAM are almost completely devoid of polySia it is also not surprising that the major neurodevelopmental defects of $Ncam^{-/-}$ animals are recapitulated in *St8siaII*, *St8siaIV* double-knockout mice ($II^{-/-}IV^{-/-}$), which are polySia-negative but retain normal levels of NCAM expression [154, 155]. In marked contrast to the *Ncam*-knockout, however, the simultaneous deletion of both polysialyltransferases generates a postnatally lethal phenotype. Although born at Mendelian ratio and without overt morphological defects, $II^{-/-}IV^{-/-}$ mice fail to thrive and more than 80% die within the first 4 weeks of age [154].

The comparative analysis of $II^{-/-}IV^{-/-}$ and $Ncam^{-/-}$ brains then demonstrated that loss of both polysialyltransferases confers a phenotype that combines two types of defects: (1) defects that develop in polySia-negative mice irrespective of the presence or absence of NCAM and (2) defects that manifest exclusively in $II^{-/-}IV^{-/-}$ mice and therefore may be caused by the appearance of polySia-free NCAM [154]. The first category comprises defective rostral migration of subventricular zone precursors and smaller olfactory bulbs as well as delamination of mossy fibers. Besides postnatal growth retardation and precocious death, the second category includes a high incidence of progressive hydrocephalus and severe anomalies of a diverse set of brain fiber tracts, which occur regardless of ventricular dilatation. Affected are commissural and non-commissural axon tracts. Most conspicuous is the complete agenesis of the anterior commissure. As shown by anterograde tracing of the anterior limb, axons of the anterior commissure are present but lack normal fasciculation, deviate early from their normal trajectory, and therefore never cross the midline [154]. Morphometric analyses also revealed hypoplasia of the internal capsule, the major gateway of fibers to and from the cerebral cortex, and of the mammillothalamic tract. This tract projects from the mammillary bodies to thalamic nuclei as part of a circuit involving thalamus,

cortex, hippocampus, and mammillary body (Papez' circuit) and is essential for spatial working memory in the mouse [163]. Furthermore, size reduction but correct midline crossing of the corticospinal tract was detected. Although resembling the hypoplasia of the corticospinal tract in *Ncam*$^{-/-}$ [164] the defect was significantly more severe in *II*$^{-/-}$*IV*$^{-/-}$ mice [154]. In *II*$^{-/-}$*IV*$^{-/-}$ mice escaping from hydrocephalus, the corpus callosum reached its normal thickness in central sections, but, as demonstrated in a later study, is significantly shorter due to a marked hypoplasia of the splenium, the posterior end of the corpus callosum [165]. In contrast, other tracts, like lateral olfactory tract, optic tract, fasciculus retroflexus, or posterior commissure appeared to be normally developed.

Remarkably, all the fatal developmental defects specifically found in *II*$^{-/-}$*IV*$^{-/-}$ but not in *Ncam*$^{-/-}$ mice could be rescued by the additional deletion of NCAM in polysialyltransferase- and NCAM-negative triple-knockouts (*II*$^{-/-}$*IV*$^{-/-}$*N*$^{-/-}$). It therefore was hypothesized that the major function of polySia is to mask NCAM and to guarantee that NCAM mediated contacts take place in a highly organized, time- and site-specific manner [154]. To substantiate the assumption that untimely expressed polySia-negative NCAM causes malformation of brain axon tracts, the available mouse models with defects in NCAM, ST8SiaII, and ST8SiaIV were used to breed mice with different levels of polySia-negative, "naked" NCAM during brain development [165]. In addition to the entirely polySia-negative, NCAM-positive *II*$^{-/-}$*IV*$^{-/-}$ and the polySia- and NCAM-negative *II*$^{-/-}$*IV*$^{-/-}$*N*$^{-/-}$ animals, mice with different combinations of functional and mutant polysialyltransferase and NCAM alleles were screened. Out of the 27 possible allelic combinations, mice of nine genotypes with different levels of polySia, NCAM, and polySia-free NCAM at postnatal day 1 were selected for morphometric evaluation at the age of 4 weeks. Axon tracts like anterior commissure, internal capsule, and corpus callosum, for which morphological deficits have been identified in the brain of *II*$^{-/-}$*IV*$^{-/-}$ mice, were analyzed. As shown in Fig. 3 by the example of the corpus callosum, the degree of the axon tract defects correlated precisely with the amounts of untimely expressed polySia-free NCAM and not with the overall polySia or NCAM level at postnatal day 1 [165]. The premature occurrence of "naked" NCAM due to a loss of the shielding functions of polySia, therefore, causes inappropriate development of major axon connections, and addition of polySia to NCAM is needed for correct brain wiring. This strengthens the view that concealing NCAM is the key regulatory mechanism that makes polySia essential for brain development. In a broader perspective, these findings indicate that cell surface glycosylation can be used as a surveillance system to control interactions of the corresponding carrier protein.

In search for the cause of the internal capsule hypoplasia the development of thalamocortical and corticothalamic fibers was analyzed [80]. During normal embryogenesis the two fiber systems grow towards each other and intermingle to form the reciprocal connections between cortex and thalamus, which account for a major part of the internal capsule. Similar to the situation for the anterior commissure, labeling of thalamocortical axons revealed that the fibers are present but misrouted in the polysialylation-deficient *II*$^{-/-}$*IV*$^{-/-}$ but not in the NCAM-negative *II*$^{-/-}$*IV*$^{-/-}$*N*$^{-/-}$ mice. After correctly crossing the primordium

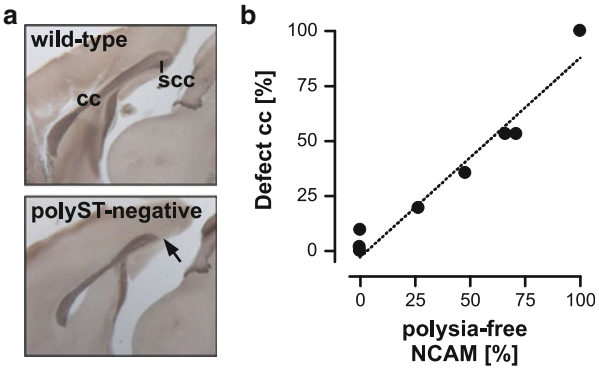

Fig. 3 Polysialylation of NCAM is essential for brain development. (**a**) In mice without functional polysialyltransferases (PolyST-negative) the splenium of the corpus callosum (scc) is markedly reduced (*arrow*), leading to an overall shorter corpus callosum (cc) as shown here in midsagital sections. (**b**) The severity of the corpus callosum defect in 4- to 6-week-old mice correlates linearly with the amounts of polySia-deficient NCAM at postnatal day 1. The strongest defect, i.e., the shortest cc, and the most polySia-free NCAM were detected in the polyST-negative *St8siaII*, *St8siaIV* double-knockout mice and set to 100%. Each of the data points stands for one of the nine mouse lines investigated (see text for details). Adapted from [165]

of the reticular thalamic nucleus the thalamocortical axons fail to turn into the internal capsule and therefore are unable to meet the corticothalamic fibers. In addition, deficiencies of corticothalamic connections contribute to the hypoplasia of the internal capsule in polysialylation-deficient mice [80]. The same study revealed a striking degeneration of the reticular thalamic nucleus (Rt) in specifically the $II^{-/-}IV^{-/-}$ mice. Apoptotic loss of Rt neurons occurred right after birth in a narrow time-window, closely matching the onset of glutamatergic innervation by thalamocortical and corticothalamic fibers under healthy conditions. Apoptosis of Rt neurons could also be induced by lesioning corticothalamic fibers on whole-brain slice cultures, suggesting that defective afferent innervation leads to anterograde transneuronal degeneration. The loss of Rt neurons in polysialylation-deficient, NCAM-positive mice, therefore, seems to be caused by the defects of thalamocortical and corticothalamic axon development.

4.3 PolySia in Oligodendrocyte Maturation and Myelination

Surprisingly, the polysialylated form of the synaptic cell adhesion molecule SynCAM 1 was recently found to be expressed by a subpopulation of NG2 cells (polydendrocytes) in the perinatal mouse brain [33]. These multifunctional precursor cells serve as the primary source of myelinating oligodendrocytes during development and myelin repair but are also able to give rise to astrocytes and neurons [166, 167]. Possible functions of polySia as a modification of SynCAM 1 have not

yet been explored. The most prominent function of SynCAM 1, however, is its potency to induce neuronal synapse formation [168]. Interestingly, NG2 cells receive glutamatergic synaptic input [169, 170]. Thus, integration of NG2 cells into neural networks might be modulated by polysialylation of SynCAM 1 [33].

PolySia on NCAM is expressed by migrating oligodendrocyte precursor cells (OPC) but down-regulated during maturation into myelinating oligodendrocytes [72, 91, 92]. During the two phases of oligodendrocyte development polySia seems to play a dual role. On the one hand, the presence of polySia promotes OPC migration in response to chemoattractive guidance cues [171–173]. On the other hand, polySia helps to keep the precursors in an undifferentiated state, while downregulation of polySia enhances differentiation into mature oligodendrocytes as shown in vitro and under pathological conditions of precursor recruitment from the anterior subventricular zone after lysolecithin-induced demyelination of the corpus callosum [174, 175]. In a complementary approach, neural precursor cells overexpressing ST8SiaIV were transplanted into the brain of hypomyelinated shiverer mice. The engineered cells displayed widespread integration and myelination in the host, but differentiated more slowly than controls [176]. Involvement of polySia as a negative regulator in the process of myelination itself has been derived from co-cultures of oligodendrocytes and neurons. In this in vitro system, removal of polySia enhanced myelin formation, but, in contrast to the studies on OPCs discussed above, the negative regulation of myelination was attributed to the presence of polySia on axons, thought to prevent attachment of the myelin-forming oligodendrocyte processes [177]. The question whether down-regulation of polySia is required for the myelination process in vivo was addressed in transgenic mice expressing the polysialyltransferase ST8SiaIV under the control of the proteolipid protein promoter [178]. In these mice, postnatal down-regulation of polySia in oligodendrocytes was abolished. Similar to the transplantation study with polySia overexpressing precursors [176], the sustained polysialylation caused a delay of oligodendrocyte maturation and myelin formation. Furthermore, the transgenic mice exhibited structural abnormalities of their myelin and axonal degeneration. Thus, myelin formation per se does not necessarily require the loss of polySia from the oligodendrocyte membrane but down-regulation of polysialylation during oligodendrocyte differentiation is a prerequisite for efficient myelin formation and maintenance [178].

4.4 Cellular Models of Polysialic Acid-Controlled NCAM Signaling

In rodents and humans, polySia is part of the neurogenic niches in the anterior subventricular zone and in the subgranular layer of the dentate gyrus [102, 104, 106, 179, 180]. As shown by endosialidase treatment in vivo, loss of polySia causes premature differentiation of neuronal precursors in both systems [181, 182]. In the dentate gyrus of ST8SiaII-deficient mice, many of the immature granule neurons

display aberrant locations and morphology, suggesting a role of ST8SiaII in their terminal differentiation [139]. Reminiscent of the in vivo data, removal of polySia from cultured subventricular zone-derived neuroblasts promotes neurite induction and maturation into olfactory bulb interneurons [183]. Interestingly, both effects were independent from changes in cell migration and could be mimicked by exposure to polySia-free NCAM. The assumed gain of NCAM function in the absence of polySia is corroborated by the finding that the degree of differentiation in cultures obtained from polySia-negative, NCAM-positive $II^{-/-}IV^{-/-}$ mice was higher than in $Ncam^{-/-}$ neuroblasts [183]. This outcome is highly compatible with the proposed role of polySia as a key regulator of NCAM interactions in brain development (see Sect. 4.2). Further experiments revealed that the effect of polySia removal depends on cell–cell contacts and that NCAM-negative and polySia-NCAM-positive neuroblasts respond equally well to polySia-free NCAM. Thus, NCAM on the cell surface is not required for these effects, suggesting the existence of heterophilic signaling. In agreement with these observations, heterophilic NCAM binding has been shown to promote differentiation of hippocampal progenitors from the embryonic brain [184]. In this study, however, the influence of polySia was not addressed.

The potency of polySia as a regulator of particularly heterophilic NCAM interactions has been clearly demonstrated in a series of in vitro studies with tumor cells [21–23, 140]. The prevailing model of NCAM-induced signaling involves association with fibroblast growth factor (FGF) receptors and predicts their activation as well as downstream signaling through the mitogen-activated protein kinase ERK1/2 pathway [185, 189]. Consistent with this model, a crucial role of ERK1/2 in polySia-regulated, heterophilic NCAM signaling was identified, leading to cell differentiation, growth arrest, and increased cell survival (Fig. 4) [21, 22]. A recent study confirms that the activation of ERK1/2 in response to a loss of polySia indeed depends on FGF receptor activity [23]. Moreover, experimentally induced loss of polySia initiates NCAM-mediated signaling at cell–cell contact sites causing reduced motility and enhanced focal adhesion at the cell-substrate interface. Surprisingly, this response was independent from FGF receptor and ERK1/2 activation but involves association of the src-family kinase $p59^{fyn}$ (Fyn) with paxillin (Fig. 4). The analysis of a set of truncated NCAM variants revealed that induction of focal adhesion is triggered by NCAM domains distinct from the FGF receptor binding site. A fragment comprising the immunoglobulin-like domains Ig3 and Ig4 is sufficient to induce focal adhesion but lacks the ability to activate ERK1/2. By contrast, the fibronectin type III repeats containing the FGF receptor binding site are sufficient to induce activation of ERK1/2 but unable to promote focal adhesion [23].

Although these studies were performed in tumor cells, the mechanisms of polySia-controlled NCAM signaling may apply to other cell models. As described above, subventricular zone-derived neuroblasts and hippocampal progenitors respond with enhanced differentiation to loss of polySia or exposure to polySia-free NCAM [182–184], and OPCs differentiate significantly faster after enzymatic removal of polySia than in the absence of NCAM [174, 175]. It should also be

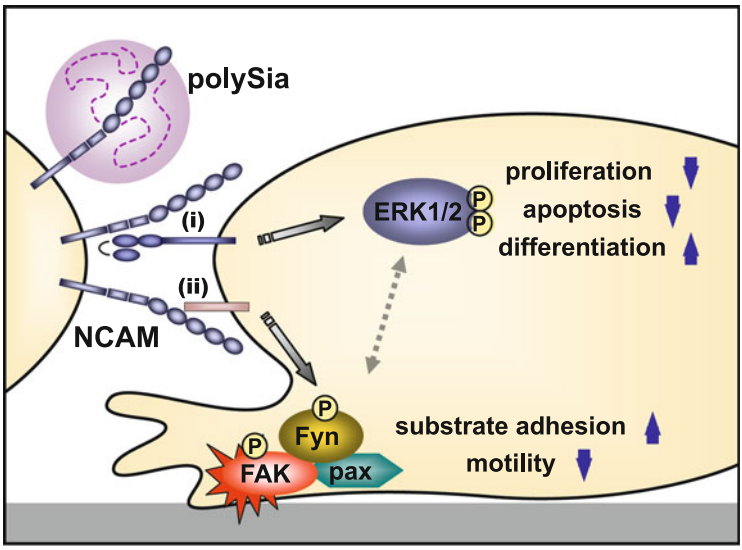

Fig. 4 Model of polySia as a negative regulator of heterophilic NCAM signaling. Removal of polySia unmasks NCAM and thereby initiates NCAM-mediated heterophilic interactions at cell–cell contacts. This involves (**i**) FGF receptor activation by the fibronectin type III repeats of NCAM leading to ERK1/2 dependent promotion of cell differentiation with reduced proliferation and enhanced survival and (**ii**) interactions of NCAM Ig3-4 domains with an unknown binding partner causing recruitment of the src family kinase Fyn to paxillin (pax) and focal adhesion kinase (FAK) to enhance focal adhesion at the cell-substrate interface. Kinase activation by phosphorylation is indicated by (P). Based on [21–23]

noted that focal adhesion kinase-dependent point contacts regulate growth cone motility [190]. Thus, polySia-dependent NCAM signaling from cell–cell to cell–substrate contacts may modulate growth cone adhesion and motility and this could contribute to the axon guidance deficits caused by the gain of NCAM functions in polysialylation deficient mice (see Sect. 4.2).

5 Polysialic Acid in Synapse Formation, Synaptic Plasticity, Learning and Memory

5.1 Formation of Excitatory Synapses

The role of NCAM in formation of excitatory hippocampal synapses is mediated by a polySia-dependent heterophilic mechanism [80]. As polySia and NCAM are expressed both pre- and postsynaptically, the original topic of investigation was to distinguish between pre- vs postsynaptic effects. This was done using so-called heterogenotypic co-cultures of $Ncam^{+/+}$ and $Ncam^{-/-}$ neurons. Comparison of the

mean amplitudes of excitatory postsynaptic currents in synaptic connections with different patterns of pre- vs postsynaptic NCAM expression revealed that the presence of NCAM presynaptically did not influence synaptic strength, whereas postsynaptic expression of NCAM increased the synaptic strength by a factor of 2. Analysis of synaptophysin immunoreactivity associated with NCAM-positive and NCAM-negative neurons revealed a twofold higher synaptic coverage of NCAM-positive cells. This was observed only in heterogenotypic cultures, i.e., under conditions when growing axons had a choice of which postsynaptic target to select, $Ncam^{+/+}$ or $Ncam^{-/-}$. There was no difference between NCAM-positive and NCAM-negative neurons in synaptic coverage in homogenotypic cultures. Thus, expression of NCAM dictates where to form synapses, but is not required for synapse formation. Since expression of NCAM and polySia in the CNS is regulated in an activity-dependent manner [191], an increase in NCAM/polySia-NCAM expression may promote experience-dependent excitatory synaptogenesis in stimulated neurons and/or dendritic subdomains [192].

Does NCAM act as a ligand or a receptor during formation of excitatory synapses? Transfection of NCAM-deficient neurons with any of three major NCAM isoforms, GPI-linked NCAM120, or transmembrane domain-containing NCAM140 or NCAM180 stimulated preferential synapse formation on all NCAM isoform-expressing neurons [82]. These experiments suggest that the extracellular domain of NCAM functions as a synaptogenic ligand. To investigate the involvement of polySia, cultures were treated with endosialidase. This treatment completely abolished preferential formation of synapses in NCAM-expressing cells. Enzymatic removal of heparan sulfates from cultured neurons, a mutation in the heparin-binding domain (HBD) of NCAM, and application of recombinant soluble extracellular domains of NCAM and polySia-NCAM similarly diminished synaptogenic activity of neuronally expressed polySia-NCAM, suggesting that interaction of NCAM with heparan sulfate proteoglycans is involved. PolySia-NCAM-driven synaptogenesis was also blocked by antagonists to FGF receptor and the NMDA subtype of glutamate receptors, but not by blockers of non-NMDA glutamate receptors or voltage-dependent Na^+ channels. Enzymatic removal of polySia and heparan sulfates also suppressed the increase in the number of perforated spine synapses associated with NMDA receptor-dependent long-term potentiation (LTP) in the CA1 region of organotypic hippocampal slice cultures [82]. Thus, neuronal polySia-NCAM in complex with heparan sulfate proteoglycans promotes synaptogenesis and activity-dependent remodeling of synapses.

In *St8siaII*-knockout mice, ectopic synapse formation of hippocampal mossy fibers has been detected together with axon mistargeting and abnormal extension of the infrapyramidal mossy fiber bundle [143]. In the mature brain, however, polySia expression on the mossy fibers depends on ST8SiaIV activity [141] and is maintained in adult *St8siaII*-knockout mice [143]. This indicates that ectopic formation of mossy fiber synapses originates from a lack of ST8SiaII during development, when both polysialyltransferases are co-localized in the dentate gyrus [83]. Recently, the role of ST8SiaII and polySia in synapse formation of hippocampal mossy fibers has been addressed by the use of a chemically modified

sialic acid precursor (*N*-propanoyl-D-mannosamine, ManNProp) [193]. ManNProp can be used by ST8SiaIV to produce unnatural propanoyl-polySia but inhibits ST8SiaII activity [194]. Treatment of hippocampal slice cultures derived from newborn mice with ManNProp resulted in aberrant mossy fiber projections forming functional glutamatergic terminals on CA1 pyramidal neurons with a typical mossy fiber synapse-like morphology [193]. Reminiscent of the phenotype of the *St8siaII*-knockout mice, in vivo application of ManNProp to newborn rats for 4 weeks yielded a significantly longer infrapyramidal mossy fiber bundle. However, unlike in *St8siaII*-knockouts, aberrant fibers were polySia-negative but NCAM-positive and entered into the CA1 pyramidal layer. Moreover, recurrent mossy fibers were observed in the ManNProp-treated rats, which aberrantly crossed the granule cell layer to terminate on neurons in the molecular layer [193]. Interestingly, this aberrant innervation pattern resembles the mossy fiber sprouting observed after kainate induced status epilepticus. Homeostatic regulation of polySia synthesis, therefore, is essential for correct outgrowth and synaptic targeting of hippocampal mossy fibers.

5.2 *Plasticity of Inhibitory Synapse Maturation*

In the visual cortex, polySia is downregulated shortly after eye opening. This decline is inversely correlated with the maturation of GABAergic innervation and hindered by visual deprivation, indicating a role of polySia in the critical period of ocular dominance plasticity [83]. Indeed, premature reduction of polySia promotes functional maturation of GABAergic synapses. Removal of polySia by application of endosialidase to cortical organotypic cultures causes precocious maturation of perisomatic GABAergic synapses as evidenced by enhanced branching of axon arbors and higher density of mature presynaptic boutons [83]. As shown by injection of endosialidase, a too early loss of polySia in the adolescent visual cortex also promotes the maturation of perisomatic GABAergic innervation in vivo and, consistent with a higher number of GABAergic synapses, increased the frequency of miniature inhibitory postsynaptic currents. In addition, a shift in occular dominance, which can normally be evoked by monocular deprivation during a critical period between P24 and P35, could be induced much earlier in endosialidase-treated mice [83]. The enhanced inhibition in response to the loss of polySia, therefore, seems to trigger precocious ocular dominance plasticity.

Preceding the decline of polySia in the mouse visual cortex, ST8SiaII and ST8SiaIV mRNA levels decrease around the time of eye opening. However, only the reduction of ST8SiaII is regulated by sensory experience [86]. Moreover, in organotypic slice cultures, developmental downregulation of ST8SiaII is reduced by blocking spike activity with tetrodotoxin or by AP5 as antagonist of NMDA receptors and enhanced by blocking $GABA_A$ receptors with bicuculline. This indicates that ST8SiaII gene expression is regulated by activity and in particular by NMDA-mediated excitation [86]. Interestingly, a similar regulation of polySia

by sensory input and activity through NMDA receptor-dependent mechanisms has been shown during postnatal development of the dorsal vagal complex in the brain stem [195]. Conversely, as detailed in the next section, polySia modulates extrasynaptic NMDA receptor signaling, pointing towards a possible feedback regulation.

Contrasting the precocious maturation of perisomatic innervation after an acute loss of polySia, mice over-expressing a soluble extracellular domain fragment of NCAM under the neuron-specific enolase promoter (NCAM-EC mice) display a reduction in perisomatic synaptic puncta formed by parvalbumin-positive cortical interneurons, indicating a decrease in the number of synaptic terminals of basket cells [198]. Further investigations of these mice revealed perturbed arborization of basket cells in the prefrontal cortex during early postnatal stages, when endogenous polysialylated NCAM is replaced by polySia-negative NCAM [85]. Consistent with the enhanced inhibition in the visual cortex after endosialidase treatment [83], a recent study demonstrates increased numbers and sizes of perisomatic basket cell synapses in *Ncam*-knockout mice [199]. Moreover, the study also provides evidence that polysialylated is required to promote ephrinA5-induced axon remodeling of basket interneurons in cortical slices. Together, these data impressively demonstrate that the balanced regulation of polySia and NCAM is essential for proper synapse development of basket cells.

5.3 Synaptic Plasticity in the Mature Nervous System

The first evidence that NCAM may play a role in synaptic plasticity was provided in 1994 by a seminal study that showed that perturbation of NCAM function significantly reduced LTP in the CA1 area of the hippocampus [200]. Polyclonal antibodies against NCAM, soluble oligomannosides that block interaction of NCAM with oligomannosidic carbohydrates carried by L1, and synthetic peptides from the fourth Ig-like domain of NCAM, which mediates interaction with L1, were used in these experiments. Further analysis of constitutive and conditionally NCAM deficient mice (NCAMff+), in which the NCAM gene was ablated in neurons after cessation of major developmental events by expression of Cre recombinase under the CaMKII promoter, showed impairment of CA1 LTP in both mutants [191, 196, 201], thus supporting the view that NCAM plays an acute role in synaptic plasticity in the CA1 region. Additionally, long-term depression (LTD) in the CA1 was impaired in constitutive and conditional *Ncam*-knockout mice [197, 201]. In the CA3 region, constitutive but not conditional NCAM-deficient mice were found to have abnormalities in lamination of mossy fiber projections and to be impaired in mossy fiber LTP, suggesting that NCAM is required for proper development of mossy fiber-CA3 synapses [201, 202]. Recording of LTP in the dentate gyrus of anesthetized mice confirmed the role of NCAM in synaptic plasticity in vivo [203] (Table 1). Overexpression of soluble extracellular domain of NCAM in NCAM-EC

Table 1 Effects of NCAM, polySia and polysialyltransferases on multiple forms of hippocampal synaptic plasticity

Condition	CA1 LTP	CA1 LTD	CA3 LTP	DG LTP
Ncam−/−	↓	↓	↓	↓
*Ncam*ff+	↓	↓	=	n.d.
Endosialidase	↓	↓	=	n.d.
*St8sia*IV−/−	↓	↓	=	=
*St8sia*II−/−	=	n.d.	=	=

↓ impaired, = normal, *n.d.* not determined

mice did not affect LTP in CA3-CA1 synapses but resulted in specific impairment of LTP in the prefrontal cortex [204].

The role of polySia in synaptic plasticity was initially studied using the enzymatic removal of polySia by endo-N, which inhibited LTP and LTD in CA1 [123, 191]. Experiments using mice deficient in ST8SiaII or ST8SiaIV provided the genetic evidence for the importance of polySia in synaptic plasticity in the CA1 [141, 143]. No involvement of these enzymes in mossy fiber LTP in the CA3 region or in the dentate gyrus LTP was revealed using either of two lines of polySia-deficient mice, despite abnormal lamination of mossy fiber projections in ST8SiaII deficient mutants [141, 143, 203].

Several early observations suggested that polySia may modulate activity of glutamate receptors and hence regulate induction of LTP. Peptides blocking interaction of proteins with the fourth immunoglobulin-like domain of NCAM reduced LTP when applied before induction of LTP but not afterwards [205], pointing to the importance of NCAM for LTP induction. Since impairment of LTP in NCAM deficient mice could be rescued by elevation of extracellular Ca^{2+} concentration, it has been speculated that NCAM influences Ca^{2+} signaling via NMDA receptors [201]. This idea was supported by the data showing a similar central location of NCAM180 and the GluN2A receptors within the postsynaptic density in untreated animals, and a similar redistribution of these molecules to the edges of postsynaptic density in animals after induction of LTP [206]. Because polySia may directly potentiate opening of AMPA subtype glutamate receptors [207], it was hypothesized that polySia may influence activity of LTP-mediating receptors via a direct interaction with the extracellular domain of receptors. This hypothesis was supported by a study [208] in which impaired CA1 LTP in hippocampal slices was rescued via application of soluble polySia or ectodomain of polySia-NCAM, but not of NCAM. A parallel in vitro study [209] reported that soluble polySia alone or attached to NCAM inhibited activation of GluN2B-containing NMDA receptors by low micromolar concentrations of glutamate in hippocampal cultures and artificial lipid bilayers (Fig. 5). These concentrations are characteristic for extrasynaptic space but much lower than synaptic concentrations of glutamate following transmitter release.

Two recent studies demonstrate that polySia-NCAM regulates synaptic plasticity by setting a balance in signaling via extrasynaptic GluN2B and synaptic GluN2A receptors [196, 197]. Consistent with the findings of Hammond and colleagues [209], isolation of NMDA receptor-mediated component in hippocampal slices

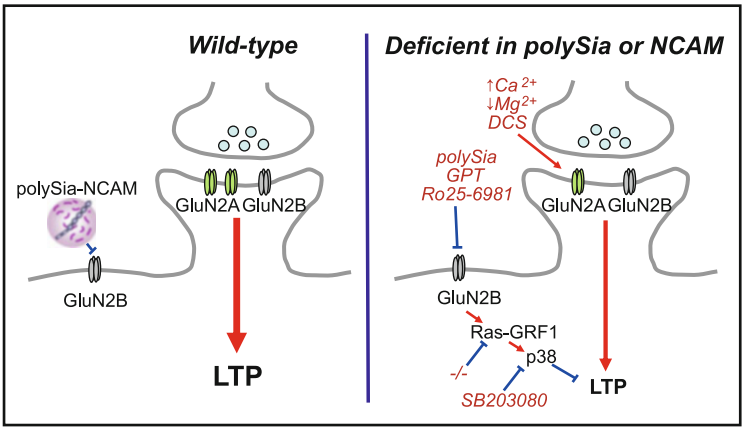

Fig. 5 Model for polySia-NCAM-mediated modulation of signaling via NMDA receptors. Endogenous and exogenous molecules are shown in *black* and *gray*, respectively. Stimulatory and inhibitory relationships are shown by *arrows with sharp and blunt ends*, respectively. In *Ncam*+/+ mice, polySia-NCAM inhibits extrasynaptic GluN2B-containing receptors and LTP is induced through activation of GluN2A receptors. In *Ncam*−/− mice, signal enhancement occurs via extrasynaptic GluN2B-containing receptors, whereas signal reduction occurs via GluN2A-containing receptors, which leads to impaired LTP. This model is supported by experiments with rescue of LTP in *Ncam*−/− mice by elevated extracellular Ca^{2+} and reduced extracellular Mg^{2+} concentrations, and application of NMDA receptor modulators DCS or Ro 25–6981, the glutamate scavenger GPT, polySia, or SB 203580 [196]. All these treatments change the signaling balance between GluN2A- and GluN2B-mediated pathways in favor of the GluN2A-mediated pathway and restore LTP in *Ncam*−/− mice. Similarly, LTP is restored in endosialidase-treated slices from *Ncam*+/+ mice by DCS, Ro 25–6981, and SB 203580, and by genetic ablation of Ras-GRF1. Furthermore, fear memory is restored by DCS and Ro 25–6981 in *Ncam*−/− mice, whereas LTD is rescued by DCS. Modified with permission from [197]

also revealed an increase in GluN2B-mediated transmission in NCAM-deficient mice and an increase in GluN2B-mediated Ca^{2+} signaling after removal of polySia [196]. In parallel, a decrease in GluN2A-mediated transmission was found. Strikingly, the suppression of extrasynaptic GluN2B signaling with Ro 25–6981 or by reduction of extrasynaptic glutamate concentrations using the glutamate scavenger GTP, or facilitation of GluN2A receptors by D-cycloserine fully restore levels of LTP in either NCAM or polySia deficient slices to wild-type levels.

The following data support the view that the mechanisms downstream of GluN2B in NCAM/polySia deficient slices involve signaling via Ras-GRF1 to the Rac effector p38 MAPK. The level of phosphorylated p38 MAPK is upregulated in NCAM-deficient mice and in endosialidase-treated slices, while it is reduced in Ras-GRF1−/− mice [210]. An inhibitor of p38 restores levels of LTP in polySia or NCAM-deficient slices to those seen in wild-type mice. The level of phosphorylated p44 is co-upregulated although to a smaller degree than p38, as expected in response to activation of Ras-GRF1, which has been shown to mediate synaptic depression through p38 MAPK [210]. PolySia deficiency does not lead to impaired LTP in

Ras-GRF1−/− mice, supporting the view that Ras-GRF1 is a transducer downstream of hyperactive GluN2B in polySia deficient neurons. Interestingly, activation of p38 MAPK signaling has also been shown to mediate impairment in LTP by tumor necrosis factor [211], by injection of the ectodomain of another cell adhesion molecule, neuroplastin [212], and by the Aβ peptide generated from the amyloid precursor protein, which is widely believed to underlie the pathophysiology of Alzheimer's disease [213]. In these cases, p38 MAPK enhances the removal of AMPA and NMDA receptors from the postsynaptic cell surface, which is likely to be the mechanism for impaired LTP in polySia/NCAM deficient mice. The mechanisms of impaired LTP in the prefrontal cortex of NCAM-EC mice are unknown. However, the study of tenascin-R deficient mice demonstrates that a deficit in perisomatic GABAergic inhibition in the hippocampus may induce metaplastic increase in the threshold for induction of LTP [214]. Whether this is also the case in the prefrontal cortex of NCAM-EC mice remains to be investigated.

5.4 Learning and Memory

The results showing the role of polySia and NCAM in hippocampal plasticity are nicely complemented by studies of hippocampus-dependent spatial and place (contextual) learning in the Morris water maze and fear conditioning paradigms. These studies demonstrated learning-associated changes in the expression of NCAM and polySia [213–218], and impaired memory after treatment with NCAM antibodies [219], in constitutively NCAM-deficient mice [152, 208, 220] and in conditionally NCAM-deficient mice [201, 221]. Temporal perturbations of NCAM or associated polySia at different phases of learning and memory lead to the same memory deficits in spatial navigation [123, 218] and contextual fear conditioning [208]. Furthermore, NCAM-EC mice are impaired in contextual and cued fear conditioning and working memory [198, 204], and genetic ablation of polysialyltransferases ST8SiaII or ST8SiaIV results in impaired fear conditioning [143, 208] or impaired spatial and reversal learning in the Morris water maze [222]. As the pre-training treatment with GluN2B antagonist Ro 25–6981 and GluN2A agonist D-cycloserine, which restored LTP in polySia/NCAM deficient hippocampal slices, also rescued hippocampus-dependent contextual fear memory in NCAM deficient mice [196, 197], acquisition of this form of learning appears to depend on polySia-NCAM mediated modulation of signaling through NMDA receptors. Another study suggests that polySia might also contribute to consolidation of memories, as mice which were injected with polySia cyclic mimetic peptide pr2 into the dorsal hippocampus (5 h after massed training in the spatial version of the water maze) showed higher levels of memory retention 24 h, 1 week and 4 weeks after the training [223].

6 Implications for Nervous System Disease

6.1 PolySia and Epilepsy

Because various plastic changes have been suggested to be functionally involved in the epileptogenesis, and polySia is the key molecule involved in plasticity, several studies addressed the polySia role in development and progression of epilepsy and associated comorbidities. There is increased expression of polySia in the hippocampus and the entorhinal cortex in human temporal lobe epilepsy [224]. Removal of polySia with endosialidase in rodents increased acute seizure susceptibility, as indicated by reduced seizure threshold [225], and lowered the number of newborn neurons following the status epilepticus (SE, induced by electrical stimulation of basolateral nucleus of the amygdala), as compared to vehicle-treated rats, thereby counteracting the SE-induced increase in neurogenesis [226]. Nevertheless, the SE induced increases in the total number of doublecortin expressing immature neurons and the fraction of doublecortin-positive cells with persistent basal dendrites was not affected by endosialidase treatment. There was also no effect of endosialidase on the number of seizures, their severity, and the duration of single seizures [226].

This is in contrast to the results obtained by intraperitoneal injection of kainate for induction of seizures, as mice deficient in ST8SiaIV showed a reduced latency to the first generalized seizure and higher lethality due to SE [227]. In the elevated plus maze paradigm, the loss of polySia in *St8siaIV*-knockout mice increased anxiety-associated behavior, suggesting a major implication of the polySia–NCAM system in the control of anxiety after SE [227]. In view of the fact that anxiety disorders represent a frequent clinical problem in epileptic patients [228], it will be of interest to evaluate further the potential of polySia–NCAM targeting for the treatment of these comorbidities.

Also in the model of mesial temporal lobe epilepsy with unilateral hippocampal injection of kainate, contralateral i.c.v. endosialidase infusion severely increased neurodegeneration in the KA-lesioned hippocampal formation [229]. A significant increase in cell death was evident in the lesioned CA3 pyramidal cell layer, accompanied by strong astrogliosis throughout the lesioned hippocampal formation. Neurodegeneration also extended to the dentate gyrus and led to early onset of focal seizures, in line with data obtained in ST8SiaIV-deficient mice and with a previous study showing that hyperthermic preconditioning-induced upregulation of polySia-NCAM has a neuroprotective effect against kainate [230]. The striking *trans*-hemispheric influence of endo-N suggests that polySia-NCAM might mediate transport of neuroprotective signals into the lesioned hippocampus. One of the signals appeared to be the binding partner of NCAM – GDNF – since injection of GDNF antibodies into the contralateral hippocampus of kainate-treated mice mimicked injection of endosialidase by enhancing neurodegeneration and decreasing expression of the GDNF family receptor α1 in the epileptic focus. Thus, polySia-NCAM-mediated modulation of GDNF signaling restrains neurodegeneration and delays onset of SE.

6.2 PolySia and Neurodegeneration

There are significantly fewer NCAM-positive neurons in the frontal cortex of Alzheimer's disease (AD) patients than in normally aging individuals, although there is little difference in the levels of NCAM in the occipital cortex and hippocampus of the two groups [231]. However, polySia is over-expressed in the outer two-thirds and the inner third of the molecular layer of the dentate gyrus in AD patients [232]. Furthermore, fiber and neuropil staining is increased in the strata oriens, radiatum, and pyramidale of the CA1 subfield. There are changes in polySia immunoreactivity in layer II and in the superficial portion of layer III of the entorhinal cortex. Thus, polySia is upregulated in hippocampal areas where neurofibrillary tangles, amyloid plaques, and neuronal loss appear, or where neurons suffer from a lack of inputs and undergo remodeling. Interestingly, acute administration of Aβ increased expression of polySia in the CA1 and DG subfields [233], but significantly decreased expression of another carbohydrate carried by NCAM, HNK-1 [234]. Moreover, HNK-1 immunoreactivity was decreased in brain tissue of a transgenic mouse model of AD.

In this context, it is remarkable that application of polySia mimetic and the combination of polySia and HNK-1 mimetics, but not the HNK-1 mimetic alone, improves functional recovery after spinal cord injury [235]. A better outcome in polySia mimetic-treated mice is associated with higher, as compared with control mice, numbers of cholinergic and glutamatergic terminals and monaminergic axons in the lumbar spinal cord, and better axonal myelination proximal to the injury site. These data suggest that polySia mimetic peptides can be efficient therapeutic tools augmenting plasticity and synaptogenesis. Furthermore, several NCAM-mimicking or -derived peptides have neuroprotective properties. For instance, systemic treatment with the FGL peptide (mimicking NCAM interaction with FGF receptors) reverses depression-like behavior in NCAM deficient mice, reduces neuroinflammation and neuroglial activation within the aged rat hippocampus and the age-related loss of synaptophysin immunoreactivity within CA3 and hilus, and attenuates Aβ induced neuropathology and cognitive impairment that are hallmarks of Alzheimer's disease [236]. The latter effects are mediated by inhibition of the GSK3beta kinase activity. The mechanisms of polySia mimetic peptides are less clear, but several putative polySia binding molecules have been identified, including BDNF, NT-3 and NT-4 [237], FGF2 [238], GluN2B-containing NMDA receptors [196], and the human-specific Siglec-11 [239], with prominent neuroprotective properties. It is particularly noteworthy that human Siglec-11 ectopically expressed on murine microglia and interacting with polySia on neurons reduces lipopolysaccharides-induced gene transcription of proinflammatory mediators, impairs phagocytosis, and alleviates microglial neurotoxicity [239].

6.3 PolySia in Demyelinating Disease and Remyelination

PolySia has been detected on chronically demyelinated axons in multiple sclerosis lesions, whereas remyelinated axons in so-called shadow plaques with partial repair were negative for polySia [240]. These data suggest that re-expression of polySia on axons could act as an inhibitor of remyelination. This is supported by the strong polySia immunoreactivity on axons of hypomyelinated white matter in a mouse model of Niemann–Pick disease type C [241] and by the persistence of polySia expression on unmyelinated fibers of the healthy rodent brain such as axons in the fimbria and in the mossy fiber tract of the hippocampal formation [77]. A hint that polySia expression interferes to at least some extent with myelin repair in vivo, was obtained by applying the mouse model of cuprizone-induced de- and remyelination to ST8SiaIV-deficient mice [242]. These mice have normal developmental myelination, and comply with the stereotyped pattern of white and gray matter demyelination described for mice fed with the neurotoxic copper chelator cuprizone [243, 244]. However, reexpression of several myelin markers and thus remyelination were accelerated in *St8siaIV*-knockout mice during the first week after withdrawal of the toxin. The effect has been assigned mainly to enhanced OPC differentiation and to a lesser extent to OPC recruitment [242]. The data are proof of the principle that targeting polysialyltransferases could be used to improve remyelination.

6.4 PolySia and Neuropsychiatric Disorders

6.4.1 Altered NCAM and PolySia Levels in Neuropsychiatric Disorders

A long standing record links dysregulation of NCAM to the pathophysiology of schizophrenia and other neuropsychiatric disorders. As reviewed in detail elsewhere [245, 246], a number of studies found increased concentrations of NCAM fragments in either serum or cerebrospinal fluid of schizophrenic patients or in post-mortem brain including samples from hippocampus and cingulate cortex. Elevated levels of NCAM fragments were also detected in cerebrospinal fluid and post-mortem brain samples of patients with bipolar disorder – for review see [245, 246]. In autism, an early study reported a decrease in a small, 70-kDa serum fragment of NCAM [247]. This finding was not reproduced by a later study, which instead found a trend towards an increase of a 105-kDa to 115-kDa NCAM immunoreactive band corresponding to the major form of NCAM typically detected in human serum samples [248]. In addition, the same study reported lower levels of specifically the 180-kDa isoform in post-mortem samples of the cerebellar cortex. In most of the studies, however, the exact nature as well as possible sources of the NCAM fragments remains elusive. Concerning polySia, one small, but prominent study found moderate to severe reductions of polySia-positive cell numbers in the hilus of the dentate gyrus in eight out of ten post-mortem brains of medicated schizophrenic patients as compared

to control brains [249]. Importantly, no significant difference was detected in the total numbers of cells in the hilus, and polySia immunoreactivity in the granular cell layer of the dentate gyrus was not apparently altered between schizophrenic and control brains. Furthermore, a recent immunohistochemical comparison of brain sections from psychiatric disorder patients indicates that polySia is not altered in the amygdala of schizophrenics but is reduced in depressed patients and increased in bipolar disorder [250].

6.4.2 Genetic Associations

Schizophrenia has a high heritability and genome-wide association studies indicate a polygenic origin with a shared genetic liability between schizophrenia and bipolar disorder [251, 252]. *NCAM1* and both polysialyltransferase genes map to chromosomal regions that harbor susceptibility loci for schizophrenia (11q23.1, 15q26, and 5q21 for *NCAM1*, *ST8SIA2*, and *ST8SIA4*, respectively) [253–255]. More telling, single nucleotide polymorphisms (SNPs) in *NCAM1* as well as in the promoter region of *ST8SIA2*, but not *ST8SIA4*, have been associated with schizophrenia [256–259]. Interestingly, risk haplotypes in the promoter region of *ST8SIA2* were identified by two independent studies in the Japanese and the Han Chinese population [256, 259]. In vitro promoter assays with one of the risk-associated variants point towards enhanced transcriptional activity [256]. Recently, analysis of a point mutation detected heterozygously in just one schizophrenic patient in the Japanese sample revealed that the E141K substitution near the sialylmotif *L* leads to reduced activity and production of shorter polySia chains [260]. Other SNPs within *NCAM1* have been found to contribute to the risk of bipolar disorder [257, 261] and a genome-wide association study in the Han Chinese population found a strong association of an SNP close to *ST8SIA2* [262]. A genome-wide scan in an Italian population indicates a common susceptibility locus for schizophrenia and bipolar disorder in 15q26 including *ST8SIA2* [263]. In this study, however, analysis of two SNPs in the promoter region of *ST8SIA2*, directly associated with schizophrenia in the Japanese sample [256], failed due to low allele frequencies in the European population and therefore, association with *ST8SIA2* was not confirmed or ruled out [263]. Moreover, an exploratory analysis of a genome-wide association study of SNPs in more than 1,500 families with autism spectrum disorders (ASD) identified a strong association signal for an intronic SNP in *ST8SIA2* in a subgroup of the ASD sample stratified by verbal status [264].

6.4.3 Animal Models

Compelling evidence suggests that schizophrenia is associated with disturbed neurodevelopment resulting in altered brain connectivity [265, 266]. In light of the particularly strong links between the polySia-NCAM system and schizophrenia

in humans, the remarkable parallels between the phenotype of NCAM- or polySia-deficient mice and pathophysiological findings in schizophrenia merit a short survey. First, ventricular enlargement, one of the most abundant abnormalities in schizophrenia [267], has been reported for mice with specific deletion of NCAM-180 [268] and variable degrees of ventricular dilatations including cases of severe hydrocephalus were observed in $II^{-/-}IV^{-/-}$ mice [154]. Second, smaller olfactory bulbs occur in schizophrenia [269] and $N^{-/-}$ or $II^{-/-}IV^{-/-}$ mice [153, 154]. Third, reductions of corpus callosum and internal capsule as found in schizophrenic patients [266, 270–273] correlate with deficits of these axon tracts in polysialylation-deficient mice [80, 165]. Importantly, the almost linear correlation of gross anatomical defects with the premature occurrence of polySia-free NCAM [165] (see Sect. 4.2 and Fig. 3) suggests that even minor imbalances of NCAM polysialylation during brain development lead to deficits of connectivity. Fourth, reminiscent of cognitive impairment in schizophrenia [274], $N^{-/-}$ as well as polysialyltransferase-deficient $II^{-/-}$ and $IV^{-/-}$ mice display deficits in synaptic plasticity, learning, or memory formation (see Sects. 5.3 and 5.4) and one study reported reduced prepulse inhibition of acoustic startle in NCAM-180 knockout mice ([268], but see [275]). These animal models therefore highlight that genetic interference with the complex coordination of NCAM polysialylation has the potential to cause a neurodevelopmental predisposition to schizophrenia and possibly other disorders.

Chronic stress is a well established model of anxiety and depressive-like behavior in rodents. Daily exposure of rats to restraint stress for 3 weeks causes an up-regulation of polySia levels in the hippocampus [276] and a transient increase of polySia-positive neurons in the dentate gyrus associated with suppression of proliferation and reduced numbers of granular cells [276, 277]. Interestingly, a recent study revealed increased vulnerability to restraint stress and depression-like behavior as well as impaired neurogenesis in the dentate gyrus of heat shock factor 1 (HSF1)-deficient mice associated with reduced polySia and polysialyltransferase mRNA levels in early postnatal stages [278]. Binding of HSF1 to the *St8siaII* and *St8siaIV* promoters suggests regulation through direct transcriptional control. Since enzymatic removal of polySia from the neonatal hippocampus also affects depression-like behavior, the data imply that polysialylation under the control of HSF1 is essential for hippocampal development and behavioral maturation [278].

A stress-induced increase of polySia-positive cells is also observed in the piriform cortex [279]. In contrast, chronic stress leads to reduced levels of polySia immunoreactivity in the amygdala [280]. This was confirmed by a recent study showing that these changes can be attributed to altered polySia levels of interneurons, and are paralleled by lower mRNA levels of ST8SiaII and reduced expression of the GABAergic marker GAD67 [113]. Conversely, polySia and GAD67 are significantly increased in the amygdala of rats subjected to social isolation rearing, indicating that this model, which causes a behavioral phenotype with schizophrenia-like traits, is not reproducing the decrease of inhibitory markers found in the amygdala of schizophrenic patients [281].

Dysfunction of inhibitory neurotransmission and connectivity in the prefrontal cortex (PFC) are involved in the pathogenesis of schizophrenia and major

depression [282–284]. The medial PFC is particularly affected and in depression shows remodeling, which may be corrected by antidepressants. As discussed before (see Sect. 3.2), a series of studies show that polySia is specifically expressed by interneurons of the adult neocortex, and this holds true for the PFC in rodents and men [107, 109]. Strikingly, chronic antidepressant treatment with the selective serotonin reuptake inhibitor fluoxetine (Prozac®) increases polySia levels within the rat PFC [285–287] but also in other cortical areas [288]. Similar results have been obtained with another antidepressant, imipramine [289]. Likewise, a dopamine D2 receptor agonist increased, but the D2 receptor antagonist and antipsychotic drug haloperidol reduced polySia in the PFC [290]. Furthermore, polySia-positive interneurons show low levels of synaptic connectivity [114] and polySia seems to be required for the dopamine D2 receptor-mediated increase in perisomatic inhibition of principal neurons in the PFC of adult rats [291]. Collectively, these findings point towards polySia as a trigger of structural plasticity of inhibitory networks in the mature PFC. As shown in mice, polySia of interneurons in the mature PFC is exclusively produced by ST8SiaIV [142]. Thus, complementary to the potential role of dysfunctional ST8SiaII expression during brain development, altered NCAM polysialylation of cortical interneurons by ST8SiaIV may contribute to the etiology of neuropsychiatric disorders at later stages.

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