THE ROLES OF EXTRACELLULAR MATRIX MOLECULES IN SYNAPTIC TRANSMISSION AND PLASTICITY IN THE MOUSE HIPPOCAMPUS

Dissertation

zur Erlangung des Doktorgrades des Fachbereiches Chemie

der Universität Hamburg

vorgelegt von Olena Bukalo

Hamburg, 2002

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Titel der Dissertation:	The roles of extracellular matrix molecules in synaptic transmission and plasticity in the mouse hippocampus
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ABSTRACT

The extracellular matrix (ECM) is a complex network of macromolecules including glycoproteins, polysaccharides and proteoglycans. In the nervous system, cell adhesion molecules (CAMs) and ECM components mediate cell-cell and cell-matrix interactions, regulating cell migration, survival, differentiation, axonal pathfinding and synapse formation. Our study demonstrates the involvement of recognition molecules, such as tenascin-R (TN-R), tenascin-C (TN-C), chondroitin sulfate proteoglycans (CSPGs), and polysialylated neural cell adhesion molecule (PSA-NCAM) in hippocampal synaptic transmission and plasticity of mice.

To investigate the contributions of these molecules, we used either enzymatic treatment of the hippocampal slices with chondroitinase ABC, removing chondroitin sulfates (CSs) from the proteoglycan core, or mice deficient in TN-R, TN-C, or polysialyltransferase PST, attaching polysialic acid (PSA) to neural cell adhesion molecule (NCAM) in mature neurons. Comparison of the effects of enzymatic treatment with chondroitinase ABC and the phenotypes of TN-C, TN-R and PST deficient mutants reveals a number of differences in the involvement of these molecules in CA1 subfield-specific synaptic plasticity.

We have shown that the treatment with chondroitinase ABC and TN-C deficiency does not interfere with basal excitatory synaptic transmission and short-term plasticity, but reduces theta-burst stimulation (TBS)-induced long-term potentiation (LTP) in the CA1 region of the hippocampus. In contrast, increased levels of synaptic transmission, as well as impairment in both short-term potentiation (STP) and LTP were attributed to TN-R mutants. N-methyl-D-aspartate (NMDA) receptor-mediated transmission appeared normal after chondroitinase ABC treatment and in TN-R and TN-C mutants. Reduction in low-frequency stimulation (LFS)-induced short-term depression (STD) and long-term depression (LTD) was demonstrated in TN-C deficient mice, whereas LTD was normal, despite decreased STD, in TN-R mutant mice. On the other hand, removal of CSs blocked LTD but did not affect STD. Synaptic plasticity in the CA3 region was not affected by TN-R and TN-C deficiency.

Despite functional interaction and colocalization of CSPGs and TN-R our data do not support the possibility of synergetic effects produced by removal of CSs and TN-R on

synaptic transmission and plasticity in the CA1 region of the hippocampus. CSPGs and TN-R could, however, share some signaling mechanisms related to the impaired expression of TBS-induced LTP in the CA1 subfields, which are similar under two conditions. TN-R and TN-C glycoproteins, two members of one gene family, are also differently involved in CA1 plasticity. On the one hand, our data show enhanced excitatory synaptic transmission and modified threshold for induction of LTP/LTD in TN-R deficient mice, correlating with the increased number of perforated synapses observed in these mutants. On the other hand, TN-C affects L-type voltage-dependent calcium channel (VDCC)-mediated currents/signaling.

During postnatal development PST mutants showed a decrease of PSA in most brain regions compared to wild-type animals. Loss of PSA in the presence of NCAM protein but in the absence of obvious histological changes allowed us to directly study the role of PSA in synaptic plasticity. Schaffer collateral-CA1 synapses, which express PSA in wild-types, showed impaired LTP and LTD in mutants. This impairment was age-dependent, following the time course of developmental disappearance of PSA. Delamination of mossy fibers in the hippocampal CA3 region, as found in NCAM-deficient mice, does not occur in PST knockout mice. Contrary to NCAM mutants, LTP in PST deficient mice was undisturbed at mossy fiber–CA3 synapses, which do not express PSA in wild-type mice. These results demonstrate an essential role for PST in synaptic plasticity in hippocampal CA1 synapses, whereas NCAM, but not PSA, is likely to be important for LTP in the hippocampal CA3 region.

As *in vitro* correlate of epileptic activity, the ability of hippocampal slices to develop multiple population spikes in response to repetitive stimulation of Schaffer collaterals were analyzed. Treatment of wild-type slices with chondroitinase ABC did not change polyspiking activity, whereas TN-R deficient mice exhibited significant increase in the area and numbers of secondary polyspikes. TN-C mutants demonstrated a slight reduction in polyspiking activity during the initial phase of activity-dependent disinhibition. A marginal reduction in the steady-state phase of the polyspiking activity was observed in PST knockout mice. Thus, recognition molecules are likely to be involved in regulation of excitability via multiple mechanisms, which could represent endogeneous processes underlying some aspects of epilepsy.

LIST OF PUBLICATIONS

The present dissertation is mainly based on the following articles and abstracts of poster and oral presentations.

Articles:

1. Eckhardt M, Bukalo O, Chazal G, Wang L, Goridis C, Schachner M, Gerardy-Schahn R, Cremer H, Dityatev A (2000) Mice deficient in the polysialyltransferase ST8SiaIV/PST-1 allow discrimination of the roles of neural cell adhesion molecule protein and polysialic acid in neural development and synaptic plasticity. J Neurosci 20: 5234-5244.

2. Bukalo O, Schachner M, Dityatev A (2001) Modification of extracellular matrix by enzymatic removal of chondroitin sulfate and by lack of tenascin-R differentially affects several forms of synaptic plasticity in the hippocampus. Neuroscience 104: 359-369.

3. Evers MR, Salmen B, Bukalo O, Rollenhagen A, Morellini F, Bösl M, Bartsch U, Dityatev A, Schachner M. Impairment of voltage-gated Ca^{2+} channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C (submitted).

4. Bukalo O, Nikonenko A, Schmidt S, Dityatev A, Schachner M. Shifted threshold for induction of long-term potentiation or depression correlates with the elevated number of perforated synapses in the CA1 region of mice deficient in the extracellular matrix glycoprotein tenascin-R (in preparation).

Abatracts:

1. Bukalo O, Saghatelyan A, Dityatev A, Schachner M (1999) Modification of extracellular matrix in tenascin-R deficient mice or by removal of chondroitin sulfate reduces long-term potentiation in the hippocampus. Phys Research 48:S59.

2. Bukalo O, Dityatev A, Eckhardt M, Gerardy-Schahn R, Cremer H, Schachner M (2000) Genetic dissection of the roles of polysialic acid and NCAM in synaptic plasticity using mice deficient in polysialyltransferase ST8SIAIV/PST-1. Abstr Soc Neurosci 26: 213.11.

3. Dityatev A, Bukalo O, Evers MR, Schachner M (2001) Cell adhesion and extracellular matrix molecules in synaptic plasticity and epileptogenesis. Abstr German Physiol Soc Pflügers Archiv 441: R116.

4. Salmen B, Bukalo O, Dityatev A, Evers MR, Schachner M (2001) Impairment of synaptic plasticity in the hippocampus of tenascin-C deficient mice. Göttingen Neurobiology Report (ed. by N.Elsner and R.Wehner), p.41.

ABBREVIATIONS

ABC	chondroitinase ABC
AC	adenyl cyclase
ACSF	artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
AP-5	2-D,L-aminophosphonovaleric acid
BSA	bovine serum albumin
CaM	Ca^{2+}/calmodulin
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine-3‘,5‘-monophosphate
CAM	cell adhesion molecule
CNS	central nervous system
CS	chondroitin sulfate
CSPG	chondroitin sulfate proteoglycan
DSD-1	dermatan-sulfate-dependent epitope
ECM	extracellular matrix
EGFL	epidermal growth factor-like repeat
E-LTP	early long-term potentiation
Endo-N	endoneuraminidase
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
fEPSP	field excitatory postsynaptic potential
FGF	fibroblast growth factor
FN-III	fibronectin type III domain
GABA	γ-aminobutyric acid
GAG	glycosaminoglycan chain
GPI	glycosylphosphatidylinositol
HB-GAM	heparin-binding growth-associated molecule
HFS	high-frequency stimulation
HNK-1	3‘sulfated glucuronic acid
HSPG	heparin sulfate proteoglycan

Ig	immunoglobulin
IPSP	inhibitory postsynaptic potential
LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
L-LTP	late long-term potentiation
MAG	myelin-associated glycoprotein
MAPK	mitogen-activated protein kinase
mGluRs	metabotropic glutamate receptors
MMP	metalloproteinase
mRNA	messenger ribonucleic acid
NCAM	neural cell adhesion molecule
NMDA	N-methyl-D-aspartate
PBS	phosphate buffered saline
PKA	protein kinase A
PKC	protein kinase C
PNs	perineuronal nets
PSA	polysialic acid
PSA-NCAM	polysialylated neural cell adhesion molecule
PSD	postsynaptic density
PP	protein phosphatase
PPF	paired-pulse facilitation
PST	polysialyltransferase ST8SiaIV
PTP	post-tetanic potentiation
RPTPζ/β	receptor-type protein-tyrosine phosphatase ζ/β
SEM	standard error of mean
STD	short-term depression
STP	short-term potentiation
STX	polysialyltransferase ST8SiaII
TBS	theta-burst stimulation
TN-C	tenascin-C

TN-R	tenascin-R
TLE	temporal lobe epilepsy
tPA	tissue plasminogen activator
VDCC	voltage-dependent calcium channel

CONTENTS

1. INTRODUCTION

Recognition molecules have been identified that are thought to associate with each other in lock-and-key type interactions. Among these are molecules of the immunoglobulin superfamily, integrins, cadherins, semaphorins, laminins, collagens, tenascins, and proteoglycans. These molecules are transmembrane glycoproteins or extracellular matrix molecules. Many of them can exist in multiple forms, either as alternatively spliced or post-translatatorly processed variants.

The spatial distribution of a recognition molecule is a key factor in its attractant or repellent function. Whether these molecules are attractants or repellents depends their environment and the signal transduction machinery triggered by varrious receptors in different cell types and at diverse developmental stages. Adding even more complexity to the function of recognition molecules, they have been shown to interact with each other in many ways. These include interactions within the membrane of the cell or in the extacellular matrix (*cis*-interaction), or between the cell surfaces of neighboring cells and the cell surface and its surrounding extracellular matrix (*trans*-interactions). Such interactions can be either competitive, e.g. that is they can block each other depending on the hierarchy of affinities, or they may enhance each other by conformational changes that can be triggered by the simultaneous occupancy of different sub-domains of recognition molecule by its cognate ligands. Thus, a very complex network of functional associations and dependencies between neuronal recognition molecules needs to be elucidated (Wheal et al., 1998).

Information in the central nervous system (CNS) is encoded through networks of neurons that are functionally connected by synapses – adhesive junctions that are highly specialized for interneuronal signalling (Peters et al., 1991). Brief periods of activity at a synapse can enhance or depress subsequent synaptic strength, a term that describes the magnitude of the postsynaptic response following a sequence of axon terminal depolarization, neurotransmitter release, binding to postsynaptic receptors, and subsequent depolarization of the postsynaptic membrane. The particular frequency of which neural impulses arriving at the axon terminal, coupled with the level of postsynaptic depolarization, dictates wheather synaptic strength is increased or

decreased, as well as the duration of the subsequent synaptic modification (Malenka, 1994). Such dynamic regulation of synaptic strength by neural activity is referred to as "activity-dependent synaptic plasticity" and is a fundamental component of normal brain function. Long-term potentiation (LTP) and long-term depression (LTD) are experimentally induced forms of synaptic plasticity in which a conditioning stimulus (usually electrical stimulation of a set of axons) leads to a rapid and sustained increase (potentiation) or decrease (depression) in synaptic strength, lasting hours to days or longer. LTP has been established as a leading model for memory formation at the cellular level (Bliss and Lomo, 1973). Activity-dependent synaptic plasticity is also crucial for establishing neural circuitry during brain development (Katz and Shatz, 1996) and for reorganizing synaptic circuitry following brain, spinal cord or peripheral nerve injury (Jones et al., 2000). Recognition molecules, which are crucially required for building and maintaining synaptic structure during brain development (Goodman, 1996; Tessier-Lavigne and Goodman, 1996; Kiss et al., 2001), also play important and diverse roles in modulating distinct aspects of synaptic plasticity in maturity (Schachner, 1997; Murase and Schuman 1999; Benson et al., 2000). Tenascins and chondroitin sulfate proteoglycans (CSPGs) are two important classes of molecules of the extracellular matrix (ECM), a complex network of macromolecules (Faissner and Steindler 1995; Jones and Jones 2000). The polysialylated neuronal cell adhesion molecule (PSA-NCAM) belongs to immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs; Field and Itoh, 1996). In the present work the mechanisms through which these molecules participate in the activity-dependent modification of synaptic strength will be discussed.

1.1. ADHESION AND EXTRACELLULAR MATRIX MOLECULES IN THE CNS

1.1.1. TENASCINS

The tenascin family constitutes a group of extracellular matrix proteins displaying a common structure (Fig. 1.1). These molecules contain a linear arrangement of four protein domains: cystein-rich amino-terminal region or tenascin assembly domain, (TA); epidermal growth factor-like repeat (EGFL); the fibronectin type III domains (FN-III), and the fibrogen globe (FG). Cystein-rich amino-terminal region mediates assembly of tenascins into multimers: hexamers of TN-C (Jones and Jones, 2000) and di- and trimers of TN-R (Norenberg et al., 1992). EGFL repeats form an exceptionally compact structure compared with that of epidermal growth factor (Cooke, 1987). The nature and numbers of FN-III domains are altered by alternative mRNA splicing. This can generate a considerable diversity in the functions of tenascins (for review, see Jones and Jones, 2000). Additionally, FN-III repeats are also susceptible to proteolytic degradation by matrix metalloproteinases (MMPs) and serine proteases (Imai et al., 1994), increasing diversity of tenascin glycoproteins. The FG has calcium-binding properties that influence the interaction with other proteins and play a role in cell attachment (for review, see Jones and Jones, 2000).

Currently, five members of the tenascin gene family (TN-C, -R, -X, -Y, and -W) have been identified in diverse species from zebrafish to humans (for reviews, see Bristow et al., 1993; Schachner et al., 1994; Hagios et al., 1996; Weber et al., 1998; Jones and Jones, 2000). Of these, TN-C and TN-R have been reported to be expressed in the CNS (Jones and Jones, 2000). The homology between FN-III repeats of these molecules is about 70% emphasizing the relatedness of both glycoproteins (Pesheva et al., 1989; Fuss et al., 1993).

TN-C and TN-R both express the HNK-1 carbohydrate epitope (Kruse et al., 1984, 1985). The HNK-1 carbohydrate, a 3'sulfated glucuronic acid recognized by a monoclonal antibody (Abo and Balch, 1981), is also carried by many neural recognition molecules, among them: NCAM, L1, F3/F11/contactin, myelin-associated glycoprotein (MAG) and P_0 (Kruse et al., 1984; Faissner et al., 1987; Gennarini et al., 1990), as well as integrins (Pesheva et al., 1987), and CSPGs (Xiao et al., 1997a). The HNK-1 carbohydrate plays pivotal roles in neural development and regeneration (for review,

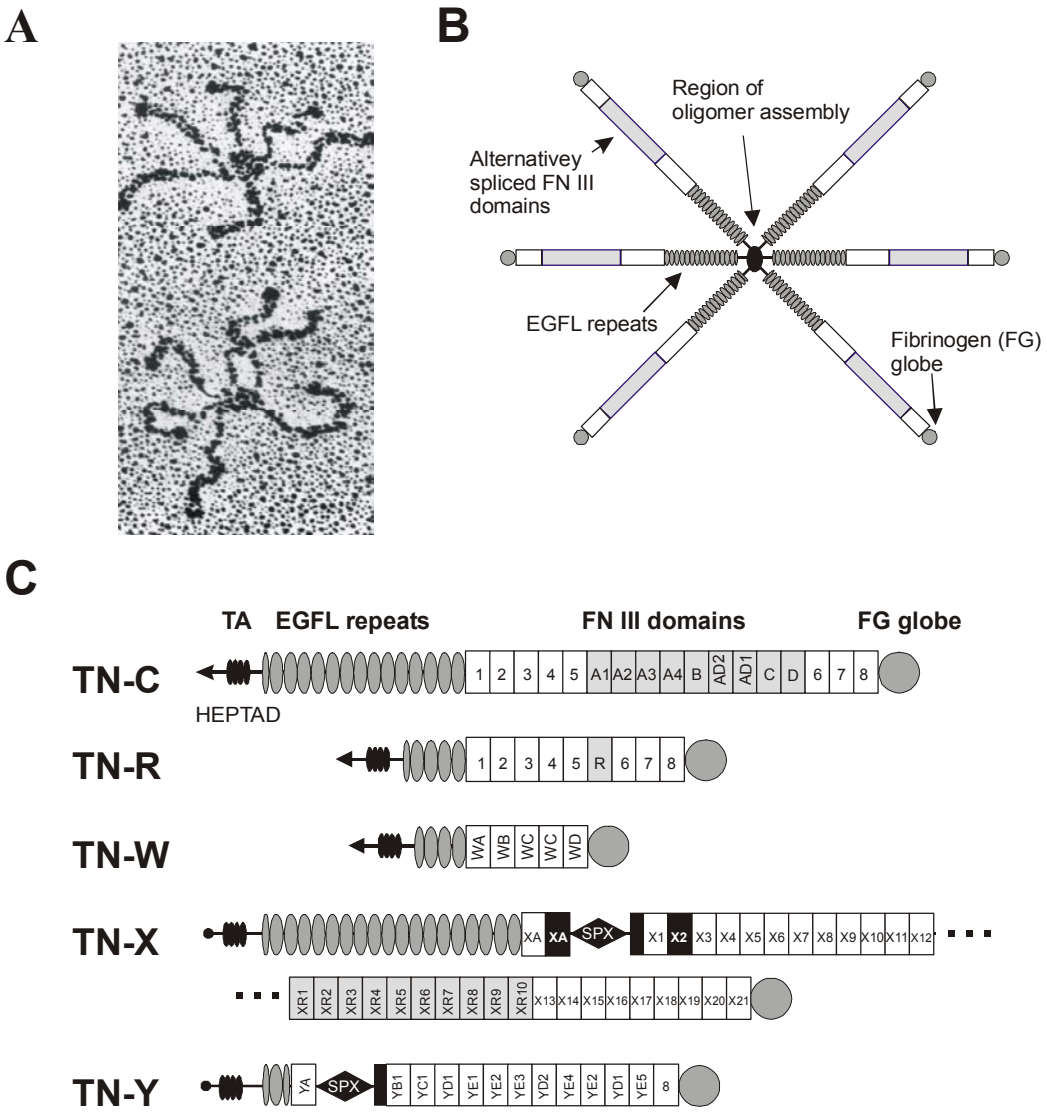


Figure 1.1. Structure of the tenascins.
A: Rotary shadowing image of two mouse TN-C hexabrachions. Each arm is approximately 100 nm in length.
B: Model of the TN-C hexabrachion. The tenascin assembly (TA) domain links six TN-C chains via the heptad repeats (see **C**).
C: Schematic diagrams of thus far described tenascins. Proceeding in a carboxiterminal direction, the domains are as follows: TA domain, an array of EGFL repeats (ovals), two types of FN-III domains: those conserved in all variants of TN-C (white rectangles) and those that are alternatively spliced (gray rectangles), and the terminal fibrogen globe (circle). The figure has been adopted from Jones and Jones (2000).

see Schachner and Martini, 1995). It has recently been reported that TN-R derived from rat brain or cultured oligodendrocytes caries chondroitin sulfate (CS) chains (Probstmeier et al., 2000).

1.1.1.1. Tenascin-C

The first identified member of the tenascin family, TN-C, was discovered independently by several laboratories. Accordingly, it was given a numbers of names including glial/mesenchymal extracellular matrix protein (GMEM) (Bourdon, 1983), myotendinous antigen (Chiquet and Fambrough, 1984a,b), hexabrachion (Erickson and Iglesias, 1984), cytotactin (Grumet et al., 1985), J1220/200 (Kruse et al., 1985), tenascin (Chiquet-Ehrismann, 1986), and neuronectin (Rettig et al., 1989).

Of the tenascins that are detectable in the nervous system, TN-C has been studied in the greatest detail. It is highly conserved through evolution and is most prominently expressed during early postnatal development. It is downregulated after maturation, but persists in restricted areas of the nervous system that exhibit neuronal plasticity, such as the hippocampus (Ferhat et al., 1996; Nakic et al., 1998), cerebellar cortex, retina, optic nerve (Bartsch, 1996), and hypothalamus (Theodosis et al., 1997). TN-C is mainly expressed by immature and reactive astrocytes (Kawano et al., 1995; Bartsch, 1996). Expression of TN-C by some nerve cell types has also been demonstrated. These include immature neurons, such as granule cells in the hippocampus, motoneurons of the spinal cord, and horizontal cells in the developing and adult mouse retina (Kawano et al., 1995; Bartsch, 1996). In the adult rat hippocampus, residual TN-C immunoreactivity is detectable in the *strata oriens* and *radiatum* of the CA1, *stratum oriens* of the CA3 region, alveus, and the molecular layer of the dentate gyrus, where it is most prominent in the hilar region (Nakic et al., 1998).

1.1.1.2. Tenascin-R

TN-R (previously designated J1-160/180 and janusin in rodents and restrictin in chicken; Norenberg et al., 1992; Fuss et al., 1993) appears to be restricted to the CNS. TN-R is synthesized by oligodendrocytes during the process of myelination (Bartsch et al., 1993; Wintergerst et al., 1993). It is detectable at contact sites between

unmyelinated axons, between myelin sheets and is highly accumulated at the nodes of Ranvier (Bartsch et al., 1993). TN-R is also expressed by small subsets of CNS neurons, interneurons and motoneurons in spinal cord, retina, cerebellum and hippocampus (Fuss et al., 1993; Wintergerst et al., 1993; Weber et al., 1999). It is localized at perineuronal nets (PNs) that surround inhibitory interneurons (Celio and Chiquet-Ehrismann, 1994; Wintergerst et al., 1996). PNs appear during the first postnatal weeks, possibly functioning in synaptic stabilization (for reviews, see Celio and Blumcke, 1994; Celio et al., 1998). TN-R immunoreactivity of PNs increases to the adult level between postnatal days 21 and 40 (Bruckner et al., 2000). The lack of TN-R leads to abnormal distribution and shape of PNs (Weber et al., 1999).

TN-R immunoreactivity in the murine hippocampus is rather similar to the expression pattern of the HNK-1 carbohydrate. At the light and electron microscopic levels, HNK-1 carbohydrate immunoreactivity surrounds pravalbumin-positive interneurons scattered in the CA1-CA3 pyramidal cell layers. In the *startum oriens* the density of HNK-1 positive interneurons is increased through CA1 to subiculum (Yamamoto et al., 1988; Ren et al., 1994). In TN-R deficient mice, the HNK-1 immunoreactivity is significantly reduced as compared to the wild-type mice (Weber et al., 1999).

1.1.1.3. Molecular interactions and functions mediated by tenascins

The tenascins bind with high affinities to several proteins and carbohydrates (reviewed by Jones and Jones, 2000). Interactions between tenascins and cell surface molecules affect cell adhesion and neurite growth. The responses can be either stimulatory or inhibitory, depending on the specific neuronal cell type studied and the assay used (choice situation on patterned substrates or homogeneous substrate). Different functions are likely mediated by separate domains. Neurite outgrowth-promoting, cell-binding, antiadhesive, and nonpermissive regions have been identified in TN-R and TN-C (Norenberg et al., 1995).

The EGFL domain primarily functions as a counteradhesive domain, it inhibits attachment of neuronal processes to cellular receptors on other neurons and glia or the ECM produced by these cells (Prieto et al., 1992; Lochter and Schachner, 1993). In TN-

R, EGFL repeats interact with phosphacan and F3/F11/contactin (Xiao et al., 1996, 1997b) and such interactions could be critical for neurite extension and repulsion.

The FN-III repeats within the tenascins have been shown to bind to Ig domains of several CAMs, including neurofascin, TAG-1/axonin-1, F3/F11/contactin, and these binding events modulate homophilic and heterophilic interactions between these Ig-CAMs (Milev et al., 1996; Weber et al., 1996; Volkmer et al., 1998). TN-C also interacts with different types of integrins, mediating cell attachment, inhibition of growth, reorganization of cytoskeleton and cell spreading (reviewed by Jones and Jones, 2000). TN-R and MAG directly bind to each other *in vitro* and MAG is a part of the signalling pathway of TN-R for cell repulsion (Yang et al., 1999). FN-III domains of TN-C interact with and are cleaved by MMPs and serine proteases, thus they are involved in tissue remodeling (Imai et al., 1994). Both, TN-R and TN-C interact with β-subunit of voltage-gated sodium channels. These interaction might provide a mechanism for Na^+-channel localization and regulation of generation and propagation of action potentials (Srinivasan et al., 1998; Xiao et al., 1999). Tenascins interact with other ECM proteins, such as CSPGs (lectican family and phosphacan; Grumet et al., 1994; Aspberg et al., 1997; Milev et al., 1997), HSPGs (heparin sulfate proteoglycans: perlecan, syndecan, glypican, heparin; reviewed by Jones and Jones, 2000), fibronectin (Chiquet-Ehrismann et al., 1991). The interaction of TN-C and TN-R with glycosaminoglycans (GAGs) present on phosphacan and the lecticans influences the extent of interactions of TN-C and TN-R with Ig-CAMs (Milev et al., 1996; Weber et al., 1996; Volkmer et al., 1998).

The multidomain and oligomeric structure of ECM glycoproteins like TN-R and TN-C suggests that they may serve to link cell surface molecules between different cells and to the ECM network.

1.1.2. CHONDROITIN SULFATE PROTEOGLYCANS (CSPGS)

CSPGs form another class of molecules that reside in the ECM. They carry covalentely bound GAG chains containing CSs. GAGs are large unbranched polymers composed of ~20-200 repeating disaccharide units, which are attached to the core proteins through a serine residue and characteristic carbohydate linkage regions (for review, see Bandtlow

and Zimmermann, 2000). CS is a disaccharide unit containing glucuronate and *N*-acetylgalactosamine and has usually one sulfated group per disaccharide, which is predominantly either in the 4^{th} or 6^{th} position on *N*-acetylgalactosamine. Minorities (<10%) of disaccharides are non-sulfated and more rarely there are disulfated or even trisulfated disaccharides. Dermatan sulfate is a structural isomer of CS in which some glucuronate is epimerised to iduronate (Fosang and Hardingham, 1996).

1.1.2.1. Members of the family

Proteoglycans belonging to the agrecan family, or lecticans, represent the largest group of chondroitin sulfate proteoglycans in the nervous system (for reviews, see Fosang and Hardingham, 1996; Yamaguchi, 2000). Aggrecan, versican, neurocan and brevican/BEHAB are the members of this family. They all contain an N-terminal Ig-like domain followed by tandem repeats of a hyaluronan-binding domain and a C-terminal region with EGFL repeats, C-type lectin-like, and complement regulatory protein-like domains (Fig. 1.2; Schwartz et al., 1999; Yamaguchi, 2000). Alternative splicing of transcripts and proteolytic processing of core proteins generate core protein variants (for versican and neurocan).

All four of these proteoglycans are present in the CNS. Aggrecan and versican are distributed in ECM of various nonneuronal tissues as well as nervous tissues, whereas neurocan and brevican show a nervous tissue-specific distribution (Fosang and Hardingham, 1996; Margolis and Margolis, 1997; Schwartz et al., 1999). In the CNS, neurocan and aggrecan are mainly synthesized by neurons. Versican is synthesized by astrocytes and oligodendrocytes. Brevican can occur as soluble isoforms secreted into the extracellular space and as membrane-bound isoforms anchored to the cell surface via a glycosylphosphatidylinositol (GPI) moiety (Yamaguchi, 1996). *In situ* hybridization studies revealed that neuronal cells express the soluble form of brevican, whereas the GPI-anchored brevican is confined to glial cells (Seidenbecher et al., 1998). Striking and distinctive changes in the concentration of the different members of the aggrecan family were observed in the developing rat brain (Milev et al., 1998a), suggesting that the individual proteoglycans and their isoforms serve unique functions during nervous tissue histogenesis and maintenance.

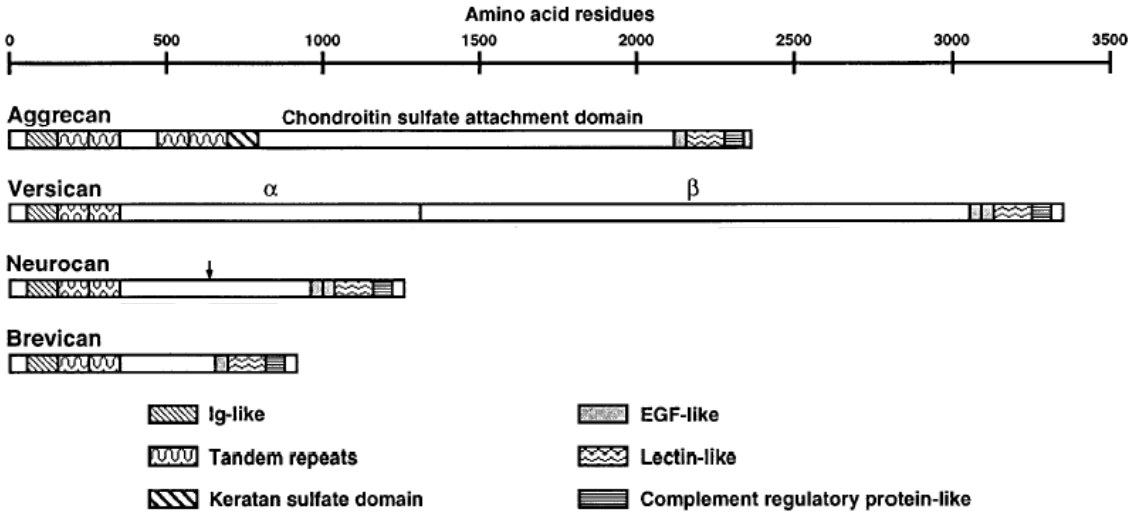


Figure 1.2. The structure of lecticans.
Summary of the primary structures and homology domains of aggrecan, versican, neurocan and brevican. Either or both of the fragments of the versican CSs attachment region designated α and β may be deleted by alternative splicing. The major proteolytic cleavage site of neurocan is indicated by an arrow. The amino acid numbering refers to that of the rat proteoglycans except in the case of versican, where it corresponds to the complete mouse sequence. The figure has been adopted from Milev et al. (1998a).

Phosphacan, also called 6B4-proteoglycan (Maeda et al., 1992) and DSD-1 (dermatan-sulfate-dependent) proteoglycan (Faissner et al., 1994), is an mRNA splicing product that represents the entire extracellular domain of the receptor-type protein-tyrosine phosphatase ζ/β (RPTPζ/β; Maurel et al., 1994; Margolis et al., 1996). Phosphacan (Fig. 1.3) contains an amino-terminal carbonic anhydrase-like sequence (CAH domain) followed by a FN-III repeat and large cysteine-free domain with no homology with other reported protein sequences (Krueger and Seito, 1992). A full-length form (RPTPζ/β or PTPζ-A) and a short form (dvRPTPζ/β or PTPζ-B), in which a part of the serine/glycine-rich region is deleted, have two intracellular phosphatase domains (Barnea et al., 1993; Nishiwaki et al., 1998). RPTPζ/β is predominantly expressed during glial cell differentiation (Engel et al., 1996; Sakurai et al., 1996). However, some studies revealed that neuronal cell types also synthesize phosphacan/RPTPζ/β at least under certain developmental or pathological conditions (Shintani et al., 1998).

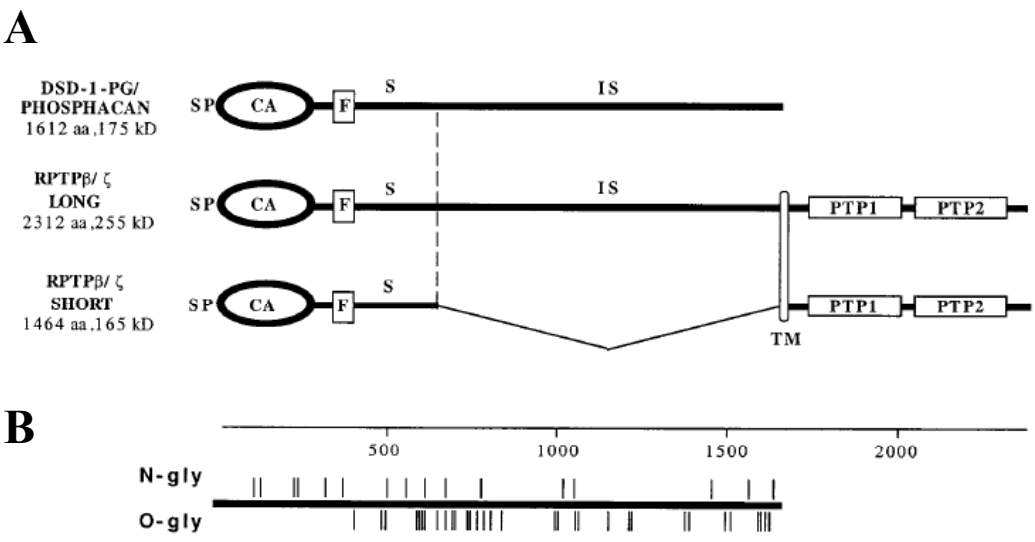


Figure 1.3. Structure of the DSD-1-PG/mouse phosphacan protein (from Garwood et al., 1999).
A: Relationship of DSD-1-PG to RPTPβ/ζ long and short forms. *SP*, Signal peptide; *CA*, carbonic anhydrase domain; *F*, FN-III domain; *S*, remaining extracellular region of short RPTPβ/ζ; *IS*, intervening sequence; *TM*, transmembrane domain; *TP*, tyrosine phosphatase domain.
B: Predicted glycosylation sites of DSD-1-PG/mouse phosphacan: N-glycosylation (*N-gly*) is shown above the line, and O-glycosylation (*O-gly*) below.

1.1.2.2. Binding partners of CSPGs

CSPGs are involved in numerous interactions with other ECM and cell adhesion molecules. Neurocan and phosphacan bind tightly to a variety of Ig-CAMs, including L1/Ng-CAM, NCAM, Nr-CAM, and TAG-1/axonin-1 (Friedlander et al., 1994; Milev et al., 1994, 1996). Neurocan binding to L1 and NCAM depends on CSs (Friedlander et al., 1994). However, CSs are not involved in binding of L1 and NCAM to phosphacan (Milev et al., 1994). CSs are important for interactions of phosphacan, but not neurocan, with TAG-1/axonin-1 (Milev et al., 1996). There is also detectable but considerably less binding of neurocan and phosphacan to F3/F11/contactin (Peles et al., 1995). Other high-affinity ligands of these proteoglycans are TN-C (Grumet et al., 1994; Milev et al., 1997) and TN-R (Aspberg et al., 1997; Milev et al., 1998b). Binding of neurocan and phosphacan to both tenascins has the same apparent dissociation constant of ~3 nM. All members of the lectican family bind to TN-R via their lectin-like domain. These interactions are mediated by the FN-III domains of the protein backbone of tenascin and

are independent of carbohydrates (Aspberg et al., 1997). In addition, lecticans are able to bind to sulfated glycolipids, such as sulfatides (Miura et al., 1999). No significant binding was observed between lecticans and a number of other cell surface and extracellular matrix proteins such as N-cadherin, fibronectin, vitronectin, laminin, merosin, thrombospondin, epidermal growth factor (EGF), and collagens I-VI (Milev et al., 1998b). Interestingly, the interaction of the neurocan with cell-surface glycosyltransferase (GalNAcPTase) coordinately inhibits both N-cadherin- and β1-integrin-mediated adhesion and neurite outgrowth (Li et al., 2000).

Phosphacan also bind to growth factors such as heparin-binding growth-associated molecule (HB-GAM)/pleiotropin, midkine, and amphoterin (Maeda et al., 1996, 1999; Milev et al., 1998b). The binding of neurocan and phosphacan to HB-GAM and amphoterin was largely abolished by chondroitinase treatment, indicating that CS is the likely mediator of their binding to these proteins (Milev et al., 1998b). Furthermore, the core protein of phosphacan has been shown to bind fibroblast growth factor-2 (FGF-2) with high affinity and to potentiate its mitogenic effect. In contrast, neurocan seems to bind FGF-2 but has no modulatory effect on its activity. Binding of phosphacan and neurocan to FGF-2 is reduced by 35 % after chondroitinase treatment of the proteoglycan (Milev et al., 1998c). Although most studies of growth factor interactions with proteoglycans have concerned HSPGs and more specifically their GAG chains, it would appear that the interactions of CSPGs with growth factors also plays a significant role in the developmental processes of the nervous system.

An overlapping localization of CSPGs and most of their binding partners is observed in the CNS (Grumet et al., 1994; Milev et al., 1994, 1996, 1998b, 1998c; Friedlander et al., 1994; Xiao et al., 1997a), suggesting that these molecular interactions occur *in situ* and are involved in various cellular events including cell proliferation, migration, cell adhesion, neurite outgrowth, and pathfinding in the brain development.

1.1.2.3. Functions of CSPGs

Recent studies imply a possible role of CSPGs in restricting structural plasticity in the adult brain. Inhibitory properties for neurite outgrowth of cerebellar granule cells were shown for purified brevican and the brain specific splice variant of versican V2 (Yamada et al., 1997; Niederost et al., 1999). The rather late onset of expression of both

molecules in the postnatal CNS (Milev et al., 1998a) suggests that their capacity to inhibit axonal growth may be linked to the stabilization of the mature neuronal network, rather than to axonal guidance processes.

Immunohistochemical analysis demonstrated that different forms of CSs were found in the adult mammalian CNS in association with PNs surrounding inhibitory interneurons, particularly in the hippocampus and cerebral cortex (Bertolotto et al., 1991; Hartig et al., 1994; Bruckner et al., 1994; Wintergerst et al., 1996; Haunso et al., 1999). PNs are lattice-like accumulations of extracellular matrix components that are associated with different types of neurons in region-specific patterns (for reviews, see Celio and Blumcke, 1994; Celio et al., 1998). The functions of this neuronal microenvironment are a matter of debate. It is discussed wheather PNs are involved in synaptic stabilization (Hockfield et al., 1990), possess a role in neuroprotection (Bruckner et al., 1999) or support ion homeostasis around highly active types of neurons (Bruckner et al., 1993, 1996; Hartig et al., 1999). It has been described in a variety of mammals that PNs develop postnatally, during the period of synaptic refinement, myelination and development of adult-like pattern of physiological activity of neurons (Wintergerst et al., 1996; Koppe et al., 1997a).

Obviously, CSPGs form major components of the nets structure, since PNs are sensitive to chondroitinase ABC treatment (Bertolotto et al., 1991; Celio and Blumcke, 1994; Koppe et al., 1997b). Both TN-C and TN-R are constituents of PNs (Celio and Chiquet-Ehrismann, 1993; Wintergerst et al., 1996). The importance of the interaction between TN-R and CSPGs is underscored by the observation that the distribution and shape of perineuronal nets in TN-R-deficient mice is clearly different from that in wild-type animals. Immunostaining for phosphacan is weak and more diffuse in the mutant when compared with wild-type mice (Weber et al., 1999; Haunso et al., 2000; Bruckner et al., 2000).

Recently, it has been demonstrated that sodium channels interact with RPTPζ/β through both its extracellular carbonic anhydrase homology domain and its intracellular phosphatase domain. The association of RPTPζ/β with sodium channels could reverse the functional effect of tyrosine phosphorylation, modulating sodium channels function (Raticliffe et al., 2000). The C-terminal sequence of RPTPζ/β binds to the PSD-

95/SAP90 family through the second PDZ domain. Furthermore, RPTPζ/β and PSD-95/SAP90 are similarly distributed in the dendrites of pyramidal neurons of the hippocampus and neocortex and RPTPζ/β is concentrated in the postsynaptic density (PSD) fraction (Kawachi et al., 1999). These results suggested that RPTPζ/β is involved in the regulation of synaptic function as postsynaptic macromolecular complexes with PSD-95/SAP90 and sodium channels. Interactions with different ligand molecules (see above) could modify synaptic formation and plasticity by regulating RPTPζ/β activity through modification of the tyrosine phosphorylation levels of channel molecules directly and/or other protein at the synapses.

1.1.3. NEURAL CELL ADHESION MOLECULE (NCAM)

NCAM is a member of a large family of cell surface glycoproteins which includes MAG, P_0, L1/Ng-CAM, CHL1, TAG1/axonin-1, F3/F11/contactin, Nr-CAM, neurofascin (for reviews, see Doherty et al., 1995; Field and Itoh, 1996). They share structural motifs related to Ig and FN-III domains (Fig. 1.4). All of these glycoproteins are expressed in the developing nervous system, where they have been postulated to play a role in axon growth or fasciculation, or both (for reviews, see Goodman et al., 1996; Kiss et al., 2001).

1.1.3.1. Structure of NCAM

The extracellular part of NCAM has five Ig-like domains, termed Ig1-5, and two FN-III homology modules. By alternative splicing three main isoforms of NCAM, namely NCAM-180, NCAM-140 and NCAM-120 (according to their approximate molecular weight) arise. NCAM-180 and NCAM-140 are transmembrane forms differing in their cytoplasmic domains, whereas NCAM-120 is attached to the membrane via a GPI anchor (for reviews, see Doherty et al., 1995; Ronn et al., 2000). In addition, soluble forms of NCAM, generated by truncation, proteolysis or shedding exist (Olsen et al., 1993).

In addition to the diversity generated by alternative splicing, NCAM can be subjected to different post-translational processing. For instance, NCAM contains O-linked carbohydrates (Walsh et al., 1989), HNK-1 epitopes (for review, see

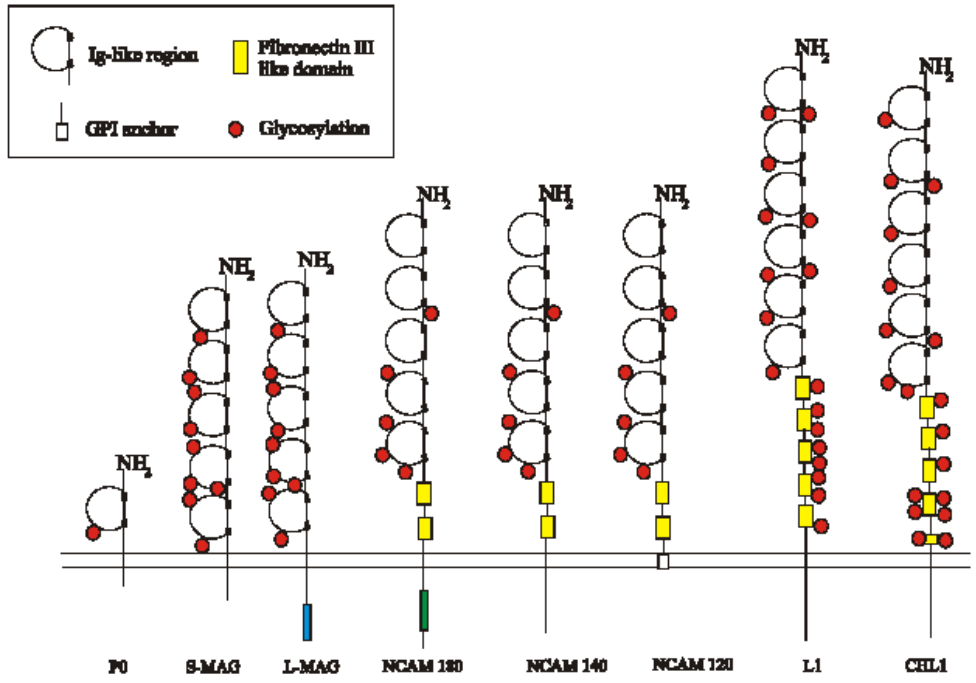


Figure 1.4. Examples of cell adhesion molecules of the immunoglobulin superfamily (Ig-CAMs).
The Ig-CAMs consist of an extracellular domain with Ig-like domains and FN-III repeats, a single transmembrane region or a GPI anchor and in most cases an intracellular domain.

Schachner and Martini, 1995), and polysialic acid (PSA). PSA is attached uniquely to NCAM through developmentally regulated process, known to modulate functional properties of NCAM (for reviews, see Rutishauser and Landmesser, 1996; Kiss and Rougon, 1997; Muhlenhoff et al., 1998).

1.1.3.2. Biosynthesis of PSA

PSA is a linear homopolymer of α2,8 linked sialic acid residues. The unique structure of this carbohydrate allows for its specific recognition by monoclonal antibodies (anti-PSA) and by a phage-derived endoneuraminidase (endo-N).

NCAM's fifth Ig domain contains three potentially polysialylated asparagine (Asn) residues at positions 404, 430 and 459 (Fig. 1.5). Although PSA is located within Ig domain 5, the fourth Ig domain, the first FN-III domain and a membrane attachment

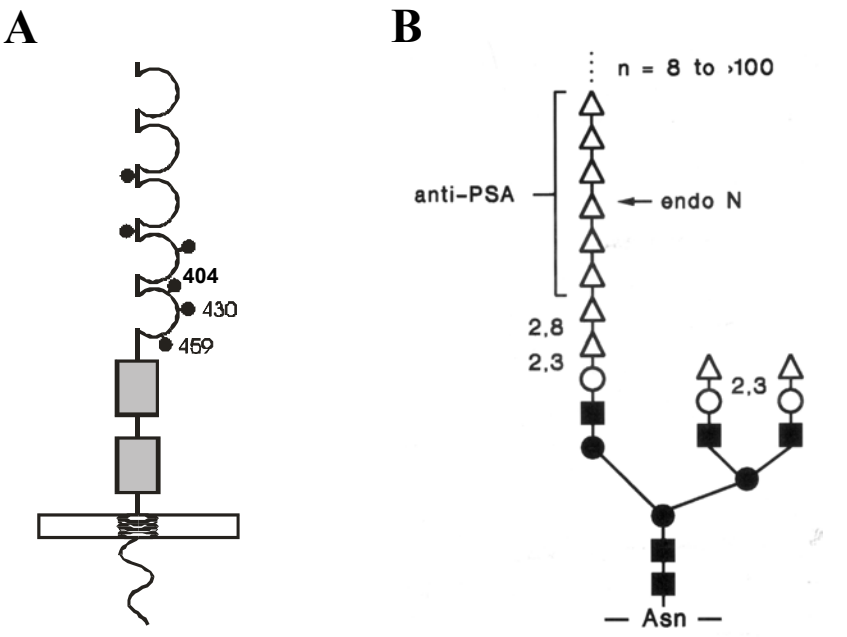


Figure 1.5. Structure of PSA-NCAM.
A: Schematic diagram showing *N*-glycosylation sites of Ig domains. The fifth Ig-like domain of NCAM contains three potentially polysialilated asparagine (Asn) residues at positions 404, 430 and 459.
B: Structure of polysialic acid attached to NCAM via a typical *N*-linked core glycosylation. The unique structure of the α2,8-linked polymer allows for its specific recognition by monoclonal antibodies and by phage-derived endoneuraminidase (endo-N). Open triangles, sialic acid; open circles, galactose; closed squares, glucosamine; closed circles, mannose. The figure **B** from Rutishauser and Landmesser (1996)

also play a role in polysialylation (Nelson et al., 1995).

Addition of PSA to NCAM takes place in the *trans*-Golgi compartment as a regular step in the biosynthetic pathway of protein glycosylation in eukaryotes (Scheidegger et al., 1994). The exact numbers of enzymes involved in PSA biosynthesis is still under discussion, but *in vitro* experiments strongly suggest that at least two polysialyltransferases, ST8SiaII (STX; Kitagawa and Paulson, 1994; Kojima et al., 1995) and ST8SiaIV (PST; Nakayama et al., 1995; Eckhardt et al., 1995) catalyze the entire reaction. PST and STX are highly homologous to each other and have 59% identity at the amino acid level (Eckhardt et al., 1995). Two enzymes are markedly different with respect to their spatial and temporal expression patterns (Kurosawa et al., 1997; Phillips et al., 1997; Hildebrandt et al., 1998; Ong et al., 1998). In mouse and rat, both PST and STX are expressed in early stages of development, reaching maximum

level just before birth. In mouse, approximately 10 days after birth, STX is dramatically decreased while PST is just moderately decreased during development (Angata et al., 1998; Nakayama et al., 1998). These enzymes seem to be the regulators of PSA-NCAM synthesis and their activity is controlled at the mRNA level (Eckhardt et al., 1995).

The expression pattern of PSA-NCAM in the developing nervous system is highly dynamic and the polysialylation of NCAM reveal to be independently of the expression of NCAM (Rougon, 1993). Whereas in the early development (in mice up to embryonic days 8 and 9) NCAM does not carry PSA, PSA-NCAM become predominant at later stages and reaches a maximum in the perinatal phase. However, postnataly the amount of PSA progressively declines, and only a minor fraction of NCAM remains in its polysialylated state (for review, see Rutishauser and Landmesser, 1996). In the adult brain, PSA-NCAM remains expressed in neuronal populations showing ongoing neurogenesis, cell migration, axonal outgrowth, and synaptic plasticity. Examples are the rostral migratory stream (Lois et al., 1996), the hippocampal formation (Seki and Arai, 1993), and the hypothalamic nuclei (Theodosis et al., 1991).

1.1.3.3. Functions of PSA-NCAM

There is increasing evidence that PSA-NCAM plays a role in nerve fasciculation, axon branching and formation of synapses (for review, see Rutishauser and Landmesser, 1996), promotes cell migration and enhances neurite outgrowth and branching during development and neural regeneration (for review, see Kiss and Rougon, 1997). The developmental expression of PSA-NCAM might contribute to the establishment of specific patterns of synaptic connections. Polysialic acid is thought to modulate the functional properties of NCAM by reducing its “self-adhesiveness”, namely homophilic binding (Hoffman and Edelman, 1983) or its binding to other cell surface molecules (heterophilic binding; Kadmon et al., 1990), except for HSPGs (Storms and Rutishauser, 1998). In other words, PSA-NCAM is believed to represent a less adhesive form of NCAM, thus facilitating the synaptic/structural reorganization that occurs during brain development and under conditions of synaptic plasticity in adults (Rutishauser and Landmesser, 1996).

Another role of PSA has been suggested on the basis of data obtained with the use of atomic force microscopy applied to image PSA and PSA-NCAM. Surprisingly, formation of filament bundles that assemble into a network has been found for oligomers of sialic acid having more than 12 residues and biochemically isolated PSA-NCAM (Toikka et al., 1998). Since the length of the PSA chains in the developing brain undergoes temporal and topical modulation, the observed length-dependent formation of filament bundle networks may offer a molecular mechanism for the modulation of cell environment. Evidences the extracellular cleavage of PSA-NCAM in a synaptic activity-dependent manner (Hoffman et al., 1998; Endo et al., 1999) suggests that PSA-NCAM might be involved in regulation of ECM during synaptic plasticity.

1.2. SYNAPTIC TRANSMISSION AND PLASTICITY IN THE HIPPOCAMPUS

1.2.1. The structure of the hippocampus

The hippocampus, named for its resemblance to the sea horse (*hippo* = horse, *kampos* = sea monster; Greek), is among the best-characterized cortical structures. Its highly regular organization is ideally suited for anatomical and physiological investigations. Neuropsychological studies suggest that the hippocampus plays a key role in certain aspects of learning and memory (for reviews, see Squire and Zola-Morgan, 1991; Deweer et al., 2001).

Interest in the hippocampus also derives from the fact that pathophysiology in this structure can have serious clinical consequences. For example, the hippocampus is a target of degenerative disorders such as Alzheimer`s disease (Carr et al., 1997). In addition, it is relatively seizure-prone and is commonly involved in temporal lobe epilepsy (TLE). Therefore, the cellular mechanisms underlying normal and abnormal forms of synchronization of hippocampal neuronal activity have been investigated intensively (Wong et al., 1984; Traub et al., 1989; McBain et al., 1999). The neuropharmacology of the hippocampus is thus relevant to the search for better anticonvulsants and other therapeutic drugs.

The hippocampus proper can be divided into four regions, which have been traditionally designated CA1 to CA4 (from the Latin *cornu Ammon*, or Ammon`s horn, because of its resemblance to a ramn`s horn; Fig. 1.6). The *dentate gyrus*, the *subiculum*, and the

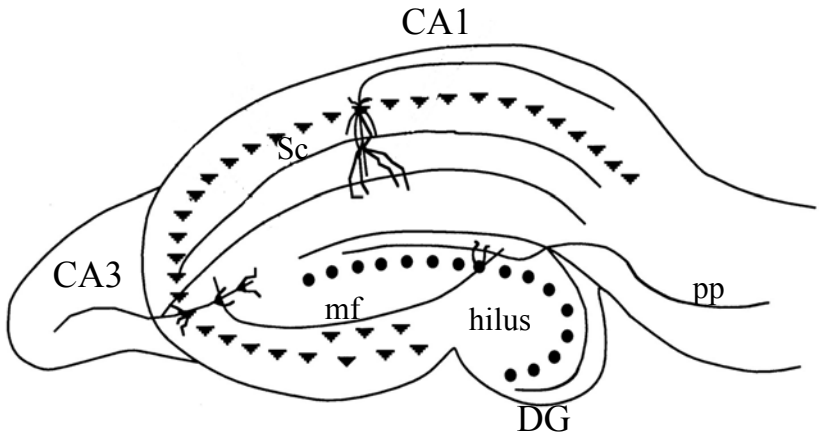


Figure 1.6. The structure of hippocampus.

For clarity, only a single neuron is shown in its entirety (i.e. somata, dendrites and axon) in each subregion. Information from the entorhinal cortex enters the hippocampus through the perforant path (pp), which terminates on the dendrites of granule cells in the dentate gyrus (DG). The axons of the granule cells – the mossy fibres (mf) – relay information to the dendrites of pyramidal cells in area CA3. Information is subsequently relayed by the axons of area CA3 neurons – the Schaffer collaterals (Sc) – to the dendrites of pyramidal neurons in area CA1.

entorhinal cortex are included in the more general term *hippocampal formation* or *hippocampal region*. The CA1 and CA3 region constitute most of the hippocampus proper. The area between the dentate gyrus and *stratum granulosum* of the CA3 region is called the *polymorphic* or *hilar region*, or simply the *hilus*.

Both the hippocampus and dentate gyrus are three-layered cortices (Brown and Zador, 1990). The principal cell type, the pyramidal cell, is the major component of the *stratum pyramidale*. Underneath to the *stratum pyramidale* is the *stratum oriens*, into which the basal dendrites of the pyramidal cells descend and which contains a number of non-pyramidal neurons. The largely acellular region superficial to the pyramidal cell layer, where the apical dendrites of the pyramidal cell layer are located, is divided into a deeper *stratum radiatum* and a more superficial *stratum lacunosum-moleculare*. In CA3, the region just above the pyramidal cell layer contains the mossy fibers from the dentate gyrus and is called the *startum lucidum*. The mossy fiber axons arise from the granule cells of the dentate gyrus. These axons are unmylienated and tend to course in

fascicles. The mossy fiber synaptic expansions ("giant boutons") are among the largest presynaptic structures in the brain (Claiborne et al., 1993).

The pyramidal cells of CA3 have highly colateralized axons that contribute to associational projection that terminate within CA3, and give rise to the major projection to CA1, the so-called Schaffer collaterals (Amaral and Witter, 1989).

The principal cells (pyramidal and granule cells) represent 90 % of hippocampal neurons, and their intra- and extrahippocampal connections convey information arriving from and heading to extrahippocampal areas. The remaining 10 % of hippocampal cells are GABA(γ-aminobutyric acid)ergic interneurons (for review see Freund and Buzsaki, 1996). These later cells form a well-organized neuronal network which controls and regulates the operation of principal cells.

Interneurons have extensive axonal arbors and innervate pyramidal cells multiple times. Such an interneuronal network modulates the membrane potential of principal cells, keeping these cells just below the firing threshold so that they fire if they received an additional excitatory input – for example from the entorhinal cortex. It has been suggested that synchronizing spatially distant principal cells is a major function of interneurons, and that co-activation of neuronal populations serves as the basis for the representation of information (for review, see Buzsaki and Chrobak, 1995). Based on their morphological and functional properties, two board classes of GABAergic neurons can be distinguished: dendritic-projecting interneurons, that control the input of principal cells and the propagation of calcium currents from the dendrite to the soma (dendritic inhibition), and interneurons that selectively innervate soma of pyramidal cells, which control the generation of sodium spikes, and thus the output of principal cells (perisomatic inhibition; Miles et al., 1996).

In the CA1 region of the hippocampus, pyramidal neurons receive both feed-back and feed-forward inhibition in response to activation of Schaffer collaterals. In the feed-forward regulatory system, afferent volleys directly activate the inhibitory neurons (first event) that in turn reduce the probability of firing of the principal cells (second event). In the feed-back system, an excitatory input discharges the principal cells (first event), whose excitatory output feeds back to the inhibitory cells through recurrent axon collaterals (second event). The inhibitory neuron(s) then may discharge and inhibit a

group of principal cells, including those that initially activated the interneuron(s) (third event; for review, see Freund and Buzsaki, 1996).

1.2.2. Basal synaptic transmission in the CA1 region of the hippocampus

The excitatory amino acid glutamate is the most prevalent transmitter in the brain, and it is very important in the physiology of the hippocampal formation. Glutamate elicits its effects through ionotropic and metabotropic receptors. Ionotropic glutamate receptors (iGluRs) mediate the fast synaptic action of glutamate. They have traditionally been classified into three types, mainly based on pharmacological and biophysical properties: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors, high-affinity kainate receptors, and NMDA (N-methyl-D-aspartate) receptors. Ionotropic GluRs are composed of subunits, and for each receptor type several subtypes exist that differ in subunits composition and functional properties (for reviews, see Sprengel and Seeburg, 1995; Vizi and Kiss, 1998).

AMPA receptors are ion channels mostly permeable to Na^+ and K^+ ions, while NMDA type channels are additionally highly permeable to Ca^{2+} ions. AMPA receptor channels are opened upon glutamate binding and are mostly responsible for mediating fast excitatory synaptic transmission. Although the affinity of NMDA receptors to glutamate is even higher then for AMPA receptors, activation of NMDA receptors also requires concurrent postsynaptic depolarization to release a voltage-dependent Mg^{2+}- block in the receptors (for review, see Ozawa et al., 1998).

Accumulating evidence indicates that the postsynaptic kainate raceptors mediate stimulatory effects, whereas presynaptic kainate receptors (autoreceptors) reduce the release of glutamate from interneurons (Vizi and Kiss, 1998).

Metabotropic receptors (mGluRs) appear to play a more important role in the control of transmitter release in the hippocampus. At least eight subtypes of mGluRs have been identified and cloned. These have been separated into three groups based on sequence homology, transduction mechanisms, and pharmacological profiles. Group I (mGluR1 and mGluR5) stimulate inositol phosphate metabolism and mobilization of intracellular Ca^{2+} through the stimulation of phospholipase C (PLC), whereas group II (mGluR2 and

mGluR3) and group III (mGluR4 and mGluR6-8) act to inhibit adenyl cyclase (AC; for review, see Ozawa et al., 1998).

Fast inhibitory neurotransmission in the hippocampus is mediated by GABAergic interneurons, which form a highly interconnected network regulating the excitability of principal neurons. GABA binds to two types of receptors, the $GABA_A$ receptors, which are ion channels mostly permeable to chloride, and $GABA_B$ receptors, which activate slow potassium currents by a G-protein-dependent mechanisms.

Under normal conditions, activations of afferent pathways to hippocampal pyramidal neurons produces a short-latency excitatory postsynaptic potential (EPSP), which may be large enough to evoke a single action potential. EPSP is rapidly followed by a biphasic inhibitory postsynaptic potential (IPSP), due to $GABA_A$ mediated chloride current ($IPSP_A$) followed by slower $GABA_B$ mediated potassium current ($IPSP_B$). The IPSPs reduce the amplitude and duration of EPSPs and thus prevent NMDA receptor-mediated currents from contributing to low frequency synaptic transmission at resting membrane potentials. At the network level patterned GABAergic inhibition coordinates the input-output efficacy of the pyramidal neurons as well as spatial and temporal integration in hippocampus (for review, see Freund and Buzsaki, 1996).

1.2.3. Relationship of disinhibition to epilepsy

When interneurons are serially connected, it is assumed that increased activity of the primary interneuron will lead to increased firing of the target of the secondary interneuron through a process generally referred to as “disinhibition” (Freund and Buzsaki, 1996).

Repetitive stimulation at relatively low frequencies has been shown to result in a gradual increase in the excitability of hippocampal pyramidal cells, as shown by transition of the neuronal firing pattern from single spikes to multiple-spike bursts, synchronized throughout the population. Coincident with the development of burst discharge there is a large reduction in the amplitude of IPSP (Thompson and Gahwiller, 1989). This activity-dependent depression could potentially have effects on excitatory transmission similar to those seen after pharmacological suppression of inhibition. Disinhibition may increase excitatory interaction reducing the voltage-dependent block

of NMDA receptors by Mg^{2+} ions and increasing the probability that an EPSP will generate an action potential in the postsynaptic cell (Wigstrom and Gustafsson, 1983).
The similarity of these bursts to epileptioform discharge, and the ability to evoke them with physiologically relevant stimulation frequencies and without pharmacological intervention, suggests that depression of the IPSP that follows repetitive stimulation, or activity-dependent disinhibition, may represent a significant endogeneous process underlying some aspects of epilepsy (Thompson and Gahwiller, 1989).

1.2.4. Activity-dependent changes in synaptic efficacy in the hippocampus

A remarkable feature of neuronal communication is its dependence on previous activity of contacting cells. Even a single pulse delivered to a neuron can modulate the efficacy of the next pulse delivered within a certain interval (usually tens or hundreds ms). If the efficacy of synaptic transmission is increased on the second pulse, the phenomenon is called paired-pulse facilitation (PPF). If it is decreased, the term paired-pulse depression (PPD) is used. PPF is usually thought to result from a transient increase in intracellular $[Ca^{2+}]$ triggered by first stimulation in presynaptic terminals (Zucker, 1973; Kuhnt and Voronin, 1994; Kamiya and Zucker, 1994). The increase in $[Ca^{2+}]_i$ results in the increase in probability of neurotransmitter release (p) in a stimulated synapse. Therefore, PPF is mostly a presynaptically mediated phenomenon (Zucker, 1973; Kuhnt and Voronin, 1994). Due to a non-linear (S-shaped) dependence of p on $[Ca^{2+}]_i$, the magnitude of PPF corresponds to p in physiological range of values in an inversely related manner: a small PPF corresponds to a high probability of release and vice versa. However, PPF is also masked by the efficacy of recurrent inhibition (Nathan and Lambert, 1991), desensitization of receptors (Wang and Kelly, 1997) and reduction in availability of vesicles in stimulated presynaptic terminals (Debanne et al., 1996). In case of inhibitory GABAergic synapses, activation of presynaptic $GABA_B$ receptors by the released GABA is a dominant factor, resulting in PPD due to downregulation of presynaptic Ca^{2+} channels and, hence, reduction in p.
Trains of stimuli could evoke longer (for several minutes) increase in synaptic efficacy due to the persistence of Ca^{2+} in the presynaptic terminal (Fisher et al., 1997). This phenomenon, called post-tetanic potentiation (PTP) is, however, often superimposed

with postsynaptic NMDA receptor-dependent modifications. Therefore, the operational term of short-term potentiation (STP) will be used in this study to refer to a transient increase in synaptic efficacy (regardless of pre- or postsynaptic origin) after titanic stimulation. Similarly, short–term depression (STD) is a transient decrease in synaptic efficacy.

In addition to above-mentioned short-term forms of synaptic plasticity, activity could also induce long-term changes in synaptic efficacy, which appear to be critical for the development of appropriate neuronal circuits and for many forms of neuronal plasticity, including learning and memory. In 1973, it was discovered that brief tetanic stimulation produced a long-lasting form of synaptic plasticity, LTP, that can lasts for hours or days in the mammalian hippocampus (Bliss and Lomo, 1973). Just before that the involvement of the hippocampal formation in memory was established by clinical data indicating that lesion of this structure in humans produce anteriograde amnesia (Milner, 1972). Many laboratories have been studying LTP as a cellular model for information storage in the brain. Although the relation of LTP to learning is not generally accepted (e.g. see Mayford et al., 1996 in favor and Zamanillo et al., 1999 against it), LTP is a widely used paradigm for long-term synaptic plasticity in a central synapse.
The first demonstration of LTP, elicited by brief repetitive stimulation, was at synapses made between perforant path fibers and granule cells in the dentate gyrus of the hippocampus (Bliss and Lomo, 1973). Subsequent studies established that LTP could be elicited at the Schaffer collateral/commisural synapses in the CA1 region of the hippocampus (Schwartzkroin and Wester, 1975) as well as at the mossy fibers synapses in the CA3 region of the hippocampus (Alger and Teyler, 1976). Numerous reviews concerning the neuronal mechanisms of both NMDA-dependent and NMDA-independent LTP have been published, which are describing in details the pre- and postsynaptic mechanisms, signal transduction pathways, and molecular mechanisms (Bliss and Collingridge, 1993; Nicoll and Malenka 1995; Malenka and Nicoll 1999). Depending on the induction protocol, several forms of plasticity can be elicited (Linden and Connor, 1995): homosynaptic (modulation is observed in the same synaptic input that was stimulated during induction), heterosynaptic (modulation is observed in

another input that was stimulated during induction), and associative (co-stimulation of two inputs is used to modulate at least one of them).

The activity-dependent decrease in the strength of synaptic transmission referred to as LTD was also described in the hippocampus (first discovered in the CA1 *in vitro* by Lynch et al., 1977) as well as in other brain structures (Ito, 1989; Linden et al., 1991). LTD is widely agreed to be a useful, if not necessary, counterpart of LTP, in part to maintain synaptic strengths within a dynamic range (Christie et al., 1994). Like its LTP counterpart, it may be NMDA-receptor dependent or independent (for review, see Martin et al., 2000).

1.2.5 Induction of LTP and LTD in the CA1 region

The majority of experimental work has focused on the LTP observed at the Schaffer collateral synapses in the CA1 region of the hippocampus. Homosynaptic LTD also could be readily induced at the same synapses. These forms of plasticity at Schaffer collateral synapses reflect Hebbian properties: specificity - generally only stimulated input is facilitated or depressed (hence the name homosynaptic LTP and LTD); cooperativity (stimulation of several afferents may be required to see potentiation or depression); and associativity (synapses are strengthened or weakened if there is both presynaptic activity and substantial postsynaptic depolarization) (reviewed by Dudek and Bear, 1992; Bliss and Collingridge, 1993; Linden and Connor, 1995).

A commonly used protocol for inducing LTP is high-frequency afferent stimulation (HFS; classically 100Hz/1s) or stimulation at theta frequency (theta-burst stimulation, TBS; usually 10 bursts of 4 shocks at 100 Hz at 200 ms intervals). Homosynaptic LTD in Schaffer collateral-pyramidal cell synapses of the area CA1 induced by prolonged (2-15 min) low-frequency (1-5 Hz) afferent stimulation. Pairing of low-frequency stimulation (LFS, 1-5 Hz) of presynaptic fibers with the depolarization of the postsynaptic neuron is also widely used protocol ("pairing protocol") for LTP and LTD induction.

There is a wide agreement that NMDA receptor-dependent LTP is triggered by the influx of Ca^{2+} through NMDA receptors during depolarization associated with repetitive synaptic activation. The requirement for postsynaptic depolarization is due to the

properties of the NMDA channel. To open these channels, the Mg^{2+} block of them must be relieved by depolarization. Once NMDA channels open, they allow the influx of Ca^{2+} that triggers synaptic strengthening (Bliss and Collingridge, 1993).

LTD is also dependent on an increase in intracellular [Ca^{2+}], and in many cases requires Ca^{2+} entry through NMDA receptors (Bear, 1995). Although the NMDA receptors may be the critical entry point for Ca^{2+} involved in the triggering LTP and LTD, under most experimental conditions, raising $[Ca^{2+}]_i$ by activation of voltage-dependent calcium channel (VDCCs) or release Ca^{2+} from internal stores (Grover and Teyler, 1992; Huber et al., 1995; Wilsch et al., 1998; Morgan and Teyler, 1999) can also cause an increase or decrease in synaptic efficacy, suggesting that the source for the increase in $[Ca^{2+}]_i$ may not be critically important (Bear and Malenka, 1994; Oliet et al., 1997). Thus, NMDA receptors activation (and postsynaptic depolarization) leads to Ca^{2+} entry and a localized increase in the free $[Ca^{2+}]_i$. This increased $[Ca^{2+}]_i$ (from either source) would then activate signaling pathways responsible for increasing or decreasing of postsynaptic strength.

1.2.6. LTP in CA3 region of hippocampus

Hippocampal CA3 pyramidal cells receive two sets of excitatory inputs, the associational/commisural afferents from other CA3 cells and mossy fiber synapses from dentate granule cells (Amaral and Witter, 1989). Mossy fiber synapses have several unusual structural features, including large terminals, multiple release sites and a proximal termination zone along the apical dendrites of CA3 neurons (Claiborne et al., 1986; Chicurel and Harris, 1992).

The first indication that mossy fiber synapses might have different functional properties than other excitatory synapses in the hippocampus came from the observation that, unlike most areas of the hippocampus, NMDA receptor binding was very low in *stratum lucidum*, the termination zone of mossy fibers (Monaghan and Cotman, 1985). Soon afterwards it was found that NMDA receptor activation is not involved in the induction of LTP at this synapse (Harris and Cotman, 1986).

Mossy fiber LTP can be induced independently of postsynaptic activity, thus, it is thought to be triggered entirely within the presynaptic terminal (Zalutski and Nicoll,

1990). The induction of mossy fiber LTP appears to be insensitive – or at least less sensitive – to buffering of postsynaptic calcium (Williams and Johnston, 1989). Finally, mossy fiber LTP expression clearly interacts with PPF, a presynaptic process (Zalutski and Nicoll, 1990).

Other reports, have suggested that the induction of mossy fiber LTP, similar to NMDA receptor-dependent LTP, depends on an increase in postsynaptic [Ca^{2+}] and is regulated by the postsynaptic membrane potential (Jaffe and Johnston, 1990; Yeckel et al., 1999). More recently, it has been suggested that NMDA-receptor-independent LTP at mossy fiber synapses should be subdivided into two forms, depending on the duration of the stimulus train used for induction: brief trains of high-frequency stimulation applied to the mossy fibers (B-HFS) induce a form of LTP that depends on an initial postsynaptic step, whereas long trains (L-HFS) elicit presynaptically induced LTP (Urban and Barrionuevo, 1996).

The simple (although not universally held – see Yeckel et al., 1999) view of LTP in this system is that high-frequency firing of presynaptic afferent fibers triggers a rise in cAMP in presynaptic terminals, which leads to a persistent enhancement of transmitter release probability (Nicoll and Malenka, 1995). Although the induction mechanisms are still unresolved, there is a general agreement that maintenance or expression of mossy fiber LTP is based on presynaptic mechanisms (Xiang et al., 1994; Castillo et al., 1997).

1.2.7. Signal transduction mechanisms in LTP and LTD

Three phases of LTP can be distinguished: initial (PTP and STP), early LTP (E-LTP) and late LTP (L-LTP). The first stage of LTP, generally referred as short-term potentiation, is independent of protein kinase activity in its induction/expression and it is a prelude to E-LTP and L-LTP. E-LTP is mediated by persistently activated protein kinases and starts at around 30 min or less and is over by ~2-3 hr. L-LTP is dependent for its induction on changes in the gene expression and lasts many hours (for reviews, see Huang et al., 1996; Abel and Kandel, 1998; Sweatt, 1999). A review of the literature generates an enormous list of candidate signal transduction molecules, which includes several Ca^{2+}-dependent protein kinases, especially Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), protein kinase A (PKA), mitogen-

activated protein kinase (MAPK), tyrosine kinases (src and fyn kinases), and protein phosphatases (PPs) (Fig. 1.7; Malenka and Nicoll, 1999; Soderling and Derkach, 2000; Dineley et al., 2001).

Overwhelming evidence implicates CaMKII as a key component of the molecular machinery of LTP and LTD in CA1 (for reviews, see Malenka and Nicoll, 1999; Fukunaga and Miyamoto, 2000; Lisman and McIntyre, 2001). An important property of CaMKII is that when autophosphorylated on Thr^{286}, its activity is no longer dependent on Ca^{2+}/calmodulin (CaM). This allows its activity to continue long after the Ca^{2+} signal returned to baseline. Autophosphorylation of CaMKII is necessary for LTP and learning (Giese et al., 1998), and serves to translocate the kinase to the PSD (Strack et al., 1997). Since autophosphorylation is controlled by PPs (Blitzer et al., 1998) and thus requires convergence between several activated signaling routes, CaMKII has been proposed to act as a molecular coincidence detector in synaptic plasticity. Both LTP and LTD are dependent on activity of CaMKII (Mayford et al., 1995). The targets phosphorylated by CaMKII in LTP include synapsin I, microtubule-associated protein 2 and AMPA-receptors (Fukunaga et al., 1996; Nayak et al., 1996; Barria et al., 1997).

Certain protocols of LTP and LTD induction involve activation of mGluRs, which may activate PKC via G-protein dependent mechanisms. The inhibitors and activators of PKC prevent LTP and potentiate synaptic transmission, respectively (for review, see Roberson et al., 1996). LTD in the CA1 is also sensitive to antagonists of mGluRs and could be chemically induced by application of mGluRs agonists (for review, see Bortolotto et al., 1999). The putative substrates of PKC include AMPA and $GABA_A$ receptors (Roche et al., 1996; Poisbeau et al., 1999). PKC can be converted to the autonomously active form by proteolitic cleavage of its regulatory domain, and such a persistent PKC activation has been suggested to be important for LTP maintenance (for review, see Roberson et al., 1996).

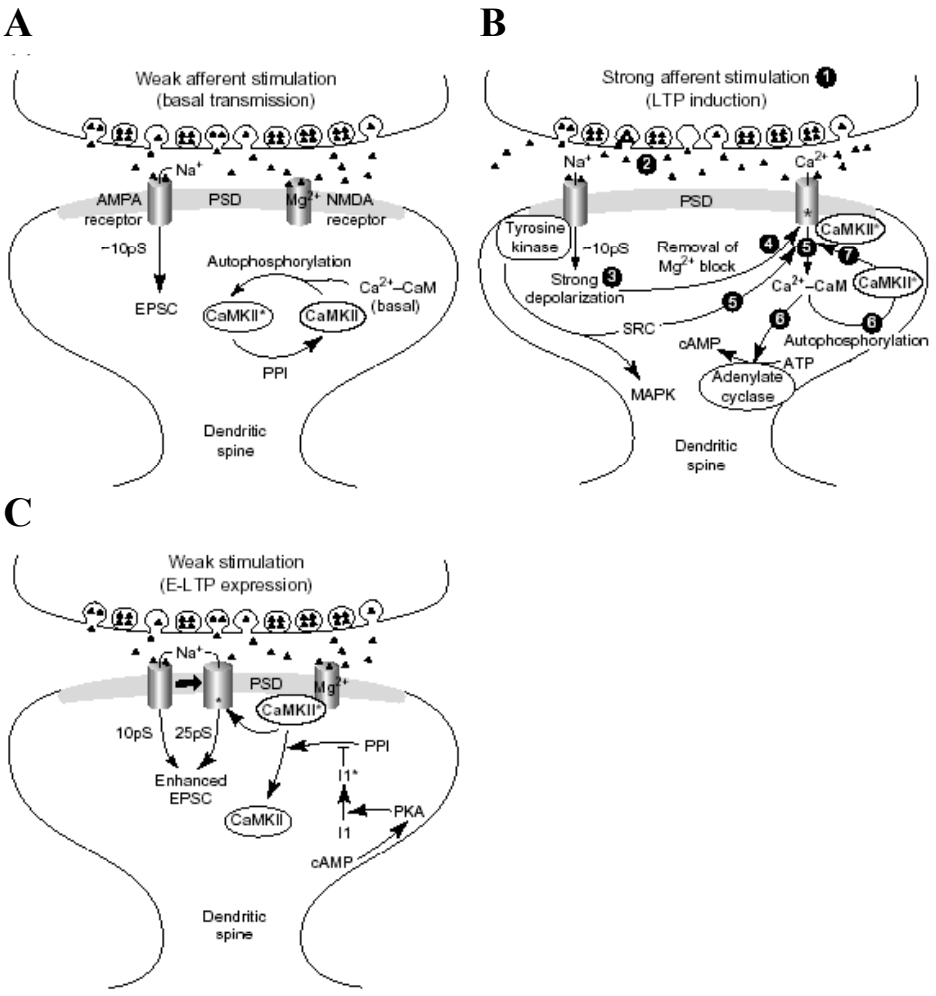


Figure. 1.7: Postsynaptic protein phosphorylation mechanisms during LTP in the CA1 region of hippocampus (from Soderling and Derkach, 2000).

A: Basal synaptic transmission via glutamate (triangles) is mediated largely by low-conductance state (~10pS) AMPA receptors that give rise to an EPSC. NMDA receptors are inactive because of voltage-dependent block of their channels by Mg^{2+}. Little of the CaMKII is in the activated, phosphorylated state (*), owing to low basal concentrations of CaM and high activity of PP1.

B: LTP induction by tetanic afferent stimulation (1) elicits enhanced glutamate exocytosis (2) and strong postsynaptic depolarization via AMPA receptors (3) to remove the Mg^{2+} block of the NMDA receptor (4). AMPA-receptor stimulation also activates associated SRC family tyrosine kinases that phosphorylate (*) and further enhance conductance of the NMDA-receptor channel, which is permeable to Ca^{2+} (5). The elevated levels of the Ca^{2+}–CaM complex stimulate AC-1 and also stimulates autophosphorylation (*) of CaMKII (6). This constitutively active CaMKII translocates to the PSD, in part through interaction with the NMDA receptor (7).

C: E-LTP expression is due, in part, to CaMKII-mediated phosphorylation of the AMPA receptor GluR1 subunit (*), which converts it largely to higher conductance states (~25 pS). The CaMKII is maintained in its autophosphorylated, constitutively active form, owing to PKA-mediated phosphorylation of inhibitor 1 (I1), which blocks the ability of PP1 to dephosphorylate CaMKII and perhaps GluR1. The predominant species and pathways are indicated in bold.

MAPK is activated upon LTP-inducing stimuli in CA1 neurons and inhibition of MAPK cascade blocks induction of LTP. MAPK substrates include phospholipase A2, an enzyme producing the putative retrograde messenger arachidonic acid, as well as proteins that regulate cytoskeletal organization (reviewed by Orban et al., 1999; Sweatt, 2001).

With respect to tyrosine kinase signalling, it is known that NMDA receptor-mediated rises in the intracellular calcium levels are enhanced by tyrosine phosphorylation (Wang and Salter, 1994) and that inhibiting tyrosine kinases prevents induction of LTP (O'Dell et al., 1991). Pharmacological evidence indicates that tyrosine kinase activity is also required for LTD (Boxall et al., 1996). The potential for involvement of src family kinases in LTP has been investigated in mice with targeted deletions of these kinases. Mutant mice lacking src gene show LTP in CA1, whereas LTP is blunted in mice lacking fyn (Grant et al., 1992). At first consideration, these observations appear to be contrary to involvement of src in this model. However, as discussed in detail elsewhere, the results in the mutant mice are likely because of compensatory changes or functional redundancy between src and fyn (for review, see Ali and Salter, 2001).

In CA3 region, induction of LTP results in the elevation of cAMP, a second messenger that activate PKA. The cAMP dependent PKA has been shown to be critical for the induction and maintenance of mossy fiber LTP (Weiskopf et al., 1994; Huang et al., 1995). It is hypothesized that increases in presynaptic Ca^{2+} stimulate AC, elevate cAMP and activate PKA (reviewed by Poser and Storm, 2001). Stimulation of NMDA receptors increases cAMP levels, thereby activating PKA, also in CA1 region (Chetkovich et al., 1991). The expression of homosynaptic LTD at the Schaffer collateral is associated with a dephosphorylation of the PKA site on AMPA receptor (Kameyama et al., 1998). cAMP-mediated transcription is implicated in the L-LTP in the Schaffer collateral, mossy fiber and the medial perforant pathways (Impey et al., 1996; Nguyen and Kandel, 1996; Abel et al., 1997).

Cytoplasmic PPs include PP1, PP2A and PP2B, also known as calcineurin (CaN) (for reviews, see Soderling and Derkach, 2000; Winder and Sweatt, 2001). CaN is a calcium sensitive phosphatase in synaptic terminals that apparently serves to downregulate synaptic transmission (Yakel et al., 1997). Inhibition of CaN produces synaptic

potentiation that occludes LTP (Wang and Kelly, 1997), or lowers the LTP induction threshold (Ikegami et al., 1996). PP1, on the other hand, downregulates CaMKII autophosphorilation, and PP1 activity is downregulated by LTP-inducing stimuli in a cAMP-PKA-dependent manner (Blitzer et al., 1998).

How can the same signal – Ca^{2+} entry through NMDA receptors - be used to trigger both LTP and LTD? It seems that some specific attributes of the Ca^{2+} signal (magnitude and duration) may determine the direction of the change in synaptic strength (Malenka, 1994; Bear and Malenka, 1994). Lisman (1989) hypothesized that Ca^{2+} levels could bidirectionally control synaptic efficacy by influencing the balance between the activity of CaMKII and PP1, which controls the phosphorylation of various proteins. In this model, small rises in $[Ca^{2+}]_i$ would favor PP1 activation and effector dephosphorilation (and thus LTD). Larger $[Ca^{2+}]_i$ rises would favor CaMKII activation and effector phosphorylation. Recent data support this model (Yang et al., 1999), but it is just a part of a much more complex process, involving many more players (for reviews, see Abraham and Tate, 1997; Fukunaga and Miyamoto, 2000).

Whereas E-LTP is dependent on persistent activity of protein kinases, L-LTP requires gene expression or new protein synthesis. Several activity-regulated genes have been identified, and their protein products may contribute to the mechanisms, maintaining L-LTP (for reviews, see Silva and Giese, 1994; Walton et al., 1999). A key messenger in the activity-dependent control of gene expression is Ca^{2+} (Gallin and Greenberg, 1995). The best studied signalling pathway in LTP from the synapse to the nucleus involves Ca^{2+}-dependent activation and phosphorylation of CREB (cAMP responsive element binding protein; Deisseroth et al., 1996), which promotes transcription from the cAMP responsive element (CRE). Activation of CREB in the postsynaptic terminal via PKA and MAPK pathways (Impey et al., 1996; Nayak et al., 1998) results in the expression of AMPA receptor genes (Nayak et al., 1998). Other transcription factors implicated in LTP include members of the *fos* and *jun* family, and NGFI-A (for review, see Walton et al., 1999).

1.2.8. Metaplasticity

It is becoming apparent that the induction of a synaptic change is also sensitive to the state generated by previous pattern of presynaptic and postsynaptic activity. The activity-dependent modulation of subsequent synaptic plasticity has been termed "metaplasticity" (Abraham and Bear, 1996; Abraham and Tate, 1997). Bienenstock et al. (1982) called the critical level of postsynaptic response at which the sign of the postsynaptic modification reverses from negative (LTD) to positive (LTP) as the modification threshold (θ_m). An additional key of assumption of this theory is that the value of θ_m is not fixed, but rather slides as a function of the history of postsynaptic activity. Thus, after a period of increased activity, θ_m slides to the right, promoting synaptic depression; after a period of decreased activity, θ_m slides to the left, promoting synaptic potentiation (Bear, 1995).

Although the induction of LTP and LTD by itself may affect subsequent induction of further plasticity, there are several metaplasticity mechanisms that involve little, if any, apparent change in excitatory synaptic transmission, yet which could have a significant impact on subsequent plasticity. These include changes in NMDA receptor-mediated synaptic transmission, GABAergic synaptic transmission, mGluR-mediated second messenger pathways, and postsynaptic membrane currents (reviewed by Abraham and Tate, 1997). Additionally, the direction of plastic changes depends on the stimulation of, and balance between, postsynaptic phosphorylation/dephosphorylation cascades (Lisman, 1989; Christie and Abraham, 1992; Abraham and Tate, 1997). Particularly, autophosphorylation of CaMKII has been implicated in metaplasticity (Bear, 1995; Mayford et al., 1995, 1996; Tompa and Friedrich, 1998). Excitingly revealing is the work of Eric Kandel and co-workers (Mayford et al., 1995, 1996), who found that transgenic mice that constitutively express active CaMKII show a systematic shift that favors LTD over LTP in the frequency range of θ_m. Their experiments, which attempt to mimic the situation that occurs in LTP, suggest that autoactivation of CaMKII plays a fundamental role in the sliding of θ_m. The fact that prior activation of mGluRs can facilitate the induction of LTP (Cohen and Abraham, 1996) points to other kinases that may contribute to a shift of the modification threshold. It has been demonstrated that PKC is involved in the regulation of LTP induction, via the metaplastic process,

whereas activation of PKA is not required for the metaplasticity at Schaffer collateral synapse (Bortolotto and Collingridge, 2000).

1.2.9. Expression of LTP and LTD

The final targets of the intracellular biochemical cascade are the subject of a major controversy (for reviews, see Kullmann and Siegelbaum, 1995; Malenka and Nicoll, 1999; Malinow et al., 2000). This issue is important for several reasons. First, once the site of the modification that is responsible for LTP is found, it should be easier to identify the relevant cellular and molecular machinery. Second, once the molecular modifications are elucidated, it will become possible to identify regulatory molecular pathways that could play modulatory roles in the acquisition or storage memories. Third, the site of modification may have an impact on how information is transmitted by neural signals. For instance, postsynaptic modifications that merely increase the strength of potential synapses by a fixed scale factor may actually decrease the signal-to-noise ratio of information (Otmakhov et al., 1993). On the other hand, presynaptic modifications are likely to interact with short-term forms of presynaptic plasticity, which may be important in dynamic mechanisms such as gain control (Abbott et al., 1997). Finally, once the signaling pathways that produce activity-induced synaptic plasticity are identified the relation between diseases that affect cognitive and mnemonic function and the molecular mechanisms of LTP can be investigated on a stronger biological basis. For these (and probably many more) reasons, establishing which parts of synapses undergo modifications during LTP is likely to be an important step toward understanding the neural basis of learning and memory as well as cognitive function in general (Malinow et al., 2000).

Recent studies using direct imaging of fluorescent, tagged glutamate receptors support the idea that LTP (Shi et al., 1999; Hayashi et al., 2000) and LTD (Carroll et al., 1999; Luscher et al., 1999) recruit, respectively, the insertion and removal of AMPA-type receptors in the postsynaptic membrane. In contrast, evidence for changes in presynaptic function comes largely from the classical electrophysiological approach of quantal analysis between pairs of synaptically connected neurons (Malinow, 1991; Stevens and Wang, 1994; Bolshakov and Siegelbaum, 1995; Stricker et al., 1999).

Additionally, enhanced presynaptic function during LTP has been shown in experiments with uptake of fluorescent dye FM 1-43 (Ryan et al., 1996; Ma et al., 1999; Zakharenko et al., 2001).

It is plausible that several mechanisms coexist to regulate synaptic strength, and that the relative importance of pre- and postsynaptic changes differs depending on the protocol of slice preparation, the age of the animal, the mode of LTP and LTD induction, or the length of time that has elapsed after induction (for reviews, see Kullmann and Siegelbaum, 1995; Malenka and Nicoll, 1999; Malinow et al., 2000).

1.3. CELL ADHESION MOLECULES IN SYNAPTIC PLASTICITY

CAMs are ligands and receptors mediating cell-cell and cell-matrix interactions by lock and key recognition events that subsequently rely signals to the cell interior (Goodman, 1996; Wheal et al., 1998). During development of the nervous system CAMs and ECM components have a vital role regulating such processes as neuronal adhesion and migration, neurite outgrowth, fasciculation, synaptogenesis and intracellular signalling (Tessier-Lavigne and Goodman, 1996; Kiss et al., 2001). The growing evidence indicates that CAMs and ECM molecules also play important and diverse roles in regulating synaptic plasticity and learning and memory (Schachner, 1997; Murase and Schuman 1999; Benson et al., 2000).

1.3.1 CAMs and synaptic plasticity

It has been shown that the regulation of the expression of some adhesion molecules such as L1 and polysialylated NCAM is regulated by neuronal activity (Itoh et al., 1995; Muller et al., 1996). Inhibition of adhesion mediated by NCAM or L1 with antibodies or oligomannosidic carbohydrate blocked LTP (Luthi et al., 1994; Ronn et al., 1995). Although LTP in CA1 and the dentate gyrus was not affected in mice lacking L1 protein (Bliss et al., 2000), deficiency in NCAM results in impaired LTP in CA1 of the hippocampus in organotypic and dissociated cultures as well as in acute slices (Muller et al., 1996; Dityatev et al., 2000; but see Holst et al., 1998). Cleavage of PSA moiety of NCAM by treatment with endo-N has also been found to cause a block of LTP and LTD in CA1 (Muller et al., 1996; Becker et al., 1996). Impaired CA3 LTP has also been

shown in NCAM deficient mice (Cremer et al., 1998). Interestingly, impaired LTP in hippocampal slices or organotypic cultures prepared from NCAM knockout mice could be recovered by addition of the neurotophic factor BDNF (Muller et al., 2000). These results suggest that one action of PSA-NCAM could be to sensitize pyramidal neurons to BDNF, thereby modulating activity-dependent synaptic plasticity or that NCAM and BDNF share intracellular signaling.

Integrin matrix receptors are assembled from a variety of subunits and commonly require a conformational change to trigger their adhesive properties (Newton et al., 1997). LTP is inhibited by peptides which block integrin mediated adhesion (Xiao et al., 1991; Bahr et al., 1997; Staubli et al., 1998). The peptide-mediated inhibition of LTP was time-dependent: the application of the antagonist peptides 10 minutes, but not 25 minutes, after LTP induction was effective in blocking LTP, suggesting that integrin function is required for a very early stage of LTP stabilization (Staubli et al., 1998).

The cadherin superfamily is a family of CAMs that mediate calcium-dependent homophilic interactions and transduce signal by interacting with cytoplasmic proteins. N- and E-cadherins have been shown to be involved in activity-dependent synaptic plasticity and synaptogenesis (Tang et al., 1998; Bozdagi et al., 2000). Arcadlin, another member of this family, has been reported to be expressed transiently in activity-dependent manner following seizures and application of arcadlin antibody blocked LTP (Yamagata et al., 1999). In contrast, a recent study of synaptic plasticity in cadherin-11-deficient mice has shown an enhanced level of CA1 LTP (Manabe et al., 2000).

1.3.2. ECM and synaptic plasticity

Among the extracellular matrix molecules both proteoglycans and glycoproteins have been implicated in synaptic plasticity. For the members of tenascin gene family, a role of TN-C in LTP has been suggested. Induction of LTP *in vivo* results in an increase in mRNA levels of TN-C in the dentate gyrus (Nakic et al., 1998). The application of NMDA and mGluR receptor antagonists during LTP induction abolished the elevation of TN-C mRNA (Nakic et al., 1998). Furthermore, TN-C as well as TN-R are involved in regulation of sodium channel localization and conductance (Srinivasan et al., 1998; Xiao et al., 1999).

A previous study from our laboratory revealed that HNK-1 carried by TN-R in the CA1 region of the hippocampus is involved in regulation of perisomatic but not dendritic inhibition of pyramidal cells via reduction of presynaptic GABA release (Saghatelyan et al., 2000). It has been demonstrated that the HNK-1 antibody relieves a constitutive inhibitory action of endogenous HNK-1 on $GABA_B$ receptors expressed by pyramidal cells, thereby activating outward K^+-currents (Saghatelyan et al., 2001).

Extracellular proteolysis is another modulatory factor implicated in synaptic plasticity (Sappino et al., 1993). TN-C is a substrate of extracellular proteases: for instance, TN-C can be cleaved by plasmin (Gundersen et al., 1997). Tissue plasminogen activator (tPA) is involved in late LTP (Qian et al., 1993; Baranes et al., 1998). Recently, cleavage of the ECM molecule laminin by the plasmin system has been suggested to be important for CA1 LTP (Nakagami et al., 2000). Thus, cleavage of other ECM components, including TN-C, could also be essential for activity-dependent plasticity.

HSPG N-syndecan (syndecan-3) and associated with it HB-GAM are expressed in activity-dependent manner and have been implicated in CA1 LTP (Lauri et al., 1998, 1999; Amet et al., 2001). An involvement of neurocan, a brain specific CSPG, in the late phase of CA1 LTP has recently been demonstrated (Zhou et al., 2001).

2. AIMS OF THE STUDY

- Despite the well-documented roles of integral membrane cell adhesion molecules in synaptic plasticity (for review see Schachner, 1997; Murase and Schuman, 1999), the contributions of their binding partners located in the ECM have not been studied. During the present study, the first reports investigating the roles of HSPGs and HB-GAM (Lauri et al., 1998, 1999; Amet et al., 2001), TN-R (Saghatelyan et al., 2001), and laminin (Nakagami et al., 2000) have appeared. Therefore, the major aim of this study was to investigate the involvement of ECM molecules - TN-R and TN-C - in the regulation of synaptic plasticity in hippocampal synapses using genetically modified mice deficient in these molecules. As a first step towards elucidation of the roles of CSPGs in synaptic transmission and plasticity, these processes were studied in hippocampal slices treated with chondroitinase ABC. Particular attention was paid to possible synergetic effects produced by removal of CSs and TN-R, since TN-R is known to be a carrier of CSs and to bind to CSPGs.
- Several studies using enzymatic removal of PSA with endoneuraminidase (endo-N) have provided first evidence that PSA is required for some forms of synaptic plasticity in the mammalian brain (Becker et al., 1996; Muller et al., 1996). Although endo-N appeared so far as a very specific enzyme, a rapid removal of PSA could trigger some ectopic signaling or structural modifications, possibly not equivalent to the absence of PSA. Therefore it was desirable to use a complementary method to confirm the role of PSA in the synaptic plasticity. It has been known that LTP is strongly affected in the Schaffer collateral-CA1 and mossy fiber-CA3 synapses in NCAM-deficient mice (Muller et al., 1996; Cremer et al., 1998). However, because both PSA and NCAM are absent from these mice throughout development and in the adulthood, it is difficult to distinguish between PSA- and NCAM-dependent phenomena on the one hand and between developmental and acute functions, on the other. Therefore, we took advantage of having mice deficient in polysialyltransferase PST that is responsible for attachment of PSA to NCAM in mature neurons. Since there is a drastic postnatal downregulation in expression of PSA in PST deficient mice, the age-dependent changes in LTP and LTD were studied in these animals.

- Most electrophysiological studies in the field of recognition molecules have concentrated on LTP in the CA1 region of the hippocampus, the most easily assessible form of synaptic plasticity *in vitro*, mostly ignoring other forms of plasticity. Since other forms of synaptic plasticity are also important for learning and memory and since a selective impairment of different forms of plasticity could facilitate further search of mechanisms by which recognition molecules regulate synaptic plasticity, several forms of plasticity have been studied in parallel in the present study. Among them were:

  TBS-induced LTP in the CA1 region of the hippocampus;

  pairing-induced LTP/LTD in the CA1 region of the hippocampus;

  LFS-induced LTD in the CA1 region of the hippocampus;

  HFS-induced LTP in the CA3 region of the hippocampus.
- Since published data demonstrated that epileptic seizures can induce changes in expression of TN-C, NCAM and CSPGs (Nakic et al., 1996; Mikkonen et al., 1998; Wu et al., 2000), the relevance of the extracellular matrix and related cell adhesion molecules (TN-C, TN-R, CSPGs, PSA-NCAM) in modulation of use-dependent disinhibition and excitability of neurons has been analyzed.

3. METHODS

3.1. Animals

For investigation of the roles of ECM molecules and related CAMs (TN-C, TN-R, CSPGs, PSA-NCAM) in the regulation of hippocampal synaptic plasticity the following animals were used.

1) 14- to 22-day-old and 2- to 6-month-old TN-R deficient mice (Weber et al., 1999) with a mixed C57BL/6J x 129Ola x 129Sv/Ev genetic background and wild-type littermate mice with corresponding genotype. Neither heterozygous nor homozygous TN-R-deficient mice showed any obvious, grossly abnormal behavioral phenotype up to an age of ~1 year, the latest time point investigated. The gross anatomy of the brain and spinal cord and the morphology of the retina and cerebellum are also indistinguishable between TN-R-deficient and wild-type mice at the light microscopic level. Although the aberrant RNA is expressed in the mutant in quantities similar to those of the TN-R mRNA in wild-type mice, neither TN-R nor a truncated form thereof could be detected by Western blot analysis using various polyclonal and monoclonal antibodies at a level of sensitivity sufficient to reveal a 1000-fold reduction of TN-R expression (Weber et al., 1999).

2) 2- to 6-month-old mice with C57BL/6J or mixed C57BL/6J x 129Ola x 129Sv/Ev genotypes were used for chondroitinase ABC treatment, as mentioned in Figure Legends;

3) 4- to 6-week-old TN-C deficient mice (recently generated by M. Evers from our laboratory) and their wild-type littermates with a mixed C57BL/6J x 129SvJ background. The TN-C mutants showed no apparent abnormalities in the gross anatomy of brain at the light microscopic and at the ultrastructural level. Northern blot analysis revealed low level expression of an aberrant TN-C mRNA. By quantitative Western blot analysis it has been demonstrated that residual TN-C comprises less than 0.05 % of the TN-C protein levels expressed in wild-type littermates. Thus, the here-described TN-C deficient mouse appears to be a true null mutant (M. Evers, unpublished observation).

4) 13- to 19-day-old, 4- to 5-week-old, and 4- to 6-month-old *pst* -/- mice (Ekhardt et al., 2000) with mixed C57BL/6J×129/Ola genetic background and age-matched groups

of wild-type C57BL/6J, 129/Ola, or C57BL/6J×129/Ola mice. No significant differences in body weight or brain size between mutant and wild-type mice up to 6 weeks of age were observed. Histochemical examination of the brain revealed no obvious abnormalities. Absence of PST expression in *pst* -/- mice was verified by Northern and Western blot analysis. Hybridization of mRNA from brains of 1-day-old, 21-day-old, and 6-month-old mice to a PST specific RNA probe gave no signals for homozygous mutants. At postnatal day four, the total amount of PSA expressed in the brain remained identical in mutants and wild-types. Strong differences were observed in brains from older animals (5– to 6-week-old), in which Western blot analysis revealed a striking reduction in PSA expression in all brain regions analyzed (olfactory bulb, medulla oblongata, hippocampus, neocortex, and hypothalamus). In adult animals (4-month-old), PSA expression dropped to relatively low, yet still detectable levels in whole brains of wild-type and heterozygous animals, but was undetectable in *pst* -/- mice (Ekhardt et al., 2000).

All efforts were made to minimize both the suffering and the number of animals used. All surgical procedures had been approved by the Committee on Animal Health and Care of the local governmental body.

3.2. Preparation of hippocampal slices

Transverse slices of hippocampi (400-µm-thick for 13- to 21-day-old mice; 350-µm-thick for 1- to 3-month-old mice; 300-µm-thick for 4- to 6-month-old mice) were used for whole cell recordings of excitatory postsynaptic current (EPSC) and focal field excitatory postsynaptic potential (fEPSP) recordings in the CA1 region. After halothane anesthesia, decapitation and removal of the brain, the hippocampi were cut with a Vibroslice (Campden Instruments) in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 250 sucrose, 25 $NaHCO_3$, 25 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 1.5 $MgCl_2$, pH 7.3. The slices were kept at room temperature in a large chamber (500 ml) filled with carbogen-bubbled ACSF, containing 125 mM NaCl instead of 250 mM sucrose, at least 2 hr before the start of recordings (modified from Edwards et al., 1990). In the recording chamber, slices were continuously superfused with carbogen-bubbled ACSF (2-3 ml/min). In experiments with $GABA_A$ receptor

antagonist picrotoxin (50 μM; Tocris, Bristol, UK), the CA3 region was routinely separated from the CA1 region by a cut to prevent bursting activity (originating in CA3) during application of blocker.

For the CA3 recordings the slices were prepared as for recordings in the CA1 region, but with some modifications. Before decapitation, mice were transcardially perfused with ice-cold ACSF, containing (in mM): 250 sucrose, 25 $NaHCO_3$, 25 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, and 6 $MgCl_2$, pH 7.3. Slices were cut according to Claiborne and colleagues (1993). Exchange of sucrose-containing ACSF to normal ACSF (with 2.5 mM $CaCl_2$ and 1.5 mM $MgCl_2$) was performed gradually using peristaltic pumps.

3.3. Enzymatic treatment with chondroitinase ABC

To digest CSs, the hippocampal slices were treated with protease-free chondroitinase ABC (chondroitin ABC lyase, EC.4.2.2.4.) from *Proteus vulgaris* (Seikagaku, Tokyo, Japan). Chondroitinase ABC was applied at a concentration of 0.2 U/ml in 4 ml ACSF, containing 0.1 % BSA for 2 hours at 37^{o}C. Sham-treated slices were incubated in ACSF without enzyme for 2 hours at 37°C. Immediately after treatment, slices were placed into the recording chamber and continuously superfused with carbogen-bubbled ASCF (2-3 ml/min), not containing the enzyme.

3.4. Immunostaining for chondroitin sulfates

After the end of electrophysiological recordings, the slices were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.3), embedded in gelatin and cut into 40-μm-thick sub-slices. Freely floating slices were treated for 1 hour with blocking solution containing 2% BSA in PBS, followed by monoclonal rat antibody 473HD overnight at room temperature. This antibody has been shown to react with the chondroitin sulfate/dermatan sulfate hybrid GAG structure DSD-1 epitope (Faissner et al., 1994; Nadanaka et al., 1998). Sections were then washed three times for 10 min in PBS containing 0.1% BSA. Secondary anti-rat IgG antibodies coupled to Cy3 (Dianova, Hamburg, Germany) (diluted 1:200) were applied for 1 hour at room temperature. Finally, sections were rinsed three times for 10 min in PBS containing 0.1% BSA and

mounted in Aqua-Poly/Mount (Polyscience, Warrington, USA). Images of stained sections were acquired by confocal laser scanning microscopy (LSM 510, Zeiss, Gottingen, Germany) using a laser with excitation length of 543 nm and a BP 585-615 filter.

3.5. Enzymatic treatment with endoneuraminidase

To remove PSA carbohydrate chain from NCAM backbone, the hippocampal slices were treated with endo-N (kindly provided by Dr. Rita Gerardy-Schahn, Hannover, Germany). The slices were incubated for 3 hr at room temperature in a small chamber with a volume of 4 ml in ACSF containing or not containing endo-N. Immediately after the treatment, slices were placed into the recording chamber and continuously superfused with carbogen-bubbled ASCF (2-3 ml/min), not containing the enzyme.

3.6. Immunostaining for PSA

After the end of electrophysiological recordings, the slices were fixed in 4% paraformaldehyde in PBS (pH 7.3), embedded in gelatin and cut into 40-μm-thick sub-slices. Freely floating slices were treated for 1 hour with blocking solution containing 2% BSA in PBS, followed by monoclonal mouse antibody 735 against PSA (Frosch et al., 1985) overnight at 4°C. Sections were then washed three times for 10 min in PBS containing 0.1% BSA. Secondary anti-mouse IgG antibodies coupled to Cy3 (Dianova) (diluted 1:200) were applied for 1 hour at room temperature. Finally, sections were rinsed three times for 10 min in PBS containing 0.1% BSA and mounted in Aqua-Poly/Mount (Polyscience). Images of stained sections were acquired by confocal laser scanning microscopy (LSM 510, Zeiss) using a laser with excitation length of 543 nm and a BP 585-615 filter.

3.7. Schaffer collateral-CA1 extracellular recordings

Focal field excitatory postsynaptic potential recordings were performed in the *stratum radiatum* with glass pipettes filled with ASCF having a resistance of 2 MΩ. Schaffer collaterals were stimulated by two bipolar electrodes placed approximately 400 μm distant from both sides of the recording electrode in the *stratum radiatum* of the CA1

region. The second stimulating electrode was used for antidromic stimulation of the independent control pathway. Basal synaptic transmission was monitored at 0.033 Hz. Stimulus-response curves in individual experiments were determined with an accuracy of 10 μA. Slope values of 20, 40, 60, 80 and 100% of the maximal responses and corresponding stimulus intensities were used for averaging of curves obtained in different slices. PPF was defined as A2/A1×100%, where A1 and A2 are the amplitudes of the fEPSPs evoked by the first and second pulse, respectively (with a 50 ms inter-pulse interval).

Field EPSPs were amplified and filtered at 1 kHz using CyberAmp 320 (Axon Instruments, Foster City, CA). Data acquisition and analysis was performed using the LTP101M program (Anderson and Collingridge, 1997).

3.8. Whole cell recordings in the CA1

Standard whole-cell patch-clamp technique was used (Edwards et al., 1990). In the recording chamber, slices were continuously superfused with carbogen-bubbled ACSF (2-3 ml/min) and maintained at a temperature of 30°C. Schaffer collaterals were stimulated using glass pipettes filled with ASCF, having a resistance of 1-2 MΩ, placed in the *stratum radiatum.* The patch electrodes (2.5-3.5 MΩ) were filled with an internal solution (pH 7.3; 295-305 mOsm/kg) containing (in mM): 135 K-gluconate, 5 NaCl, 5 KCl, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.2 Na_3GTP, 10 glucose. Additionally, 5 mM of QX-314 (Tocris) was added to the recording solution to block voltage-dependent Na^+ currents (Connors and Prince, 1982). EPSCs were recorded at a control frequency of 0.033 Hz and at holding potential of –60 mV, stimuli were paired within a delay of 50 ms. The amplitude of EPSCs was measured by taking the average of a 2-3 msec window around the peak of the EPSC relative to the baseline, and the stimulation strength was adjusted usually to provide currents of ~100 pA. Data were collected with software Pulse Pulsefit (Heka Elektronik, Lambrecht/Pfalz, Germany). Serial resistance as well as cell resistance and capacitance were routinely measured during the experiments. There was no systematic difference between neurons with different genotypes in these three parameters.

3.9. Induction of LTP in the CA1 region by theta-burst stimulation

Homosynaptic LTP was induced by TBS applied orthodromically and recorded extracellularly in the *stratum radiatum* of the CA1 region. A TBS consisted of 10 bursts delivered at 5Hz. Each burst consisted of 4 pulses delivered at 100 Hz. Duration of pulses was 0.2 ms, and five TBSs were applied to induce LTP (Muller et al., 1996). The stimulation strength was in the range of 40-70 μA to provide fEPSPs with an amplitude of 50% from the subthreshold maximum. The mean slope of fEPSPs recorded 0-10 min before TBS was taken as 100 %. The values of LTP were calculated as increase in the mean slopes of fEPSPs measured 50-60 min after TBS.

3.10. Induction of LTP and LTD in the CA1 pyramidal cells by pairing of presynaptic stimulation and depolarization of postsynaptic cells

In the whole cell mode, LTP or LTD were induced by pairing of afferent stimulation of Schaffer collaterals at 1 Hz for 100 sec with postsynaptic membrane depolarization (to 0 mV, -10 mV or -20 mV). Care was taken to avoid so-called "washout" of LTP due to a dialysis of the cells via patch pipette. Therefore, induction of LTP by pairing was performed within 7 min after disruption of cell plasma membrane. To combine data from different experiments, the amplitudes of EPSCs were normalized to the averaged value obtained during the 5 min interval before applying the pairing protocol. The values of LTP and LTD represent mean amplitude of EPSCs measured 25-30 min after pairing.

3.11. Recording of composite and NMDA receptor-mediated responses evoked by theta-burst stimulation

The NMDA receptor antagonist 2-D,L-aminophosphonovaleric acid (AP-5, Tocris) was added to the perfusion solution at a concentration of 50 μM to block the NMDA-mediated component of total after-burst depolarizations induced by a single train of 4 pulses applied to Schaffer collaterals at 100 Hz after at least 10 minutes of stable baseline recordings in the presence of AP-5. The NMDA receptor-mediated component was evaluated as a difference of burst-evoked fEPSPs recorded before and after application of AP-5.

3.12. Induction of LTD in the CA1 region

Homosynaptic LTD was induced by two trains applied at 1 Hz for 10 min with a 10 min interval between them (Kerr and Abraham, 1995) and recorded extracellularly in the *stratum radiatum* of the CA1 region. Duration of pulses was 0.2 ms and stimulation strength was set to produce fEPSPs with amplitude of 30-40% of the suprathreshold response during baseline and LTD recordings. The stimulation strength was increased to the 60-70% level when 1 Hz stimulation was delivered. The mean slope of fEPSPs recorded 0-10 min before first train of LFS was taken as 100 %. The values of LTD were calculated as decrease in the mean slopes of fEPSPs measured 50-60 min after second train of LFS.

3.13. Recording of polyspike activity in the CA1 region

Schaffer collaterals were stimulated by bipolar platinum electrode placed approximately 400 μm distant from the recording electrode in the *stratum radiatum* of the CA1 region. The recording glass electrode filled with ASCF, having a resistance of 2 MΩ, was placed in the *stratum pyramidale.* Duration of stimulation pulses was 0.2 ms and stimulation strength was set to produce population spike of the maximal amplitude. Afferent stimulation of Schaffer collaterals at 1 Hz 30 sec was used to induce polyspiking activity (Luthi et al., 1997). In all these experiments the CA3 region was routinely separated from the CA1 region by a cut to avoid bursting activity originating from the recurrent excitation of CA3 region.

In experiments with pharmacological disinhibition of the CA1 region, a repetitive stimulation was applied after 15-20 min perfusion of $GABA_A$ receptor antagonist picrotoxin (50 μM; Tocris).

3.14. Induction of LTP in mossy fiber-CA3 synapses

The stimulating electrode was placed close to the internal side of the granule cell layer. The recording electrode was placed in the *stratum lucidum*. Both, recordings and stimulations were performed with glass pipettes filled with ACSF and having a resistance of 2 MΩ. The LTP-inducing HFS consisted of trains of stimuli applied at 100

Hz during 1 sec and repeated four times with an interval of 20 sec. To evoke LTP exclusively in mossy fiber synapses, which are known to undergo LTP in a NMDA receptor-independent manner, the AP-5 (50 μM; Tocris) was applied 15 min before and during HFS. To confirm that the field EPSPs recorded were evoked by the stimulation of mossy fibers and not by the associational/commissural pathway, an agonist of metabotropic glutamate receptors (L-CCG1, 10 μM; Tocris) was applied at the end of each experiment (Cremer et al., 1998).

To demonstrate dependency of the recorded LTP on PKA, slices were incubated for 2 hours in a 4 ml chamber in the presence of Rp-cAMPS (100 μM; Biolog, Bremen, Germany), a membrane-permeable competitive inhibitor for PKA, that was also included in the ACSF used for perfusion of slices at a concentration of 15 –20 μM (Weisskopf et al., 1994). The mean amplitude of fEPSPs recorded 0-10 min before HFS was taken as 100 %. The values of LTP were calculated as increase in the mean amplitude of fEPSPs measured 50-60 min after HFS.

3.15. Statistical analysis

All values are reported as mean ± SEM (standard error of mean). Student's *t* test and non-parametric U-test were used to assess statistical significance using Sigma Plot 5.0 and Systat 9.0 software (SPSS, Chicago, IL). Differences between >3 groups were tested for significance using one-way ANOVA or nonparametric Kruskal-Wallis one-way analysis of variance. Chi-square test was used to compare empirical distributions.

4. RESULTS

4.1. THE ROLES OF EXTRACELLULAR MATRIX NOLECULES IN SYNAPTIC PLASTICITY

4.1.1. Immunohistochemical control of treatment with chondroitinase ABC

To remove CSs from the tissue, the hippocampal slices were incubated with chondroitinase ABC (Koppe et al., 1997b). Efficacy of treatment was confirmed by immunostaining with monoclonal antibody 473HD for CSs performed for every slice after the end of the physiological recordings. In non-treated slices CSs were expressed in the CA1 area of the hippocampus, with accumulated immunoreactivity in the pyramidal cell layer and the *strata orients* and *lacunosum-moleculare* (Fig. 4.1A). Enzymatically treated slices were morphologically intact. The slices selected for electrophysiological analysis showed complete loss of CSs immunostaining (Fig. 4.1B and C), except for astrocyte-like cells having CSPGs intracellularly (Aquino et al., 1984; Fig. 4.1D).

4.1.2. Basal synaptic transmission and paired-pulse facilitation in the CA1 region

To determine weather TN-R, TN-C and CSs play a role in modulation of basal synaptic transmission, the basic properties of synaptic transmission were studied using stimulus-response curves.

Analysis of synaptic responses evoked by low-frequency stimulation of Schaffer collaterals showed that more prominent responses were activated in the CA1 region of TN-R mutants in comparison to wild-type littermates at the same stimulation strength (Fig. 4.2A). Measurements of the initial slope of fEPSPs evoked by stimulation with different stimulation strengths confirmed the significant difference between the genotypes (Fig. 4.2B). In TN-R mutants less current intensity was necessary to evoke fEPSPs of the same slope when compared to wild-type mice ($P<0.05$): 108.0±6.8 μA (10 slices from 8 animals) was necessary to elicit supramaximal fEPSPs in *tn-R* +/+ mice, whereas such responses were produced in *tn-R* -/- mice with a stimulus strength of 76.3±7.6 μA (8 slices from 7 mice). Treatment of slices from wild-type or TN-R deficient mice with chondroitinase ABC did not affect basal synaptic transmission (Figs. 4.2C and D): stimulation strengths to elicit supramaximal responses in sham- and chondroitinase ABC-treated slices were 76.3±7.6 μA (8 slices from 7 mice) and

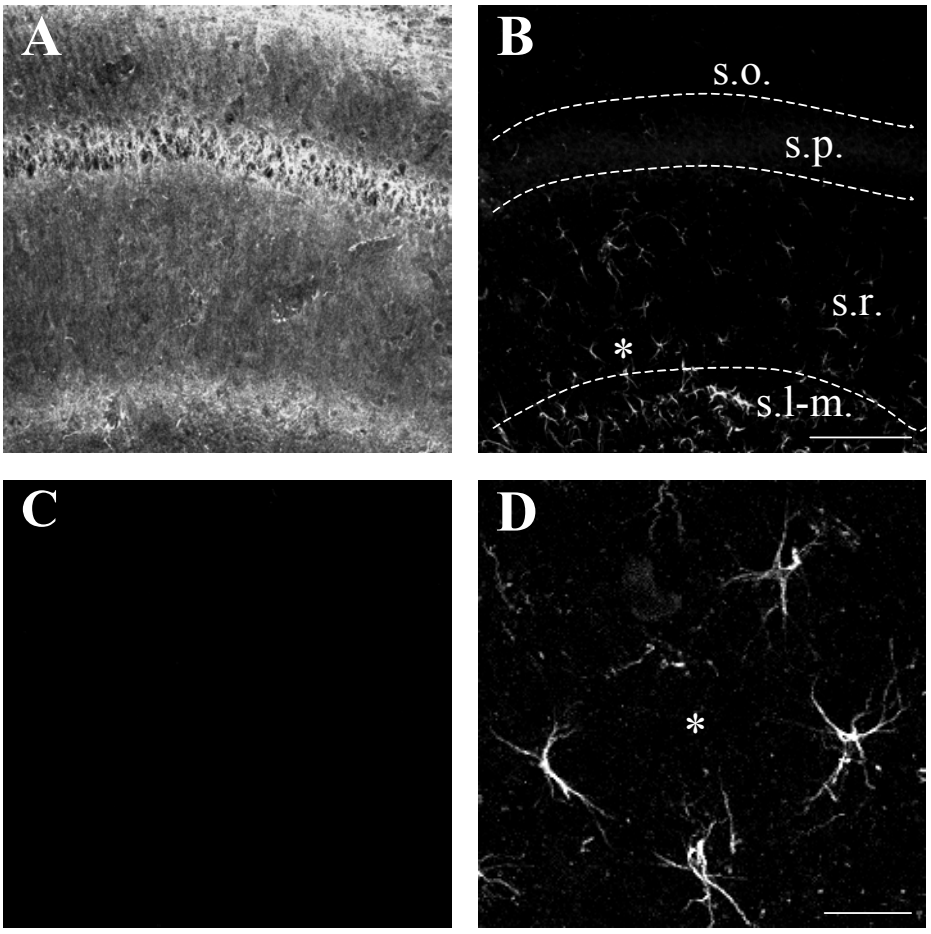


Figure 4.1. Immunostaining of chondroitin sulfates in the CA1 region of the hippocampus

A: Immunostainings with monoclonal rat antibody 473HD in hippocampal slices not treated with chondroitinase ABC.

B-C: After treatment of slices with chondroitinase ABC some immunostaining apparently remained detectable in astrocyte-like cells. The region marked by asterisk in **B** is shown at higher magnification in **D**. Scale bar (100 μm) and outlines of cell layers shown in **B** are valid for **A-C**. In **D** scale bar, 20 μm.

C: When the primary antibodies were omitted and only the secondary antibodies were applied to hippocampal slices, no immunostaining was detectable.

Images were taken with identical acquisition parameters from slices prepared from the same animal and simultaneously processed for immunostaining. S.o, s.p., s.r., s.l-m. mark the *strata oriens, pyramidale, radiatum* and *lacunosum-moleculare*, respectively.

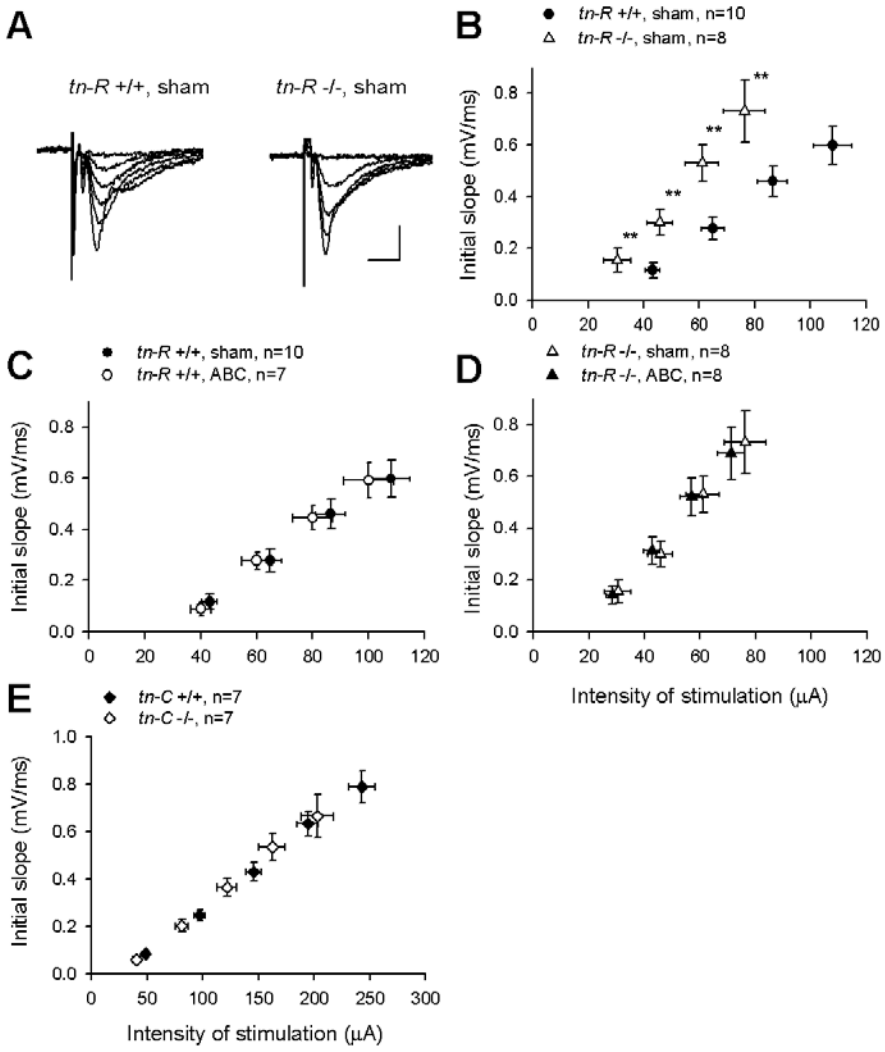


Figure 4.2. Basal synaptic transmission is increased in TN-R knockout mice, but is not altered after treatment with chondroitinase ABC and in TN-C mutants

A: Traces representing fEPSPs evoked with different stimulus strengths (from 20 μA by steps of 10 μA) in sham-treated slices from wild-type C57BL/6J x 129Ola x 129Sv/Ev and TN-R deficient mice. Bars, 10 ms and 500 μV.

B: Stimulus strength-response curves for TN-R mutants (*tn-R -/-, sham*) and wild-type mice (*tn-R +/+, sham*). Note that to evoke fEPSPs with the same slope, less stimulus strength was required in seven *tn-R* -/-, than in eight *tn-R* +/+ mice. Stimulation with the same stimulus strengths produced more prominent responses in *tn-R* -/- than in wild-type mice. **$P<0.01, unpaired *t* test, significantly different in comparison to control sham-treated slices from *tn-R* +/+ mice. *n* provides the number of slices, valid also for **C** to **E**.

C, D: Treatment with chondroitinase ABC did not shift the stimulus intensity-response curve in six wild-type (*tn-R +/+, ABC*; **C**) and in eight mutant (*tn-R -/-, ABC*; **D**) mice as compared to sham-treated slices (*tn-R +/+, sham*; **C** and *tn-R -/-, sham*; **D**).

E: Input-output curves for slopes of fEPSPs evoked by stimulation of Schaffer collaterals at different stimulation strengths. No genotype-specific difference in the stimulus strength-response curves for four TN-C deficient (*tn-C -/-*) and three wild-type (*tn-C +/+*) mice.

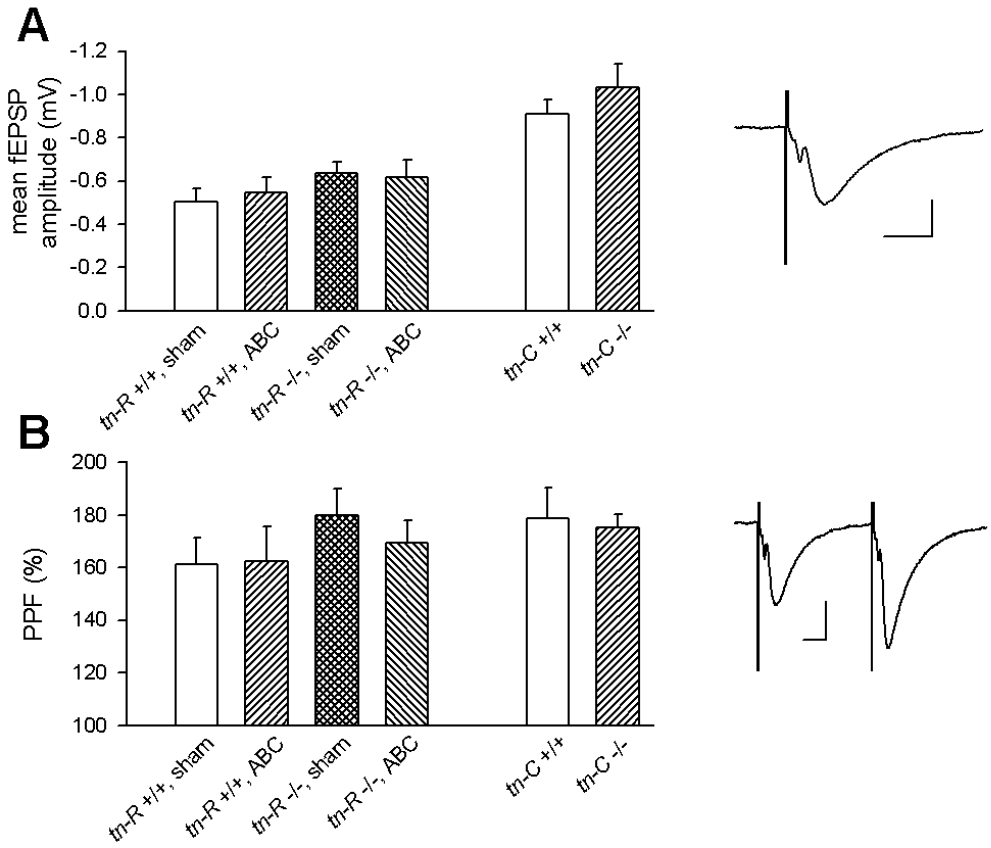


Figure 4.3. The mean amplitude of responses and the paired-pulse facilitation are not affected in slices treated with chondroitinase ABC and from TN-R and TN-C knockout mice

A: Cumulative data showing the absence of significant effects of chondroitinase ABC treatment (ABC) on the amplitude of fEPSPs evoked by half-maximal stimulus intensity (an example is given in the inset). Data represent mean fEPSP amplitudes + SEM measured in slices from eight wild-type (*tn-R +/+, sham* or *tn-R +/+, ABC*) and seven TN-R deficient (*tn-R -/-, sham* or *tn-R -/-, ABC*) mice, which were sham- or chondroitinase ABC-treated, and in slices from four TN-C mutants (*tn-C -/-*) and three wild-type littermates (*tn-C +/+*). Measurements were taken 10 min after stabilization of the baseline response. Bars, 10 ms and 500 μV.

B: Cumulative data showing that PPF was not altered by treatment with chondroitinase ABC or in *tn-R* -/- mice as compare to *tn-R* +/+ mice that were not treated with chondroitinase ABC. Normal PPF was also observed in *tn-C* +/+ and *tn-C* -/- mice. The values represent a ratio between the slopes of fEPSPs evoked by the second and first pulses with an interstimulus interval of 50 ms (an example is given in the inset). Bars, 10 ms and 500 μV.

71.1±4.8 μA (8 slices from 8 mice), respectively. Stimulus-response curve for fEPSPs evoked by stimulation of Schaffer collaterals was not different in 7 slices from 3 *tn-C* +/+ and in 7 slices from 3 *tn-C* -/- mice (Fig. 4.2E).

The mean amplitudes of responses being 50% of the supramaximal levels were not significantly different between genotypes (0.5±0.1 mV versus 0.6±0.1 mV in *tn-R* +/+ and *tn-R* -/- mice, respectively, P>0.1) and were not changed after treatment with chondroitinase ABC (0.5±0.1 mV versus 0.6±0.1 mV in *tn-R* +/+ and *tn-R* -/- mice, respectively, P>0.1) as compared to sham-treated slices. There was no genotype specific difference between *tn-C* +/+ and *tn-C* -/- mice in terms of mean amplitude of fEPSP (0.9±0.1 mV and 1.0±0.1 mV, respectively, P>0.1; Fig. 4.3A). PPF of fEPSPs with interstimulus intervals of 50 ms was about 170% and was not different in all experimental groups (Fig. 4.3B).

4.1.3. Theta-burst stimulation-induced long-term potentiation in the CA1 region

We investigated hippocampal synaptic plasticity in hippocampal slices deficient in TN-R, TN-C or CSs, starting with the most widely studied form of plasticity, LTP in the CA1 region.

TBS of Schaffer collaterals evoked long-term potentiation of fEPSPs in the CA1 region (Fig. 4.4A). In wild-type C57BL/6J x 129Ola x 129Sv/Ev mice responses recorded during 50-60 min after TBS had slopes of 142.2±7.7% of baseline levels (10 slices from 8 animals). Stimulation of the control pathway, to which TBS was not applied, evoked fEPSPs of the same magnitude before and after induction of LTP in the second pathway, showing input-specificity of induced LTP. In 8 slices from 7 TN-R mutants, LTP was significantly smaller (117.3±2.7%, P<0.05) than in wild-type mice. Treatment of slices from wild-type mice with chondroitinase ABC also reduced LTP (120.5±5.1%, 7 slices from 6 animals, P<0.05; Fig. 4.4B). Enzymatically treated and sham-treated slices from TN-R mutants showed similar levels of LTP (Fig. 4.4D): 118.1±3.7% (8 slices from 8 mice) and 117.3±2.7% (8 slices from 7 mice), respectively (P>0.5). Transient potentiation immediately following TBS (or short-term potentiation, STP) was significantly smaller in TN-R deficient mice than in wild-type littermates

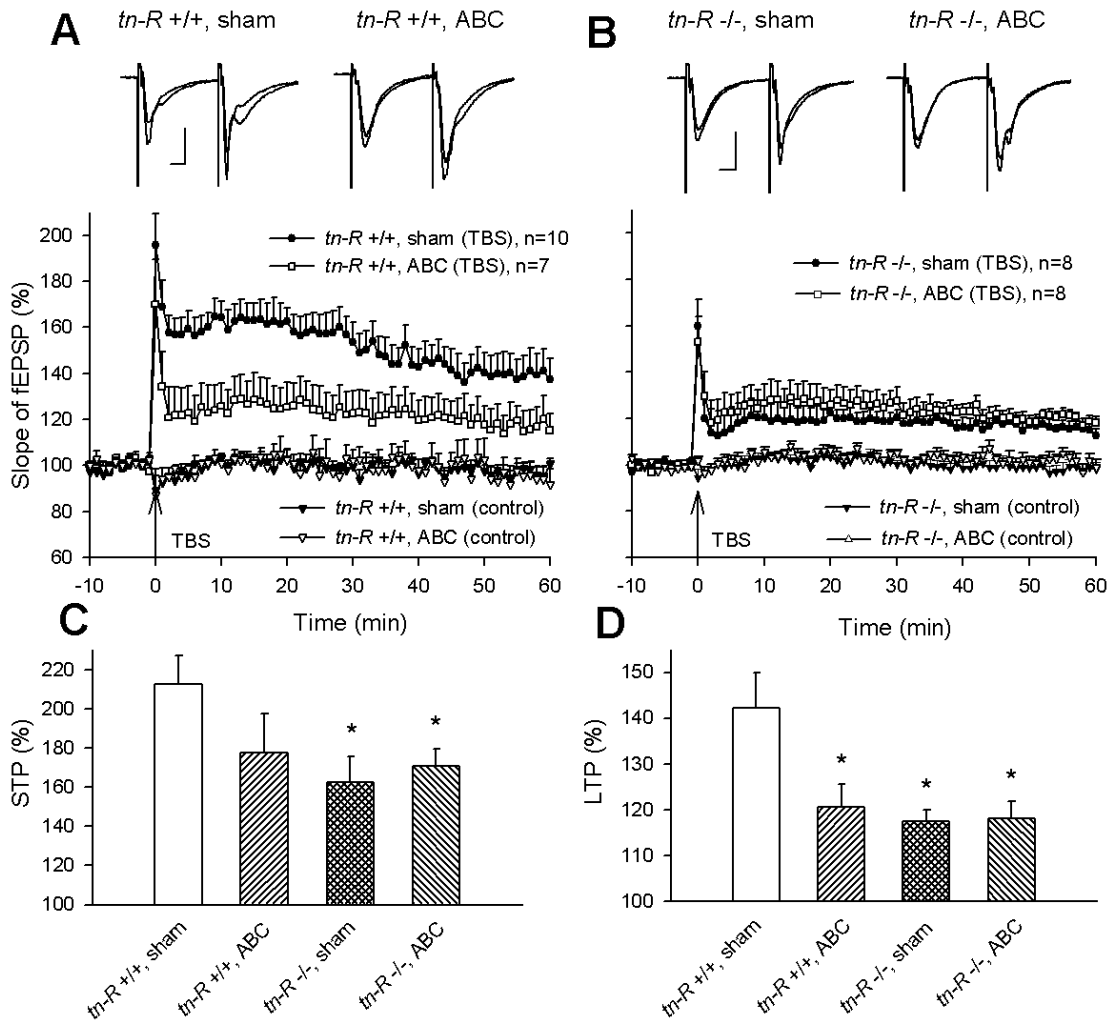


Figure 4.4. Impaired TBS-induced LTP in the CA1 region of hippocampus in TN-R knockout mice and after treatment with chondroitinase ABC

A, B: TBS-induced LTP was recorded in sham- or chondroitinase ABC-treated hippocampal slices from eight wild-type C57BL/6J x 129Ola x 129Sv/Ev (*tn-R +/+, sham* or *tn-R* +/+, *ABC*; **A**) and seven TN-R deficient (*tn-R* -/-, *sham* or *tn-R* -/-, *ABC*; **B**) mice. Data shown represent mean + SEM of slopes of fEPSPs evoked in the control pathway (*control*) and the pathway to which TBS was applied (*TBS*). Traces on the top provide fEPSPs (average of 10 sweeps) collected before and 50-60 min after TBS administration. *n* provides the number of slices. Bars, 10 ms and 500 μV.

C: Cumulative data showing that STP was significantly reduced in *tn-R* -/- mice, but not after chondroitinase ABC treatment. *P<0.05, unpaired *t* test, significantly different in comparison to control sham-treated slices from *tn-R* +/+ mice.

D: Cumulative data showing a reduction in LTP in slices from *tn-R* -/- mice and after treatment with chondroitinase ABC in *tn-R* +/+ mice. *P<0.05, unpaired *t* test, significantly different in comparison to control sham-treated slices from *tn-R* +/+ mice.

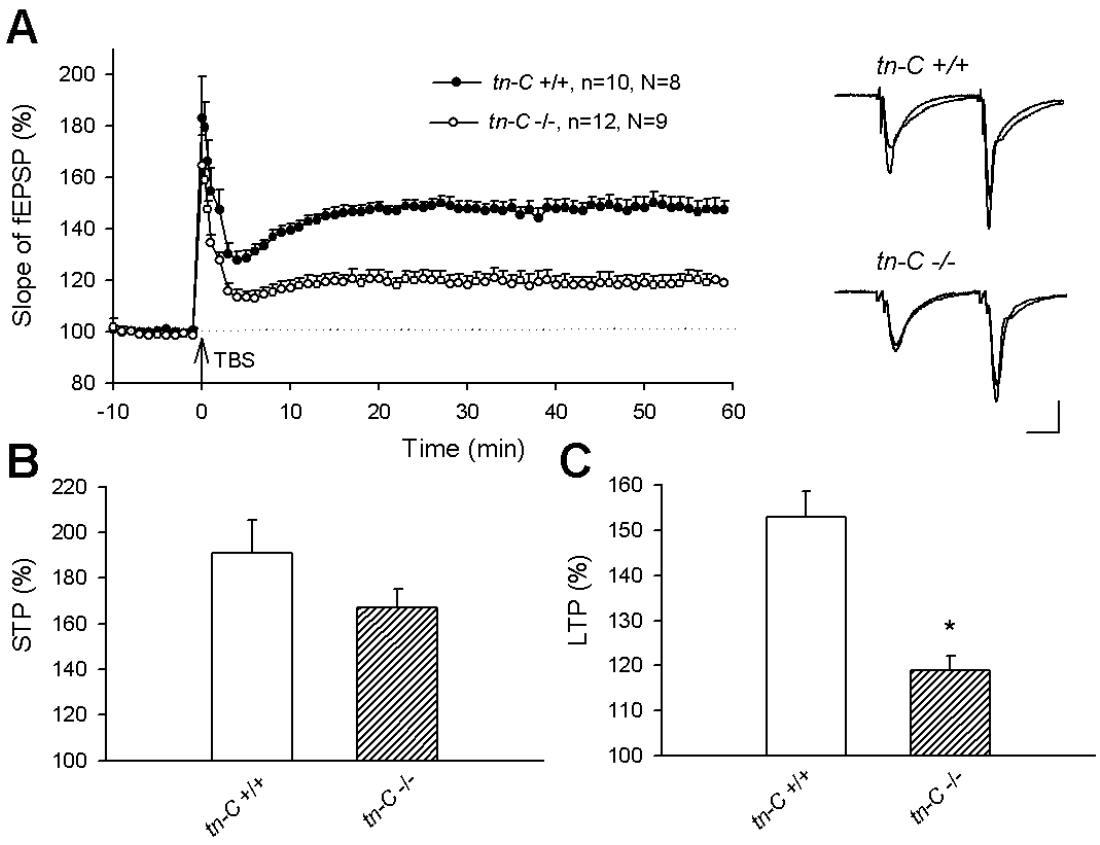


Figure 4.5. TBS-induced LTP in the CA1 region is impaired in TN-C deficient mice

A: TBS of Schaffer collaterals (marked by arrow) evoked a high increase in the slopes of fEPSPs recorded in the CA1 region of slices from wild-type mice (*tn-C* +/+). In slices from TN-C deficient mice (*tn-C* -/-), the potentiation appeared lower than in wild-type mice. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 60 min after TBS. Scale bars, 20 ms and 500 μV.

B: Cumulative plots representing not significantly different levels of STP in slices from *tn-C* +/+ and *tn-C* -/- mice (P>0.1, unpaired *t* test).

C: Cumulative data showing the impairment in LTP in slices from *tn-C* -/- mice. *P<0.05, unpaired *t* test, significantly different in comparison to slices from *tn-C* +/+ mice.

(162.3±13.2% versus 212.5±14.7%, P<0.05). This reduction in STP was not significantly different in the TN-R deficient mutants after incubation with chondroitinase ABC when compared to sham-treated slices (P>0.5; Fig. 4.4C).

No significant difference in STP was observed in TN-C deficient mice (167.3±8.0%, 12 slices from 9 animals) as compare to wild-type littermates (191.1±14.2%, 10 slices from 8 animals, P>0.1; Fig. 4.5B). Whereas TBS reliably produced potentiation in all slices from wild-type mice, TN-C mutants showed significantly reduced LTP (Fig. 4.5A). The level of LTP seen 50-60 min after TBS was 152.9±6.0% in *tn-C* +/+ and 119.1±3.0% in *tn-C* -/- mice (Fig. 4.5C).

4.1.4. NMDA receptor-mediated synaptic transmission in the CA1 region

One of the explanations for reduced LTP in slices treated with chondroitinase ABC and from TN-R and TN-C deficient mutants would be that they have impaired NMDA receptor-mediated transmission. To estimate the NMDA receptor-dependent component, we computed the difference between fEPSPs evoked by single theta-burst before and after application of NMDA receptor antagonist, AP-5.

The examples of TBS-evoked responses recorded before and during application of the NMDA receptor antagonist AP-5 are shown in Figs. 4.6A-C. The slowly decaying component of the responses proved to be mostly NMDA receptor-mediated (Fig. 4.6D) since this response was almost completely eliminated after perfusion of slices with AP-5. There were no differences in the magnitude of the AP-5-sensitive component that was around 6-7% in all groups studied (P>0.1; Fig. 4.6D), implying that NMDA receptor-mediated transmission is not impaired in TN-R and TN-C mutants nor after chondroitinase ABC treatment. Thus, impairment of LTP appeared to be not related to reduced function of NMDA receptors.

4.1.5. Theta-burst stimulation-induced long-term potentiation in the CA1 region under blockade of $GABA_A$ receptors

The difference in the amount of LTP expressed in the presence and absence of $GABA_A$ receptor antagonist would provide an indirect measure of contribution of inhibition in regulation of synaptic plasticity. Moreover, it has been demonstrated that

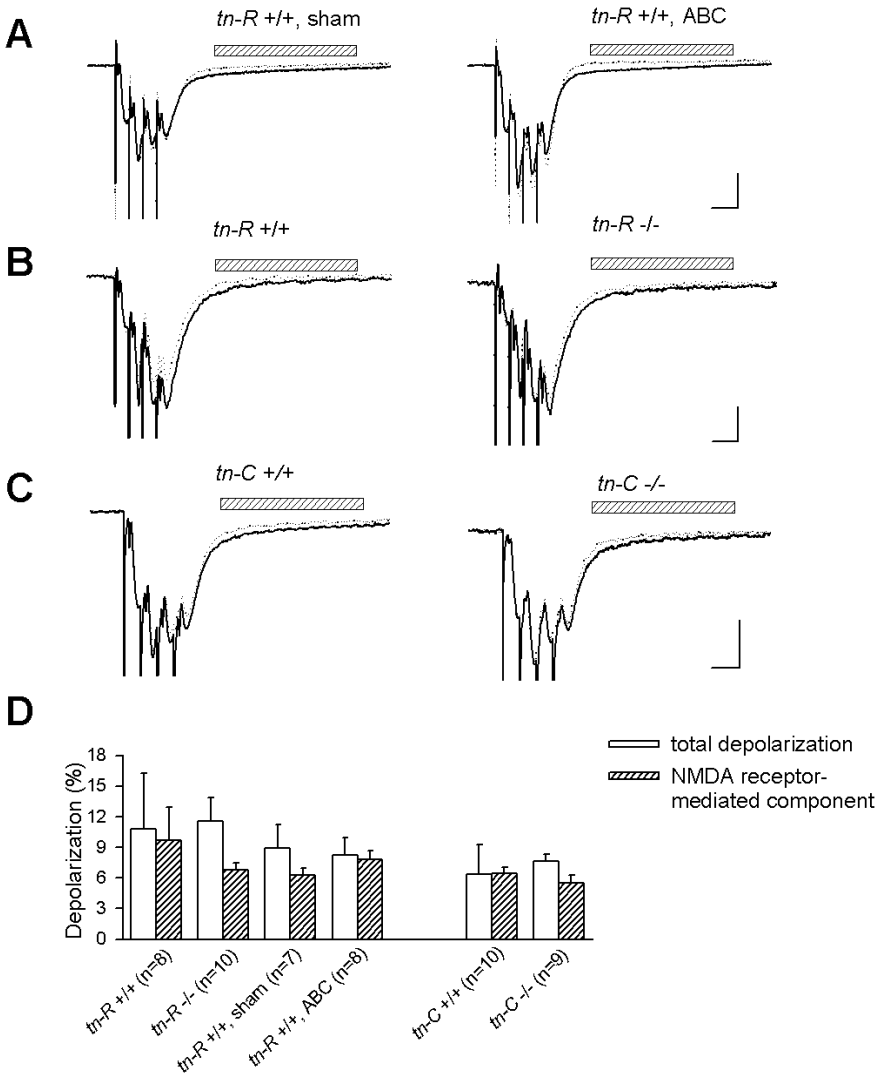


Figure 4.6. Normal composite and NMDA receptor-mediated responses evoked by TBS in TN-R and TN-C mutants and after treatment with chondroitinase ABC

A, B, C: Examples of field responses evoked by TBS applied to Schaffer collaterals in CA1 and recorded before (black line) and after (gray line) application of the NMDA receptor antagonist AP-5 (50 μM). Recordings were performed in sham- (*tn-R +/+, sham*) or chondroitinase ABC-treated (*tn-R +/+, ABC*) slices from three C57BL/6J mice (**A**) and in slices not treated with chondroitinase ABC from five TN-R mutants (*tn-R -/-, sham*) and five control C57BL/6J x 129Ola x 129Sv/Ev (*tn-R +/+, sham*) mice (**B**). The examples of such fEPSP recorded without or in the presence of AP-5 in three wild-type (*tn-C* +/+) and three TN-C deficient mice (*tn-C* -/-) are shown in **C**. Horizontal bar indicates the time interval which was used for measurements of NMDAR-mediated component, calibration bars 20 ms and 1 mV.

D: Cumulative data showing mean values + SEM of total after-burst depolarizations and the underlying NMDA-mediated component calculated as the difference in depolarization recorded before and after application of AP-5 and analyzed for the time period indicated by hatched bars in **A**-**C**. *n* provides the number of slices. Values were normalized using the peak amplitude of the first response in the burst. No significant differences between all groups studied were found ($P>0.05$, unpaired *t* test).

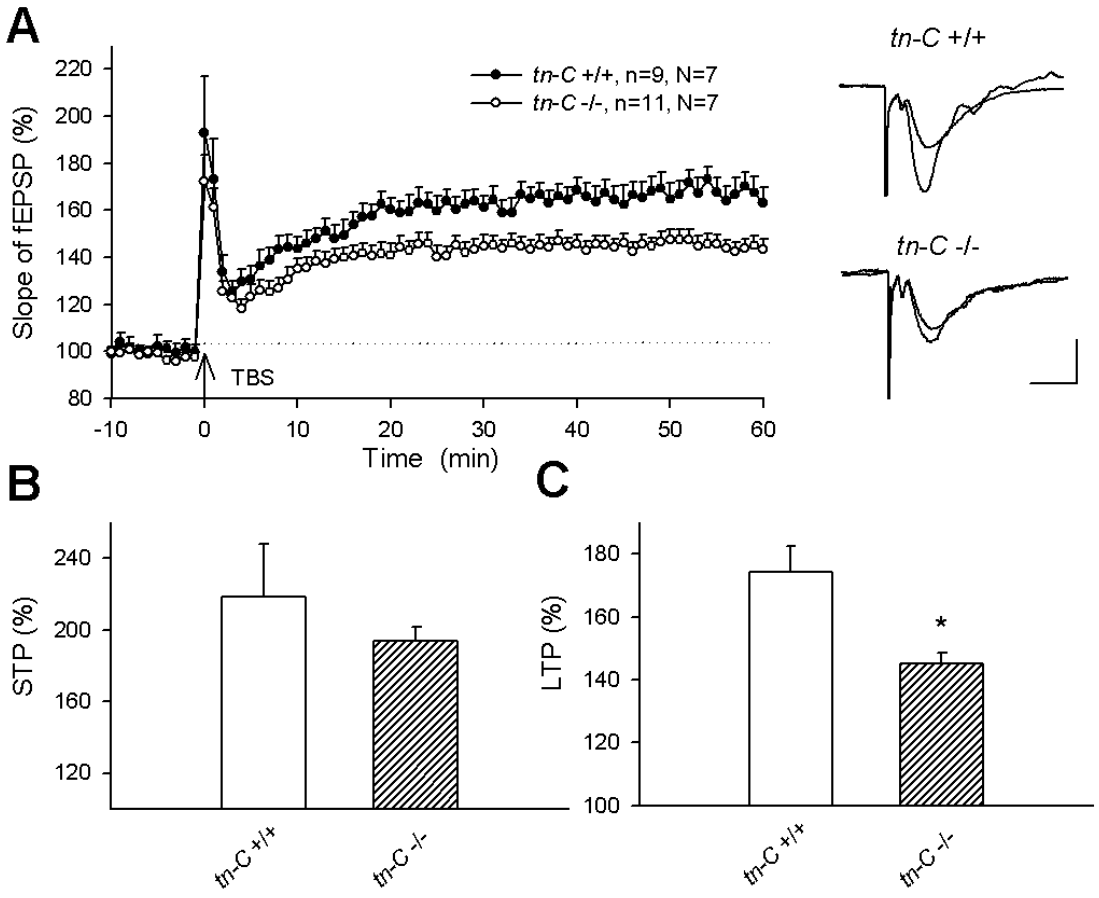


Figure 4.7. Block of $GABA_A$-receptor mediated inhibition does not fully rescue TBS-induced LTP in disinhibited CA1 region in TN-C deficient mice

A: TBS of Schaffer collaterals (applied in the presence of picrotoxin) evoked a high increase in the slopes of fEPSPs recorded in the CA1 region of slices from wild-type mice (*tn-C* +/+). In slices from TN-C deficient littermates (*tn-C* -/-), the potentiation appeared significantly lower than in wild-type mice. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 60 min after TBS. Scale bars, 10 ms and 500 μV.

B: Cumulative plots representing not significantly different levels of STP in slices from *tn-C* +/+ and *tn-C* -/- mice (P>0.1, unpaired *t* test).

C: Cumulative data showing reduced LTP recorded in the presence of picrotoxin in slices from *tn-C* -/- mice. *P<0.05, unpaired *t* test, significantly different in comparison to slices from *tn-C* +/+ mice.

pharmacological blockade of synaptic inhibition gives an apparent facilitation of LTP induction and results in a large increase in the maximal amount of potentiation, that could be produced (Wigstrom and Gustafsson, 1983; Mott and Lewis, 1991; Hsu et al., 1999). Therefore, we recorded CA1 LTP in the presence of picrotoxin, hoping to rescue LTP in TN-R and TN-C mutants, and in slices digested with chondroitinase ABC.

Within 5-10 min after superfusion of the hippocampal slices with picrotoxin (50 μM) the increase of amplitude of fEPSP and spiking activity in the pyramidal cells were observed. After at least 10 min of stable baseline recording during application of antagonist TBS was applied to Schaffer collaterals. The levels of LTP in the presence of picrotoxin were increased in 11 slices from 7 TN-C mutant mice to 145.1±4.1% (Fig. 4.7A). However, this level of LTP remained significantly lower than LTP seen in wild-type littermates (167.5±6.2%, 11 slices from 7 mice; Fig. 4.7C), indicating that mechanisms distinct from GABAergic inhibition mediate most, if not all, reduction of LTP in TN-C deficient mutants. STP values were higher in wild-type mice than in mutants but not significantly (193.0±24.1% versus 172.3±11.3%; Fig. 4.7B).

The levels of STP and LTP in slices incubated with chondroitinase ABC was significantly increased during $GABA_A$ receptor blockade (245.0±14.3% and 173.8±14.9%, 6 slices from 4 animals; Fig. 4.8A-C), as compared to STP and LTP recorded without picrotoxin (Fig. 4.4). These values were not significantly different from sham-treated slices (210.4±18.7% and 173.0±11.9%, 6 slices from 4 animals, $P>0.05$).

No genotype-specific difference in terms of STP and LTP recorded in picrotoxin has been observed in TN-R mutants. STP magnitude was 199.4±18.2% and 210.4±18.7% correspondingly in 6 slices from 4 *tn-R* +/+ and in 6 slices from 4 *tn-R* -/- mice (Fig. 4.9B). The levels of LTP were increased in the presence of picrotoxin in TN-R deficient mice (157.3±11.4%, $P<0.01$), reaching the levels observed in wild-type slices (155.3±5.3%, $P>0.05$; Fig. 4.9A, C).

Thus, the reduced CA1 LTP could be rescued in slices deficient in CSs and TN-R, but not in TN-C, by a pharmacological blockade of $GABA_A$ receptor-mediated transmission.

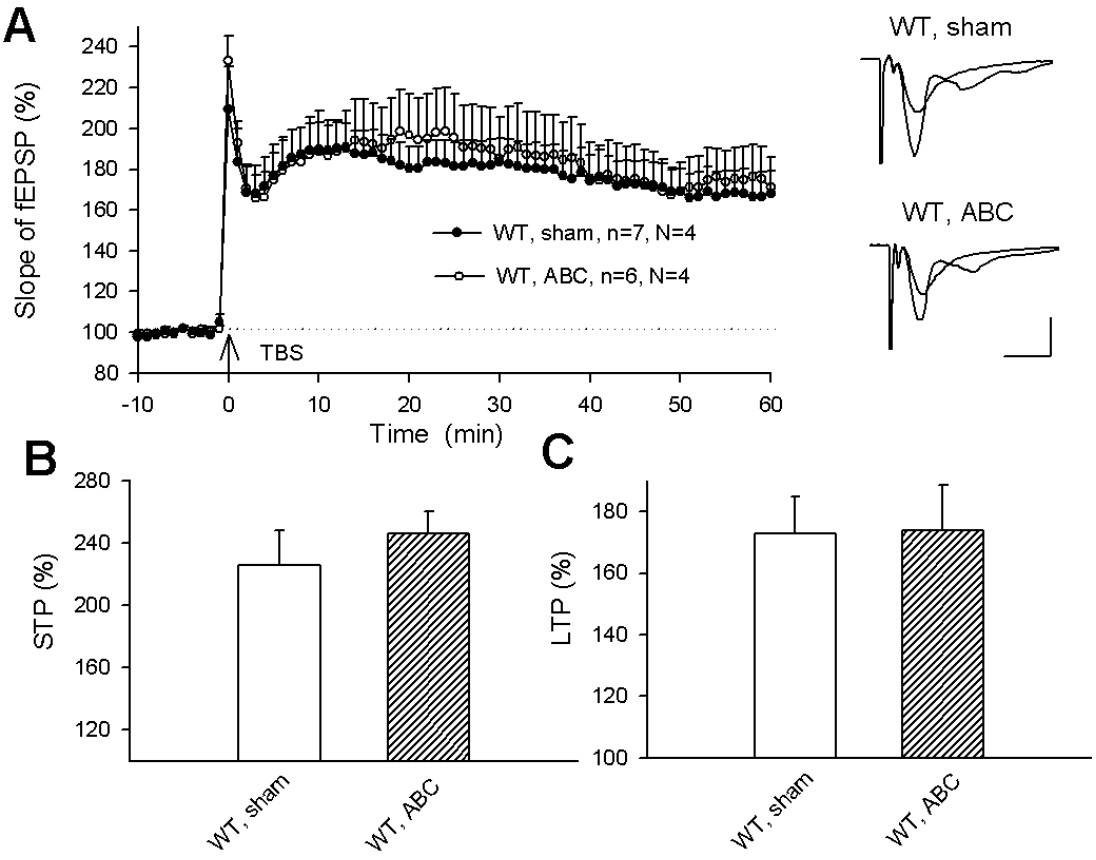


Figure 4.8. Rescued TBS-induced LTP in the CA1 region in slices treated with chondroitinase ABC during $GABA_A$ receptor blockade

A: TBS-induced LTP was recorded in sham- (*WT, sham*) or chondroitinase ABC-treated hippocampal slices (*WT, ABC*) from wild-type C57BL/6J mice in the presence of picrotoxin. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 60 min after TBS. Scale bars, 10 ms and 500 μV.

B, C: Cumulative plots representing not significantly different levels of STP (**B**) and LTP (**C**) in sham- and chondroitinase ABC-treated slices during disinhibition of CA1 region (P>0.1, unpaired *t* test).

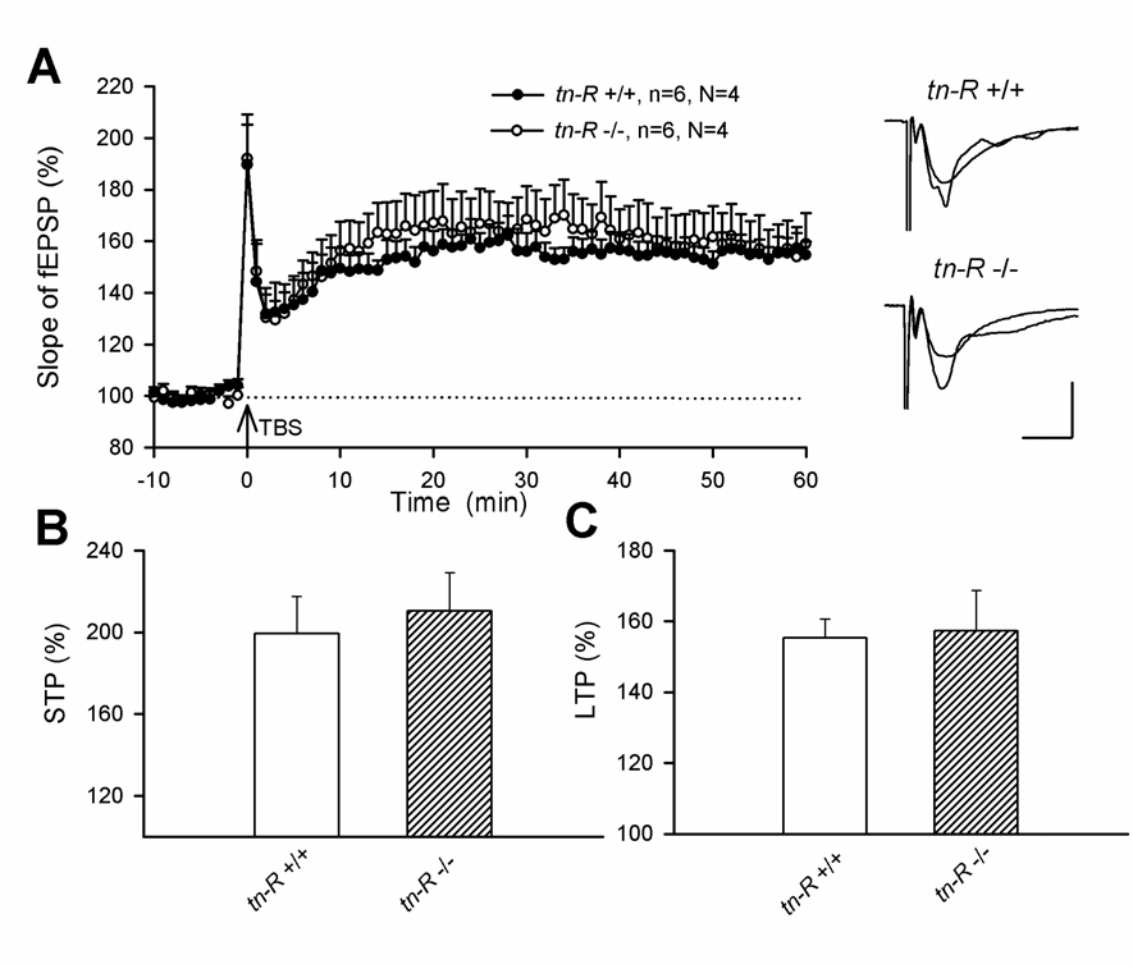


Figure 4.9. Block of $GABA_A$-receptor mediated inhibition rescues TBS-induced LTP in disinhibited CA1 region in TN-R deficient mice

A: TBS of Schaffer collaterals (applied in the presence of picrotoxin) evoked a high increase in the slopes of fEPSPs recorded in the CA1 region of slices from wild-type (*tn-R* +/+) and from TN-R deficient mice (*tn-R* -/-). Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 60 min after TBS. Scale bars, 10 ms and 500 μV.

B, C: Cumulative plots representing not significantly different levels of STP (**B**) and LTP (**C**) in slices from *tn-R* +/+ and *tn-R* -/- mice in the presence of picrotoxin (P>0.5, unpaired *t* test).

4.1.6. Long-term depression in the CA1 region

Another NMDA receptor-dependent form of long-term plasticity in CA1 is LTD that can be evoked by two trains of LFS delivered with a 10 min interval. This protocol evoked reliable depression of the fEPSP slope in wild-type mice with mixed C57BL/6J x 129Ola x 129Sv/Ev genetic background (71.6±4.3%, 8 slices from 5 mice; Fig. 5.10A) and in C57Bl/6J mice (78.0±3.3%, 8 slices from 7 mice; Fig. 4.10C). No significant LTD was induced in the control pathway. Normal LTD was also recorded in TN-R mutants (83.0±6.2%, 8 slices from 5 mice, $P>0.1$; Fig. 4.10B), whereas it was strongly impaired after the treatment with chondroitinase ABC in wild-type mice (91.7±4.8%, 8 slices from 6 mice, $P<0.05$; Fig. 4.10D). Interestingly, transient short-term depression (STD) observed immediately after induction of LTD was significantly reduced in TN-R deficient mice (78.1±6.5%) as compared to wild-type mice (59.5±4.0%, $P<0.05$), whereas it appeared normal after treatment with chondroitinase ABC (70.8±3.8%,) as compared with sham-treated slices (64.3±5.6%, $P>0.1$; Fig. 4.10C). Thus, there is a reduction in STD and normal LTD in TN-R mutant mice, whereas removal of CSs blocks LTD but does not affect STD.

During LFS a transient facilitation was observed with the similar levels of facilitation and time-course for both wild-type and TN-C deficient mice. The mean level of STD was significantly lower in mutants than in wild-types (48.9±3.5% versus 31.2±3.7%; Fig. 4.11B). A long-term reduction of the fEPSP slope by more than 15 % was seen in 7 out of 8 slices prepared from 6 wild-type mice. On average, fEPSP slopes were reduced 50-60 min after induction of LTD to 72.0±5.5% (Fig. 4.11A,C). In TN-C mutant mice no reduction bigger than 13% was induced. The mean slope of fEPSPs measured 50-60 min after the second LFS was 99.4±3.5% (8 slices from 4 animals). Thus, there is a reduction in STD and abolishment of LTD in TN-C deficient mice.

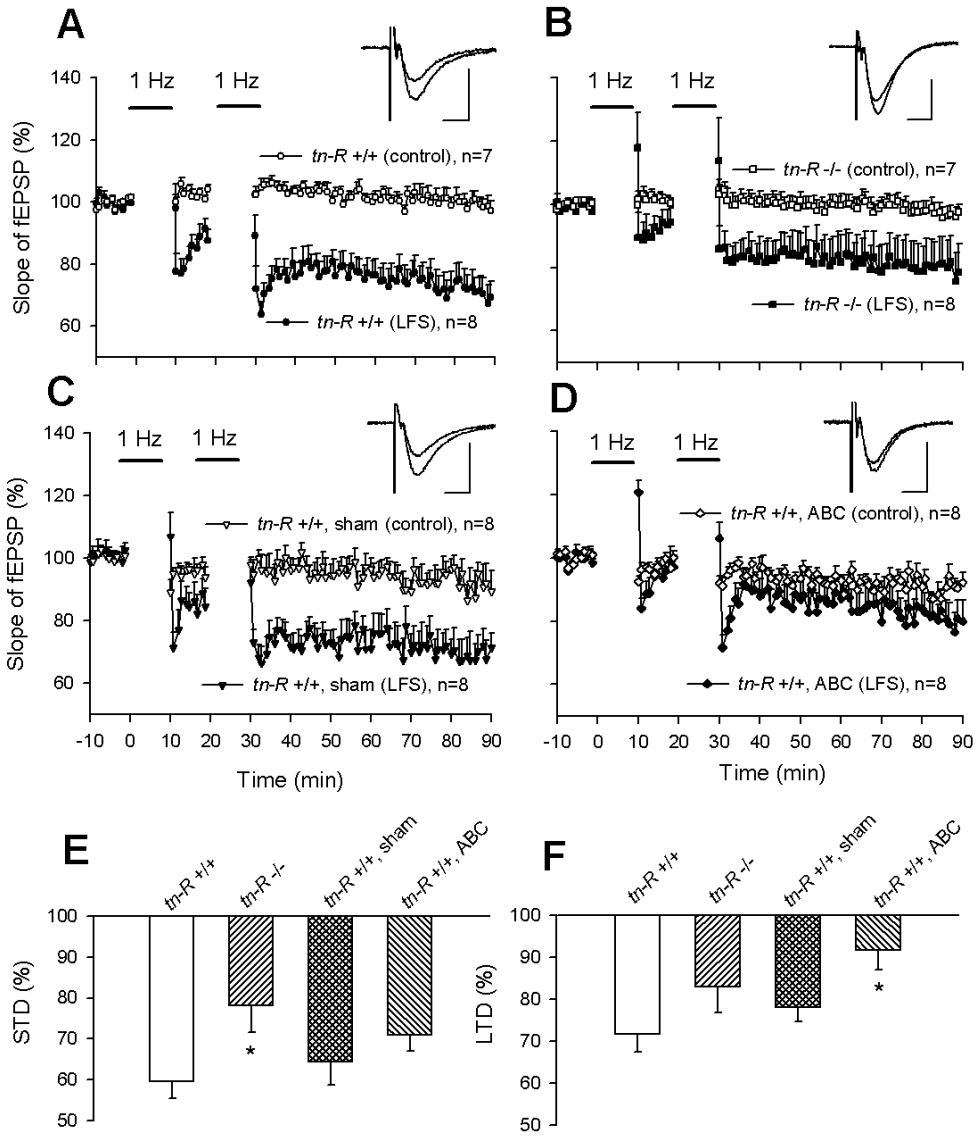


Figure 4.10. LTD was impaired in wild-type mice after treatment with chondroitinase ABC, but not in TN-R knockout mice

A, B: Similar levels of CA1 LTD produced by two trains of LFS (*1 Hz*, indicated by horizontal bars) were seen in slices from five C57BL/6J x 129Ola x 129Sv/Ev wild-type (*tn-R* +/+; **A**) and five TN-R deficient mice (*tn-R* -/-; **B**). **A-D:** Data shown represent mean + SEM of slopes of fEPSPs evoked in the control pathway (*control*) and the pathway to which LFS was applied (*LFS*). Traces on the top of each panel illustrate a depression of fEPSP in 60 min after LFS, n gives the number of tested slices. Bars, 10 ms and 500 μV.

C, D: LTD was reduced in slices from *tn-R* +/+ mice treated with chondroitinase ABC (*tn-R +/+, ABC*) (**D**) in comparison to non-treated slices (*tn-R +/+, sham*) (**C**). *n* provides the number of tested slices from seven C57BL/6J wild-type mice.

E, F: Cumulative data showing a significant reduction of STD in *tn-R* -/- mice (**E**) and of LTD by treatment with chondroitinase ABC in *tn-R* +/+ mice (**F**). *P<0.05, unpaired *t* test, significantly different in comparison to control slices from *tn-R* +/+ mice.

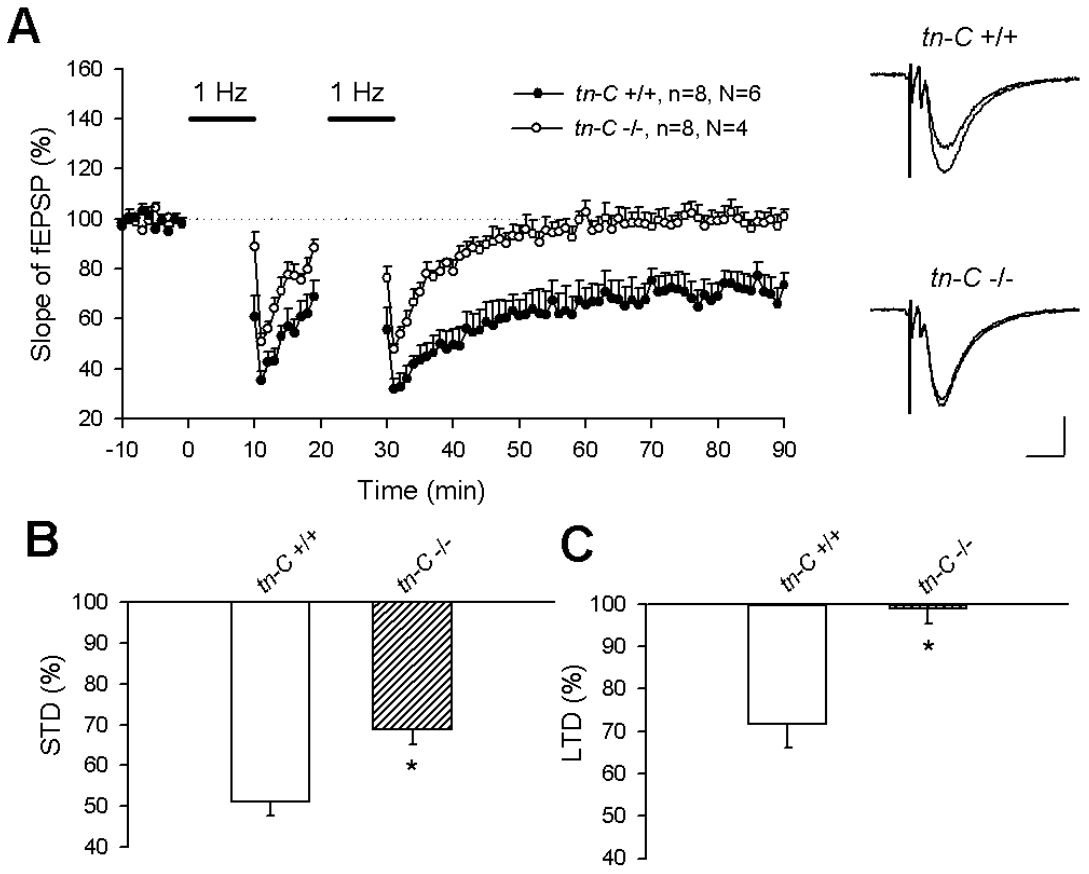


Figure 4.11. LTD in the CA1 region is impaired in TN-C deficient mice

A: Two trains of LFS (*1 Hz*, indicated by horizontal bars) of Schaffer collaterals reliably decreased the slopes of fEPSPs in slices from wild-type mice (*tn-C* +/+). In slices from TN-C deficient mice (*tn-C* -/-), the slope returned to the baseline. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. The panels on the right show fEPSPs in wild-type *tn-C* +/+ and *tn-C* -/- mutant mice before and 60 min after induction of LTD. Scale bars, 10 ms and 500 μV.

B,C: Cumulative plots representing levels of STD (**B**) and LTD (**C**) from all experiments. Lower values of both STD and particularly LTD are evident for TN-C mutants when compared to wild-type littermates. *P<0.05, unpaired *t* test, significantly different in comparison to control slices from tn-*C* +/+ mice.

4.1.7. Pairing-induced long-term potentiation and long-term depression in the CA1 region

Since in TN-R mutants both reduced and normal LTP, depending on the induction conditions, could be observed, LTP in CA1 region was induced using whole cell patch clamp recording technique. This method has an advantage as compared to field recordings, providing tighter control of postsynaptic depolarization for induction of LTP. Pairing of LFS of Schaffer collaterals with postsynaptic depolarization to 0 mV produced a stable increase in postsynaptic current recorded during 30 min. The levels of potentiation were 193.1±25.5% (6 slices from 5 animals) and 185.7±23.3% (6 slices from 5 animals) in *tn-R* +/+ and *tn-R* -/- mice, respectively (Fig. 4.12). To prove that the changes in the synaptic strength occur only when LFS is paired with cell depolarization, the following control recordings were performed. First, LFS was applied when cell membrane potential was kept at –60 mV (close to the cell's resting membrane potential). No potentiation was observed in this case (99.2±7.3%, 4 slices from 3 mice). Second, the pyramidal cell was depolarized to 0 mV without the stimulation of Schaffer collaterals. Such a procedure also induced no potentiation of EPSCs (95.9±5.0%, 4 slices from 2 mice). Thus, as expected, only an association of pre- and postsynaptic activity led to LTP.

To exclude the possibility that increased temperature in the recording chamber (30°C) could facilitate LTP induced by pairing protocol, we applied TBS to Schaffer collaterals and recorded changes in fEPSP in slices prepared from the same animals (14- to 22-day-old) and at the same recording conditions as for whole cell recordings. Still, TBS could produce a robust potentiation in wild-type (162.8±9.9%, 11 slices from 8 animals) and reduced LTP in TN-R deficient mice (124.0±7.9%, 6 slices from 5 animals; Fig. 4.13A), similar to that found in recordings performed at the room temperature. In contrast, the STP levels were not significantly different between genotypes (250.1±11.6% and 254.5±25.0%; Fig. 4.13B) at 30°C, whereas they were different at the room temperature.

Pairing protocol (to 0 mV) provided much higher levels of LTP than TBS in wild-type mice. To investigate whether a weaker induction protocol would reveal a difference between genotypes, we performed another set of experiments, in which cells were

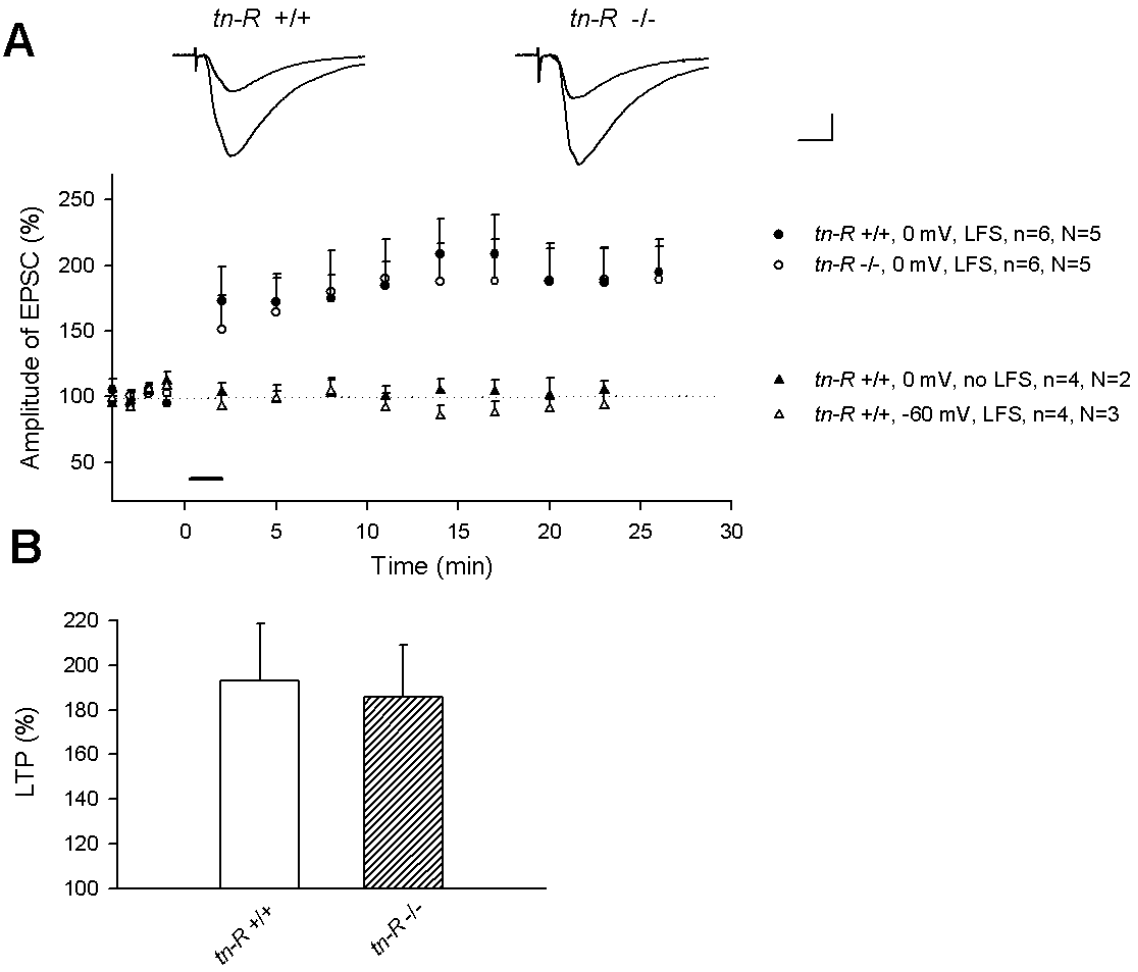


Figure 4.12. Normal LTP induced by pairing to +0 mV in the CA1 pyramidal cells of TN-R deficient mice

A: The pairing of LFS (1 Hz) of Schaffer collaterals with cell depolarization to 0 mV (marked by bar) induced a strong potentiation of responses both wild-types (*tn-R +/+, 0 mV, LFS*) and TN-R mutants (*tn-R -/-, 0 mV, LFS*). No potentiation was produced by depolarization of cells to 0 mV without LFS (*tn-R +/+, 0 mV, no LFS*) or by LFS not paired with depolarization (*tn-R +/+, -60 mV, LFS*). The mean amplitude of EPSCs evoked at 0.033 Hz in pyramidal-like cells voltage-clamped at -60 mV during 5 min before pairing was taken as 100 %. Data depict mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the top show EPSCs recorded before and 30 min after LTP induction in *tn-R* +/+ and *tn-R* -/- mice. Scale bars, 10 ms and 100 pA.

B: Cumulative plots representing not significantly different levels of LTP in slices from *tn-R* +/+ and *tn-R* -/- mice (P>0.5, unpaired *t* test).

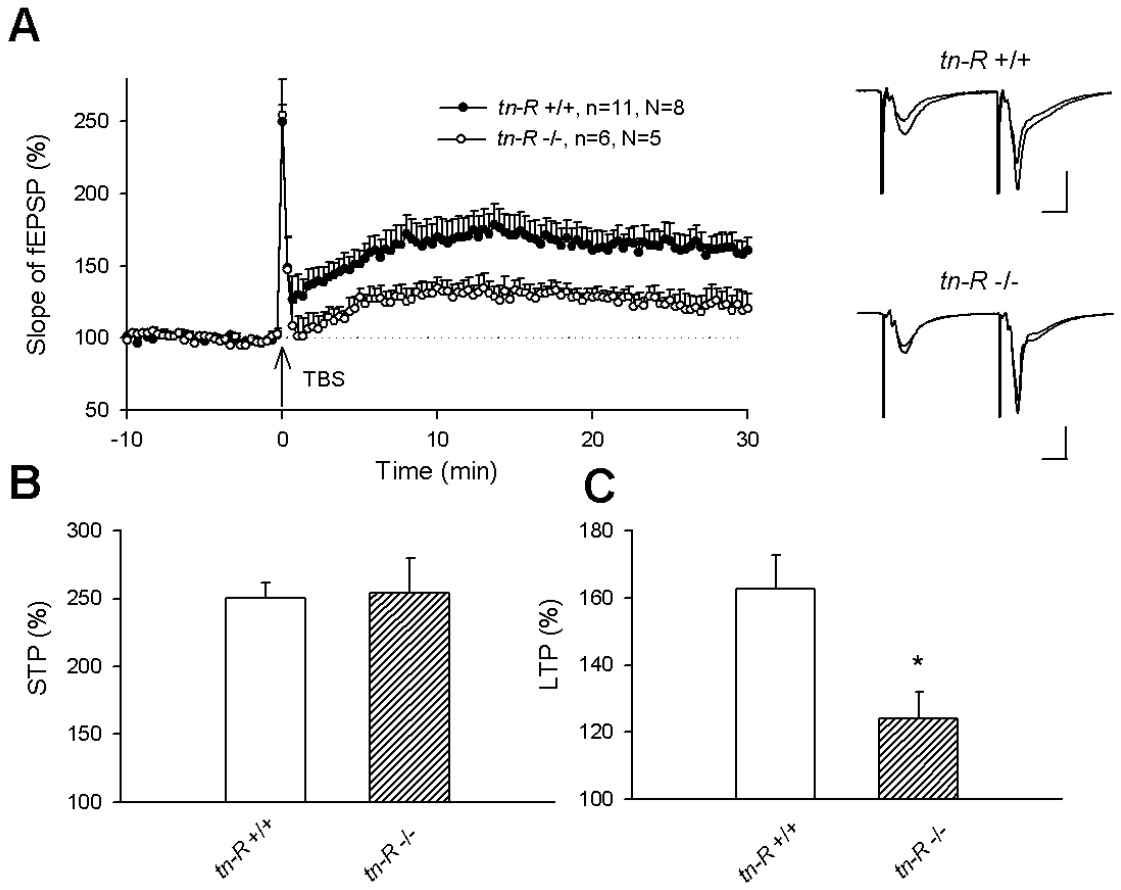


Figure 4.13. Impaired TBS-induced LTP in the CA1 region of young TN-R deficient mice at 30^{o} C

A: TBS of Schaffer collaterals (marked by arrow) evoked a high increase in the slopes of fEPSPs recorded in the CA1 region of slices from wild-type mice (*tn-R* +/+). In slices from TN-R deficient mice (*tn-R* -/-), the potentiation appeared lower than in wild-type mice. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 30 min after TBS. Scale bars, 10 ms and 500 μV.

B: Cumulative plots representing not significantly different levels of STP in slices from young *tn-R* +/+ and *tn-R* -/- mice (P>0.5, unpaired *t* test).

C: Cumulative data showing the impairment in LTP in slices from young *tn-R* -/- mice. *P<0.05, unpaired *t* test, significantly different in comparison to slices from *tn-R* +/+ mice.

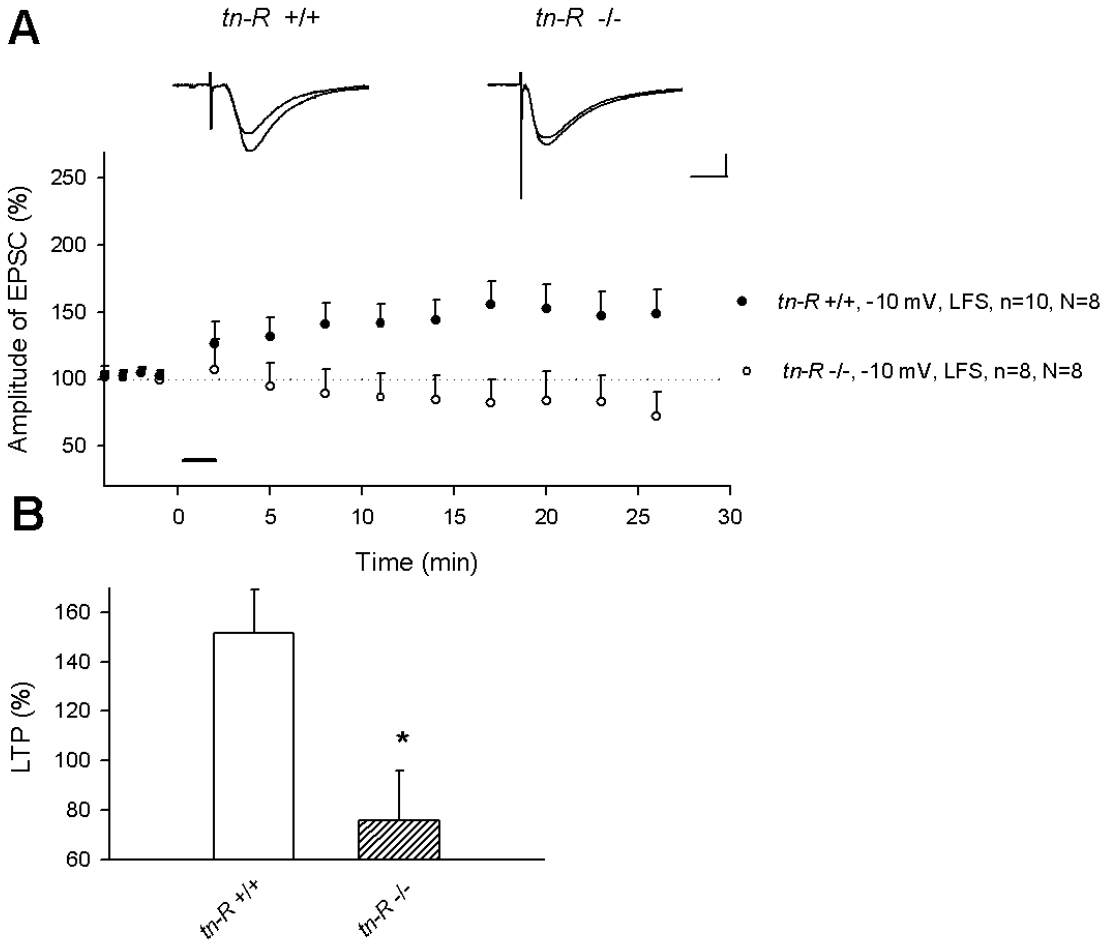


Figure 4.14. LTP induced by pairing to –10 mV in CA1 pyramidal cells is abolished in TN-R mutants

A: The pairing of LFS (1 Hz) of Schaffer collaterals with cell depolarization to -10 mV (marked by bar) induced a potentiation of responses in wild-type (*tn-R +/+, -10 mV, LFS*), whereas no potentiation was produced by this protocol in slices from TN-R deficient mice (*tn-R -/-, -10 mV, LFS*). Data depict mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the top show EPSCs recorded before and 30 min after pairing in *tn-R* +/+ and *tn-R* -/- mice. Scale bars, 10 ms and 50 pA.

B: Cumulative plots representing the impairment in LTP in slices from *tn-R* -/- mice. *P<0.05, unpaired *t* test, significantly different in comparison to slices from *tn-R* +/+ mice.

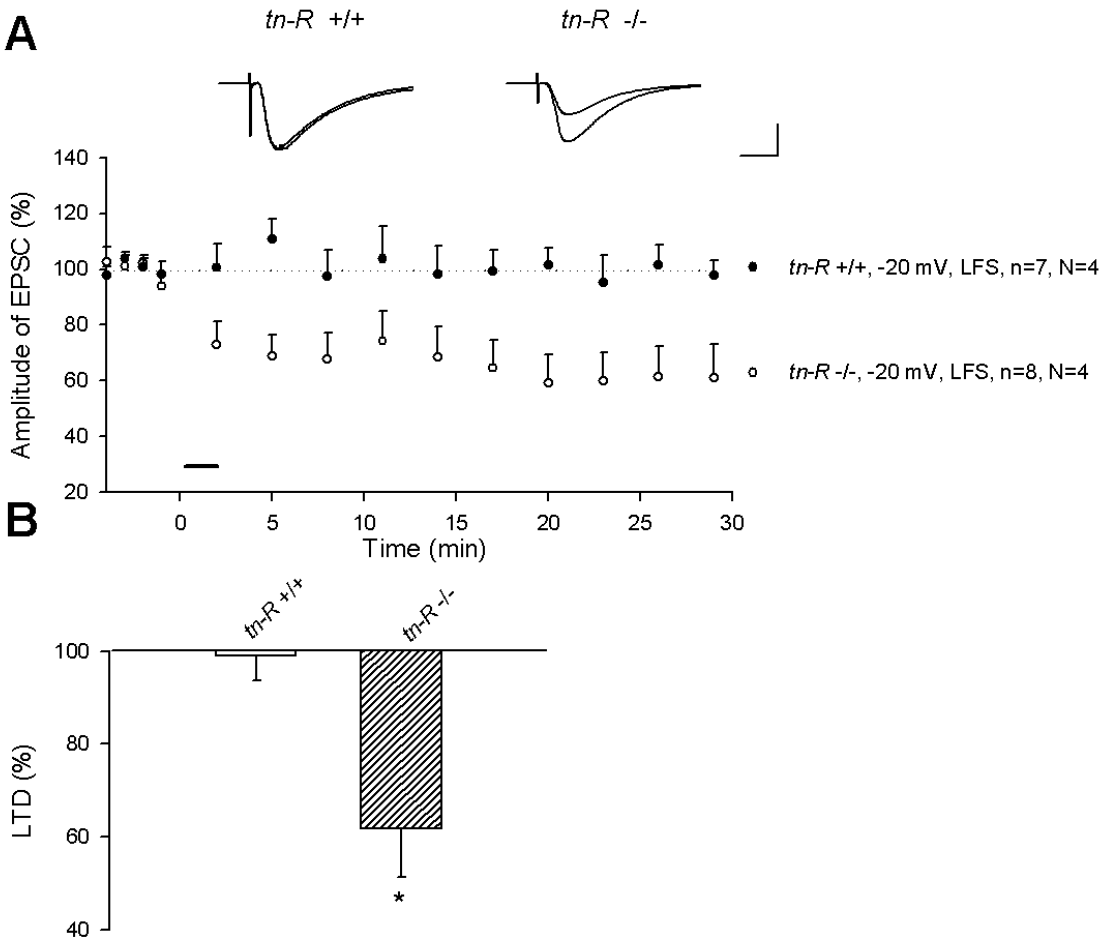


Figure 4.15. Pairing to –20 mV induced LTD in the CA1 region in TN-R deficient mice and no long-term modification of synaptic plasticity in wild-type mice

A: The pairing of LFS (1 Hz) of Schaffer collaterals with cell depolarization to -20 mV (marked by bar) induced no changes in the amplitude of responses in wild-type (*tn-R +/+, -20 mV, LFS*), whereas strong depression was produced by this protocol in slices from TN-R deficient mice (*tn-R -/-, -20 mV, LFS*). Data depict mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the top show EPSCs recorded before and 30 min after pairing in *tn-R* +/+ and *tn-R* -/- mice. Scale bars, 10 ms and 50 pA.

B: Cumulative plots representing LTD in slices from *tn-R* -/- mice and a lack of significant long-term modulation of synaptic transmission in slices from *tn-R* +/+ mice. *P<0.05, unpaired *t* test, significantly different in comparison to slices from *tn-R* +/+ mice.

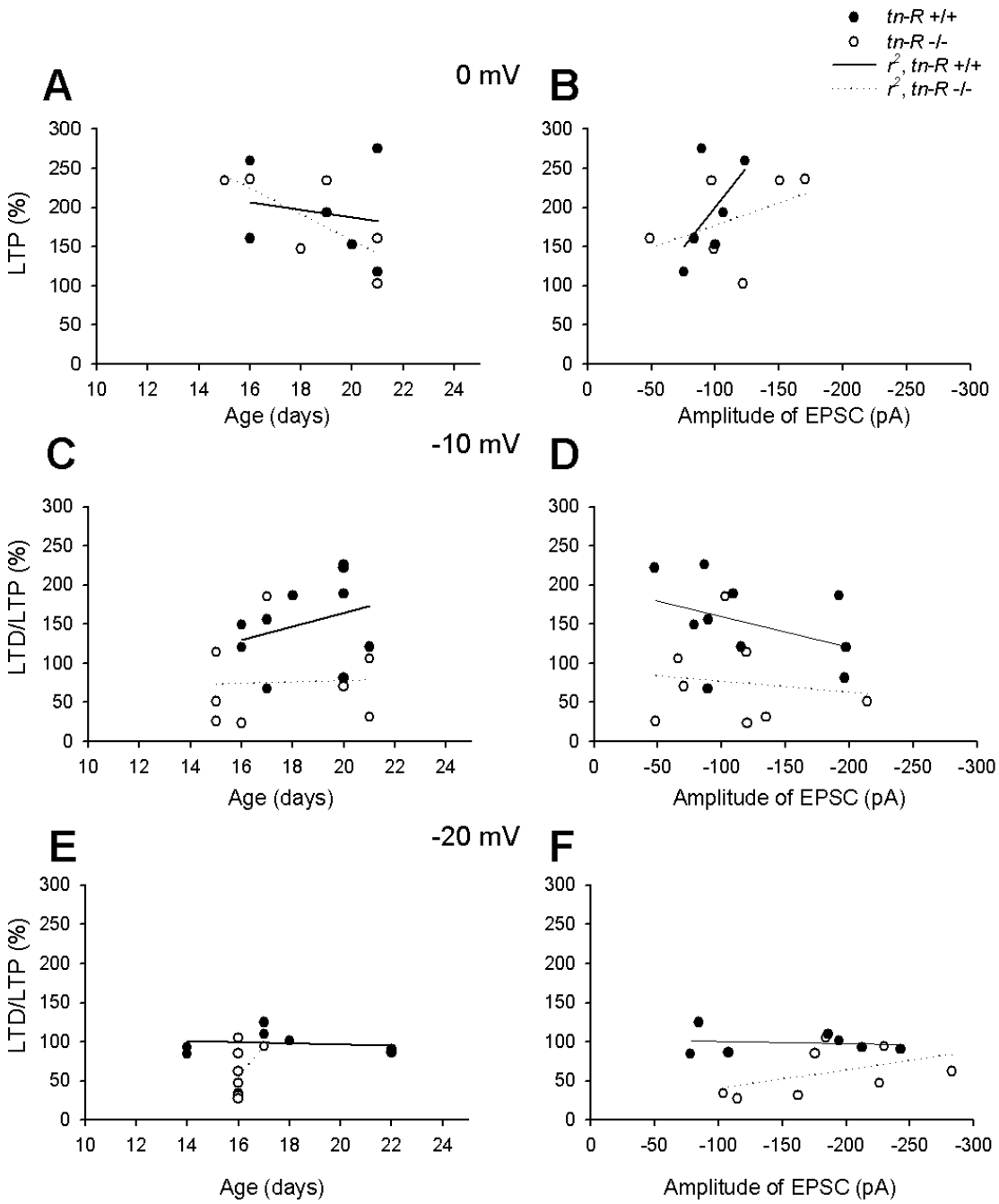

tn-R +/+
tn-R -/-
r², tn-R +/+
r², tn-R -/-
A
0 mV
B
C
-10 mV
D
E
-20 mV
F
LTP (%)
LTD/LTP (%)
Age (days)
Amplitude of EPSC (pA)

Figure 4.16. No correlation between the magnitude of responses and age of animals with pairing-induced LTP and LTD
A: The magnitude of LTP induced by pairing to 0 mV was not correlated with age of wild-type (*tn-R* +/+) and TN-R deficient mice (*tn-R* -/-). The coefficient of correlation r^2 was 0.03 and 0.54 in *tn-R* +/+ (n=6) and *tn-R* -/- (n=6), respectively.
B: The magnitude of LTP induced by pairing to 0 mV was not correlated with the amplitude of EPSC before pairing. The coefficient of correlation r^2 was 0.03 in wild-type (*tn-R* +/+) and 0.19 in TN-R deficient mice (*tn-R* -/-).
C: The magnitude of LTP/LTD induced by pairing to -10 mV was not correlated with age of wild-type (*tn-R* +/+) and TN-R deficient mice (*tn-R* -/-). The coefficient of correlation r^2 was 0.02 and 0.08 in *tn-R* +/+ (n=10) and *tn-R* -/- (n=8), respectively.
D: The magnitude of LTP/LTD induced by pairing to -10 mV was not correlated with the amplitude of EPSC before pairing. The coefficient of correlation r^2 was 0.16 in wild-type (*tn-R* +/+) and 0.02 in TN-R deficient mice (*tn-R* -/-).
E: The magnitude of LTP/LTD induced by pairing to -20 mV was not correlated with age of wild-type (*tn-R* +/+) and TN-R deficient mice (*tn-R* -/-). The coefficient of correlation r^2 was 0.02 and 0.2 in *tn-R* +/+ (n=7) and *tn-R* -/- (n=8), respectively**.**
F: The magnitude of LTP/LTD induced by pairing to -20 mV was not correlated with the amplitude of EPSC before pairing. The coefficient of correlation r^2 was 0.02 in wild-type (*tn-R* +/+) and 0.2 in TN-R deficient mice (*tn-R* -/-).
100% corresponds to the baseline level of EPSC amplitude, i.e. no LTP or LTD was induced, valid for **A**-**F**

depolarized to –10 mV instead of 0 mV. As has been reported previously, pairing to -10 mV also produced LTP in the slices from wild-type mice, although of lower magnitude (Ngezahayo et al., 2000). This stimulation protocol produced an increase of EPSC in 8 of 10 slices in 8 wild-type mice. The mean level of potentiation, measured 25-30 min after LTP induction was 151.7±17.4% (Fig. 4.14), whereas in TN-R-deficient mice no significant long-term changes in the amplitude were observed (76.0±19.9%, 8 slices from 8 animals). No significant correlation between the amplitude of EPSCs or age of animals with the levels of LTP induced by pairing to –10 mV or 0 mV was found (Fig. 4.16A-D), showing that genotype-specific differences in LTP levels are not related to these factors.

Thus, TN-R mutants exhibit increased threshold for induction of LTP, but strong induction protocols elicits normal LTP in these knockout mice.

Lowering the level of depolarization during pairing to –20 mV resulted in no significant long-term modulation of synaptic transmission in wild-type mice (Fig. 4.15A; 98.7±5.5%, 7 slices from 4 mice), confirming the previously published data (Ngezahayo et al., 2000). However, in TN-R deficient mice, a strong LTD to levels lower than 60% of baseline was observed in 5 of 8 slices from 4 mice. The mean amplitude of EPSCs measured 25-30 min after pairing was 60.6±10.8% in TN-R mutants (Fig. 4.15). No significant correlation between the amplitude of EPSCs before pairing or age of animals with the levels of LTD was revealed (Fig. 4.16E,F). Thus, TN-R deficient mice exhibit genotype-specific reduced threshold for induction of LTD in the CA1 region of the hippocampus.

4.1.8. Long-term potentiation in the CA3 region

Further, another form of hippocampal plasticity, namely mossy fiber LTP, that has mechanisms clearly distinct from CA1 LTP and LTD, were analyzed. Field EPSPs evoked in CA3 pyramidal cells by mossy fiber stimulation are known to be fast, to exhibit paired-pulse facilitation and potentiation during 0.33 Hz stimulation. These criteria were taken to search for responses that were further pharmacologically identified using L-CCG1 as an agonist of type II mGluRs, which is known to downregulate synaptic transmission in CA3 mossy fiber synapses (Cremer et al., 1998; Maccaferri et al., 1998; Yeckel et al., 1999).

LFS (0.33 Hz) potentiated fEPSPs to approximately 250% in wild-type and TN-C mutant mice (Fig. 4.17A). L-CCG1 similarly diminished the amplitude of fEPSPs in both genotypes (Fig. 4.17B). The NMDA receptor antagonist AP-5 did not affect the amplitude of selected fEPSPs neither in wild-type nor in TN-C deficient mice (Fig. 4.17C). HFS performed in the presence of AP-5 induced a strong increase in fEPSP amplitude (Fig. 4.17C). PTP during the first 1 min after HFS was 878.4±101.0% in *tn-C* +/+ (7 slices from 7 animals) and 955.9±128.5% in *tn-C* -/- (7 slices from 6 animals) (Fig. 4.17D). The mean potentiation measured 50-60 min after induction of LTP in wild-type and TN-C deficient mice was 200.6±15.7% and 205.3±11.5%,

correspondingly (Fig. 4.17E), resembling reported profiles of LTP in CA3 (Maccaferri et al., 1998; Yeckel et al., 1999). There was no genotype specific difference in short- or long-term potentiation (P>0.5). To confirm that we really recorded PKA-dependent mossy fiber LTP, HFS was applied after incubation and perfusion of slices prepared from wild-type mice with PKA antagonist Rp-cAMPS. This treatment strongly diminish both PTP and LTP of recorded fEPSP (411.8±38.8% and 115.1±9.6%, respectively, 7 slices from 5 mice; Fig. 4.17C).

LFS stimulation of mossy fiber provides similar facilitation of fEPSP in wild-types and TN-R mutants (Fig. 4.18A). The application of L-CCG1 reduced the amplitude of fEPSPs by 80% in both genotypes (Fig. 4.18B). The amplitude of selected fEPSPs was stable during 15 min perfusion of AP-5 both in *tn-R* +/+ and in *tn-R* -/- mice (Fig. 4.18C). In 7 slices from 7 wild-type mice HFS induced robust PTP and LTP (758.1±78.5% and 183.0±25.6%, respectively). The level of LTP was 214.6±25.9% and transient potentiation was 930±133.7% in TN-R mutant mice (6 slices from 5 animals, Fig. 4.18D,E), which were not significantly different from corresponding values in wild-type littermates (P>0.1).

Thus, we conclude that NMDA receptor-independent PKA-mediated LTP in mossy fiber - CA3 synapses is normal in TN-R and in TN-C deficient mice.

Figure 4.17. Normal LTP in the CA3 region of TN-C mutants

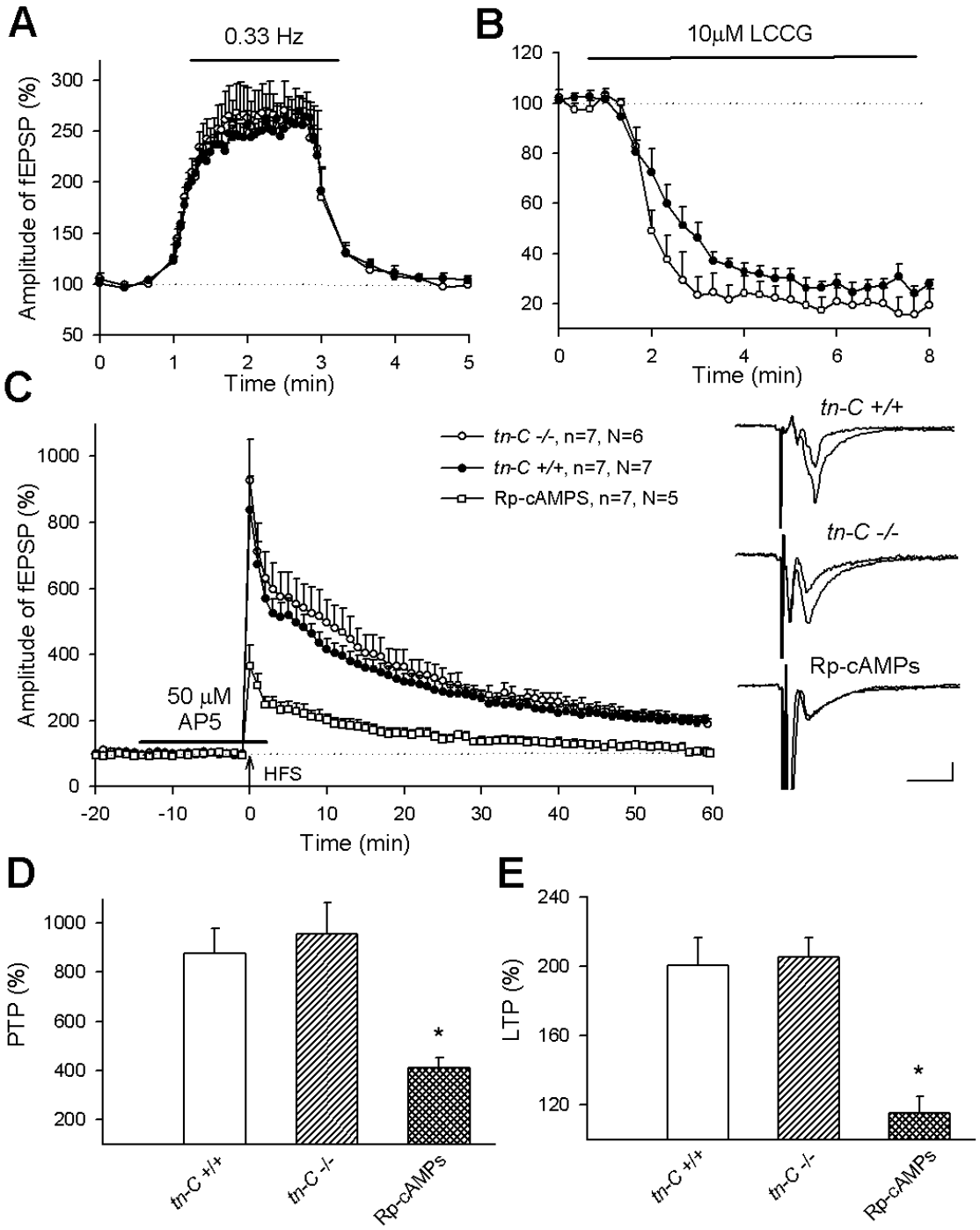
A: Stimulation of mossy fibers with a frequency of 0.33 Hz similarly increased the amplitudes of fEPSPs in slices from wild-type (*tn-C* +/+) and TN-C deficient mice (*tn-C* -/-).

B: Application of the type II metabotropic glutamate receptor agonist L-CCGI (10 μM) similarly reduced the amplitude of fEPSPs in slices from *tn-C* +/+ and *tn-C* -/- mice.

C: HFS of mossy fibers (indicated by arrow) evoked a similar increase in amplitude of fEPSPs in slices *tn-C* +/+ and *tn-C* -/- mice. The potentiation was impaired in slices treated with a competitive inhibitor for PKA, Rp-cAMPS. The time interval of the application of the NMDA receptor antagonist AP-5 is shown by a horizontal bar. Right panels show averaged fEPSPs recorded before and 60 min after induction of LTP in *tn-C* +/+ and *tn-C* -/- mice. Scale bars, 10 ms and 100 μV.

D: Cumulative plot representing similar level of PTP in slices from *tn-C* +/+ and *tn-C* -/- mice. The incubation of slices from *tn-C* +/+ mice with Rp-cAMPS significantly reduced PTP. The values represent the maximal amplitude of fEPSP measured during the first minute after HFS. *P<0.05, unpaired *t* test, significantly different in comparison to slices from tn-*C* +/+ mice.

E: Cumulative data showing LTP from all experiments. Note similar values of LTP in *tn-C* +/+ and *tn-C* -/- mice and that Rp-cAMPS diminished LTP. *P<0.05, unpaired *t* test, significantly different in comparison to slices from tn-*C* +/+ mice.


A
0.33 Hz
Amplitude of fEPSP (%)
Time (min)
B
10µM LCCG
Time (min)
C
tn-C -/-, n=7, N=6
tn-C +/+, n=7, N=7
Rp-cAMPS, n=7, N=5
Amplitude of fEPSP (%)
50 µM AP5
HFS
Time (min)
tn-C +/+
tn-C -/-
Rp-cAMPs
D
PTP (%)
tn-C +/+
tn-C -/-
Rp-cAMPs
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tn-C +/+
tn-C -/-
Rp-cAMPs

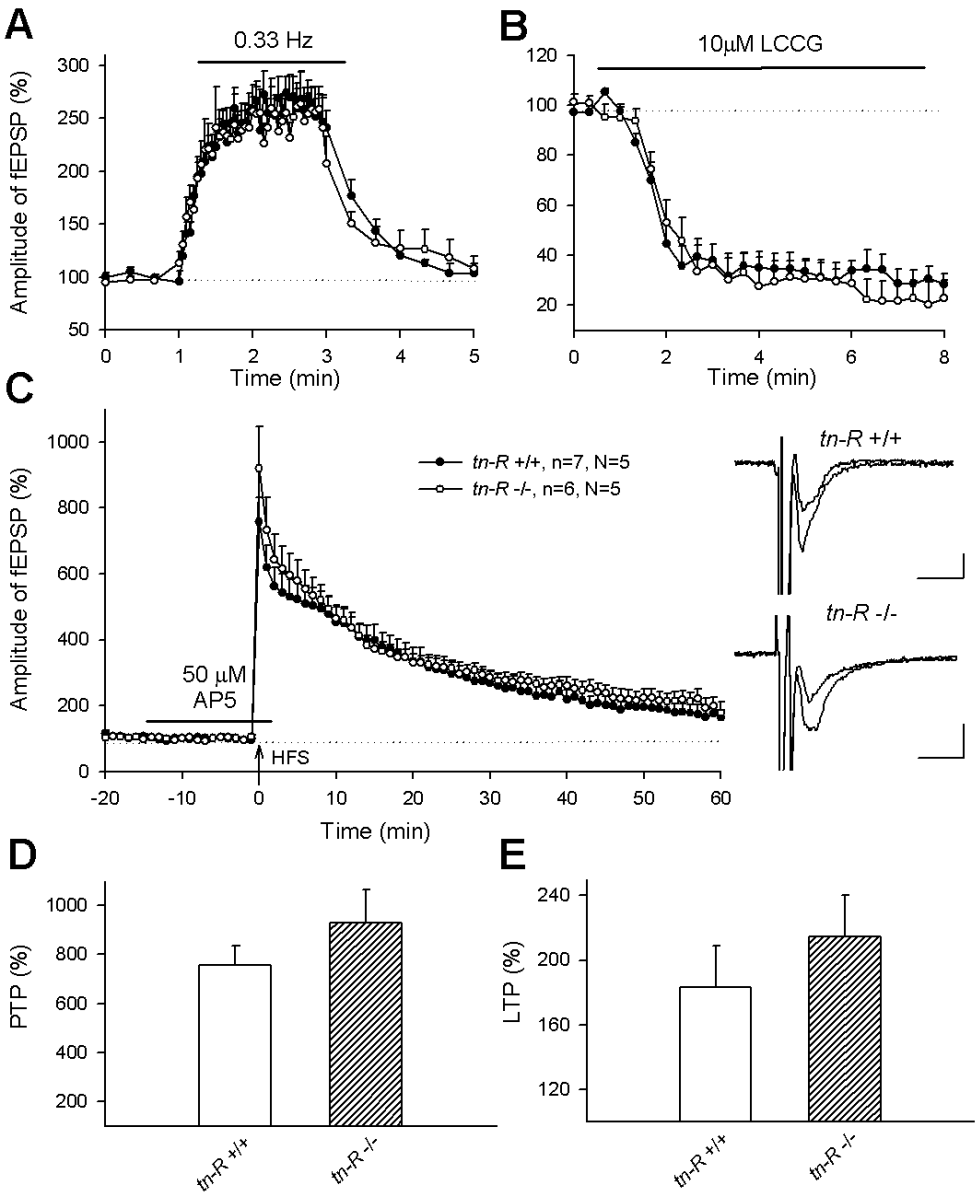


Figure 4.18. No genotype-specific difference in LTP in the CA3 region of TN-R knockout mice

A: Stimulation of mossy fibers with a frequency of 0.33 Hz similarly increased the amplitudes of fEPSPs in slices from wild-type (*tn-R* +/+) and TN-R deficient mice (*tn-R* -/-).

B: Application of the type II metabotropic glutamate receptor agonist L-CCGI (10 μM) similarly reduced the amplitude of fEPSPs in slices from *tn-R* +/+ and *tn-R* -/- mice.

C: HFS of mossy fibers (marked by arrow) evoked a similar increase in amplitudes of fEPSPs in slices from *tn-R* +/+ and *tn-R* -/- mice. The time interval of the application of the NMDA receptor antagonist AP-5 is shown by a horizontal bar. Right panels show averaged fEPSPs recorded before and 60 min after induction of LTP in *tn-R* +/+ and *tn-R* -/- mice. Scale bars, 10 ms and 100 μV.

D, E: Cumulative plot representing not significantly different levels of PTP (**D**) and LTP (**E**) in slices from wild-type and TN-R deficient mice (P>0.5, unpaired *t* test).

4.2. THE ROLE OF PSA-NCAM IN SYNAPIC PLASTICITY

4.2.1. PSA expression in PST deficient mice

In the adult brain, two cell populations are known to express particularly high amounts of PSA and exhibit phenotypic abnormalities in NCAM-deficient and endo-N-treated animals: neuroblasts migrating in the rostral migratory stream to the olfactory bulb (Rousselot et al., 1995) and granule and pyramidal neurons in the hippocampal formation (Seki and Arai, 1993). The immunostainig method was used to investigate the age-dependent PSA expression in the hippocampus of PST deficient mice and age-matched wild-types.

In the hippocampus of 2-week-old wild-type mice, a strong expression of PSA was detected in the innermost layer of the dentate gyrus granule cells and in mossy fiber axons in the hilus region (Fig. 4.19A). From 1 to 6 months of age, PSA expression in this area was somewhat reduced, although still substantial (Fig. 4.19B,C). In wild-type animals of all ages, PSA immunoreactivity was also observed in the CA3 subfield (Fig. 4.19A-C), which represents the mossy fiber termination field on the apical dendrites of pyramidal neurons.

In the hippocampus of 2-week- and 1-month-old PST mutant, PSA expression in the dentate gyrus and hilus was reduced compared to the wild-type, but nevertheless still clearly detectable on the innermost layer of dentate gyrus granule neurons (Fig. 4.19D,E). In contrast, at 6 months of age, PSA expression was virtually undetectable in the dentate gyrus and hilus regions (Fig. 4.19D) as well as in the entire CA3 subfield (Fig. 4.19F).

PSA expression in the CA1 region was considerably lower than in CA3 and had a punctuate appearance as shown before (Muller et al., 1996). Expression was highest in 2-week-old wild-type mice (Fig. 4.20A). In age-matched mutants (Fig. 4.20B) PSA immunoreactivity was strong, although slightly reduced compared to the controls. At 6 months there was still prominent PSA expression in the wild-type mice (Fig. 4.20C), whereas the CA1 region in mutants was negative at this level of detection (Fig. 4.20D).

Thus, age-dependent reduction of PSA expression was observed in hippocampal formation of PST deficient mice.

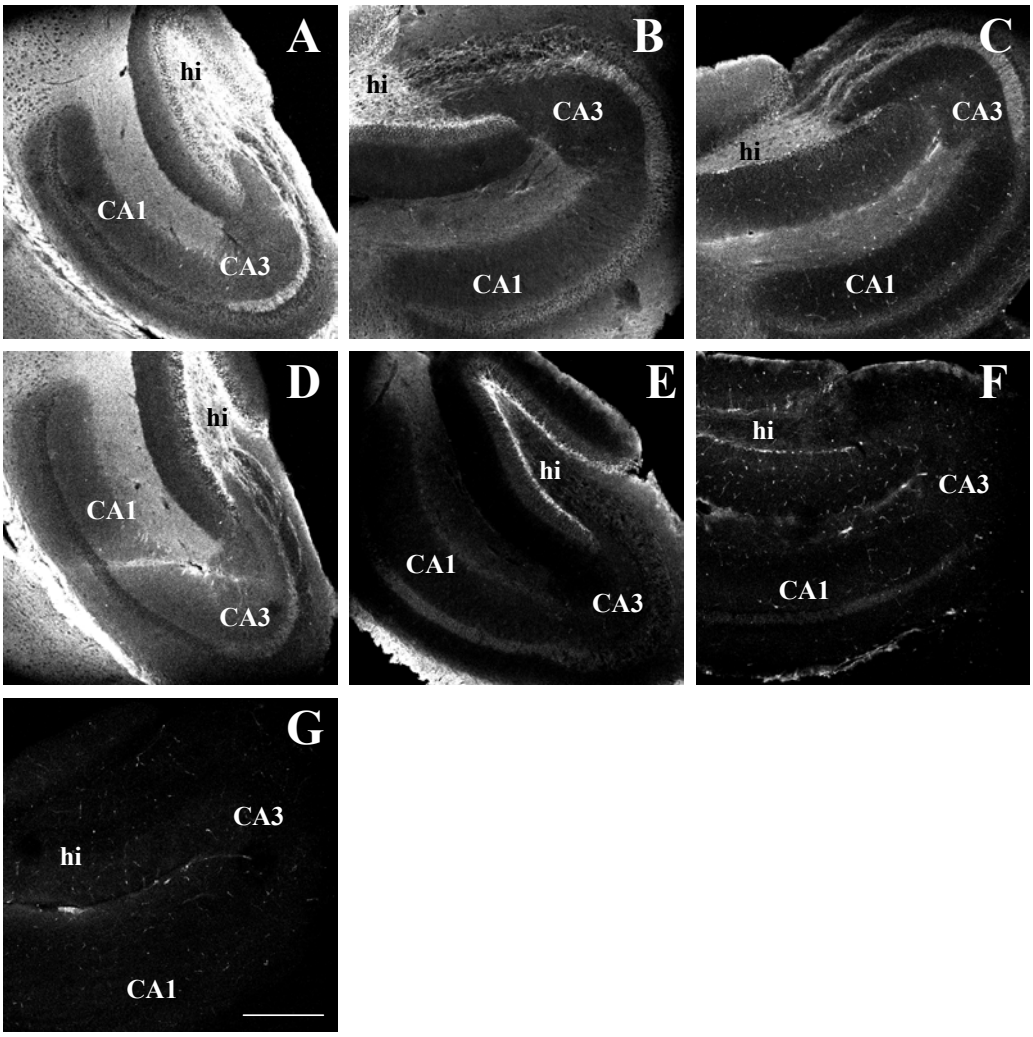


Figure 4.19. Altered expression of PSA in the hippocampus of PST deficient mice
A: Immunohistochemistry with PSA antibody (clone 735) revealed high levels of PSA expression in the dentate gyrus and hilus regions of 2-week-old wild-type mice.
B-C: In 1-month-old (**B**) and 6-month-old (**C**) wild-type animals, PSA expression is somewhat fainter but still considerable. In all situations PSA appears to be mostly expressed to the innermost layer of dentate gyrus granule neurons and their axonal projections, the mossy fibers traveling in the hilus region. The entire CA3 area, representing the mossy fiber termination field on the pyramidal neurons still expresses large amounts of PSA (**B, C**).
D: The expression of PSA in hippocampus of 2-week-old PST deficient animals is comparable with age-matched wild-type controls (**A**).
E: In 1-month-old mutants, PSA expression in the hilus of dentate gyrus is reduced. Nevertheless, many granule cells as well as individual mossy fibers are still positive for PSA.
F: At 6 months of age, expression is almost undetectable in both dentate gyrus and mossy fibers in *pst* -/-. The CA3 area appears entirely devoid of immunoreactivity in mutant animals.
G: When the primary antibodies were omitted and only the secondary antibodies were applied to hippocamdpal slices, no immunostaining was detectable. Scale bar 200 μm, is valid for **A-G**. Images were taken with identical acquisition parameters from slices prepared from the same animal and simultaneously processed for immunostaining. *CA1, CA3* and *hi,* mark hippocampal CA1, CA3 region and hilus, respectively.

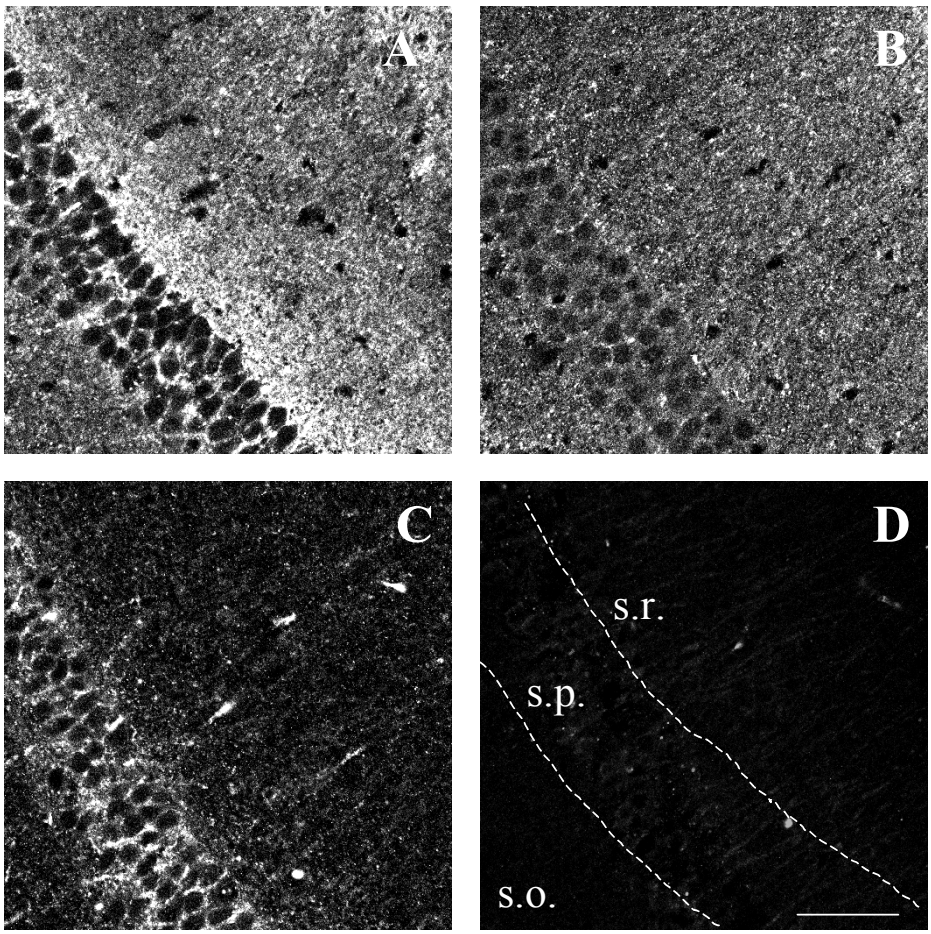


Figure 4.20. Reduced expression of PSA in the CA1 region of adult PST deficient mice

A: Immunohistochemistry with PSA antibody (clone 735) revealed high levels of PSA expression in the CA1 region of 2-week-old wild-type mice.

B: In age-matched PST deficient mice the expression of PSA in stratum pyramidale appears weaker than in wild-types (**A**). However, in *strata radiatum*, where Schaffer collaterals form synapses on CA1 pyramidal neurons, the appearance of punctate immunostaining is comparable in mutant and wild-type animals.

C, D: In 6-month-old wild-type mice (**C**) the intensity of immunostaining for PSA is weaker than in younger wild-type mice (**A**), but still much more prominent than in 6-month-old PST mutants (**D**). Scale bar (50 μm) and outlines of cell layers shown in **D** are valid for **A-C**

Images were taken with identical acquisition parameters from slices prepared from the same animal and simultaneously processed for immunostaining. S.o, s.p., s.r. mark the *strata oriens, pyramidale,* and *radiatum* respectively.

4.2.2. Theta-burst stimulation-induced long-term potentiation in the CA1 region of hippocampus

One consequence of NCAM deficiency is the impairment of LTP and LTD in the CA1 and LTP in the CA3 region of the hippocampus (Muller et al., 1996; Cremer et al., 1998). To determine the effects of a loss in PSA, we analyzed LTP and LTD in the hippocampus of *pst* -/- mice with mixed C57BL/6J and 129/Ola genetic background (C57BL/6J×129/Ola) and age-matched groups of wild-type C57BL/6J, 129/Ola, and C57BL/6J×129/Ola mice.

TBS reliably produced STP and LTP in all slices from wild-type animals independently of their genetic background. The mean levels of LTP seen 50-60 min after TBS were 137.8±2.7% (14 slices from 13 animals) for C57BL/6J mice, 140.6±4.6% (8 slices from 7 animals) for 129/Ola mice, and 152.8±9.0% (8 slices from 4 animals) for C57BL/6J × 129/Ola mice (Fig. 4.21C). Levels of STP for these three strains of mice, were 217.8±14.1, 242±21.9, and 189.8±27.3%, respectively. There was no significant difference between strains in levels of LTP or STP ($P>0.1$, ANOVA). Perfusion of slices 10 min before and during TBS with an antagonist to NMDA receptors (50 μM AP-5) resulted in a complete block of LTP (103.2±0.8%; n=3; C57BL/6J mice). Field EPSPs evoked by stimulation of the control pathway were not significantly potentiated after TBS (mean slopes of fEPSP recorded 50-60 min after TBS in slices from three wild-type strains were in the range of 96.6-100.3%; $P>0.5$), showing input specificity of the recorded LTP. Mutant mice exhibited normal levels of basic synaptic transmission measured as the amplitude of fEPSP at 50% stimulation strength and paired-pulse facilitation with 50 msec interstimulus interval (data not shown). In 13 slices from 8 PST mutants STP value was similar to that observed in wild-type mice (191.2±16.7%, $P>0.1$), but mutants showed impaired LTP in comparison to wild-type mice in all genotypes (123.9±4.6%; $P< 0.05$).

Because of the age-dependent decline of PSA expression (Figs. 4.19 and 4.20), we decided to compare levels of LTP in PST mutants and wild-types of different ages (Fig. 4.21C). In wild-type mice, there were no significant changes in the levels of LTP during development ($P>0.1$). In 1-month-old mutant mice, LTP was similar to that in 4- to 6-month-old mutants and significantly smaller than in 1-month-old wild-type mice

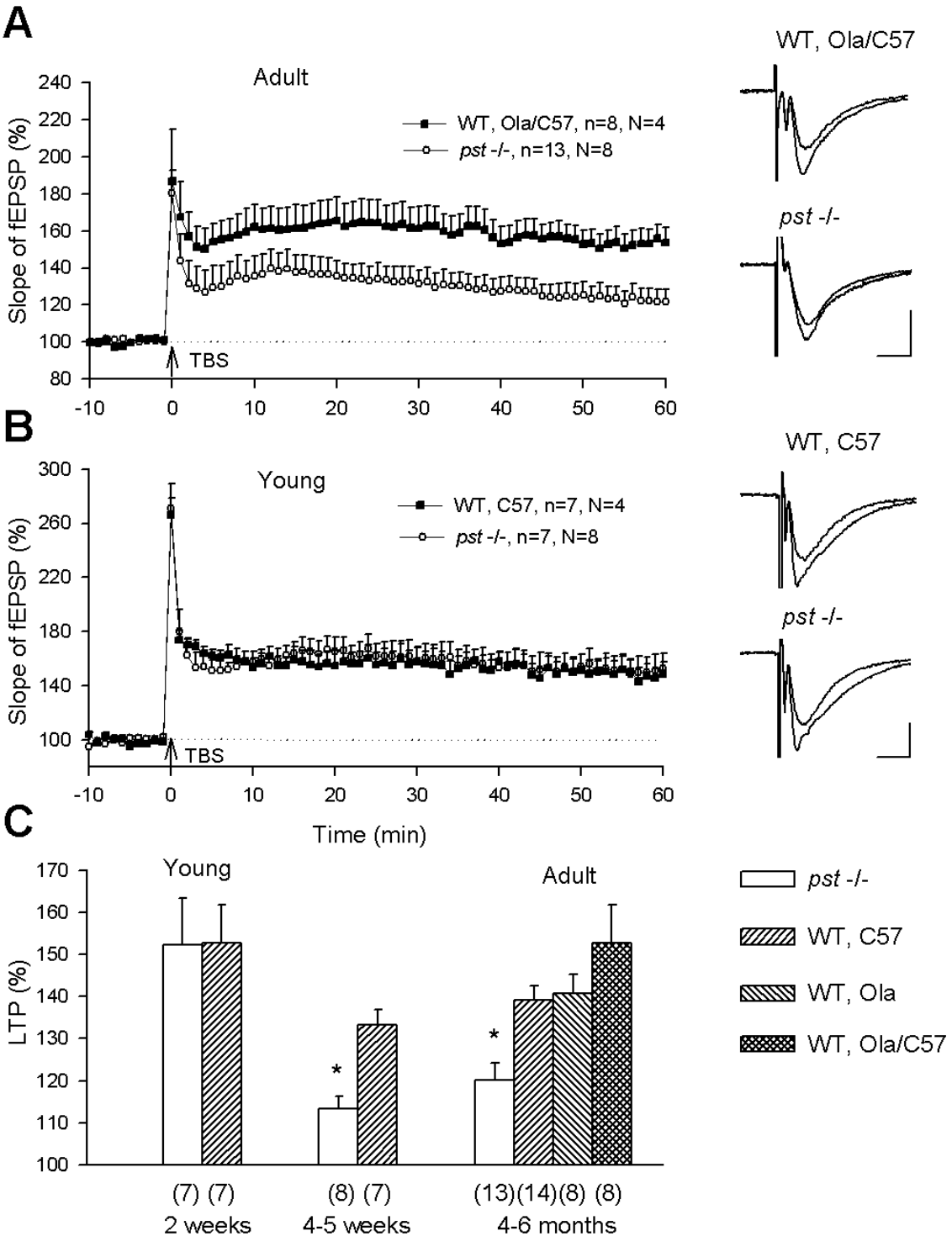

A
Adult
WT, Ola/C57, n=8, N=4
pst -/-, n=13, N=8
Slope of fEPSP (%)
TBS
WT, Ola/C57
pst -/-
B
Young
WT, C57, n=7, N=4
pst -/-, n=7, N=8
Slope of fEPSP (%)
TBS
Time (min)
WT, C57
pst -/-
C
Young
Adult
LTP (%)
(7) (7)
2 weeks
(8) (7)
4-5 weeks
(13)(14)(8) (8)
4-6 months
pst -/-
WT, C57
WT, Ola
WT, Ola/C57

Figure 4.21. TBS-induced LTP in the CA1 region is impaired in adult but normal in young PST knockout mice

A: LTP in slices from 4- to 6-month-old mice. TBS (indicated by arrow) of Schaffer collaterals evoked a high increase in the slopes of fEPSPs recorded in the CA1 region of slices from wild-type C57BL/6J × 129/Ola mice (*WT, C57/Ola*). In slices from PST deficient mice (*pst* -/-), the potentiation immediately followed by TBS appeared normal, but then the slope declined to a level significantly lower than in wild-type mice Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested mice. Panels on the right show fEPSPs recorded before and 60 min after TBS. Calibration: 10 msec, 500 μV.
B: LTP in slices from 17- to 19-day-old mice. Data represent mean + SEM from wild-type (*WT, C57*) and PST deficient mice (*pst* -/-). STP and LTP induced by TBS in mutant mice appeared normal (P>0.5, unpaired *t* test). All abbreviations as in **A**.
C: Developmental changes in LTP in the CA1 region of *pst* -/- mice. In mutants, there is a reduction in LTP, measured as the mean slope of fEPSPs recorded 50–60 min after TBS, between the second and fourth week of age. The level of LTP in wild-type mice remains the same after the second week of postnatal development and is independent of genetic background of the strains used. The numbers in parentheses indicate the number of tested slices. *P<0.01, unpaired *t* test, significantly different from C57BL/6J wild-type mice.

(113.5±2.9%, 8 slices from 4 animals versus 133.3±3.7%, 7 slices from 4 animals; P<0.01). However, in 17- to 19-day-old mutant mice, high LTP was induced that was not significantly different from LTP in the age-matched wild-type group (Fig. 4.21). This level of LTP was higher than that in two groups of 4- to 5-week-old and 4- to 6-month-old mutant mice (P<0.01; Fig. 4.21C). Thus, reduction in LTP develops in a rather narrow time window, between the second and fourth week of postnatal development when downregulation of PSA expression in the hippocampus occurs.

4.2.3. Long-term depression in the CA1 region

To produce LTD in hippocampal slices prepared from mice, two trains of 600 pulses were delivered with a 10 min interval. This stimulation induced a long-term reduction of the fEPSP slope by >10% from the baseline in 10 of 11 slices prepared from 4- to 6-month-old C57BL/6J mice (6 animals). On average, fEPSP slopes were reduced 50-60 min after induction of LTD to 77.4±3.5% (Fig. 4.22B). Similar levels were recorded in 8 slices from 6 129/Ola and in 8 slices from 4 C57BL/6J×129/Ola mice (Fig. 4.22C). This LTD was NMDA receptor-dependent because the levels of LTD were strongly reduced (93.8±2.9%; n=3; C57BL/6J mice) when LTD was induced in the presence of

the NMDA receptor antagonist AP-5. LTD was also input-specific, because fEPSPs evoked by stimulation of the control pathway were not affected. The levels of fEPSP slope in slices from 129/Ola and C57BL/6J×129/Ola mice were 99.3±4.3% and 101.7±2.3%, respectively (P>0.5). In 4 adult mutant mice LTD was induced only in 1 of 6 slices, and the mean slope of fEPSPs was not decreased (103.5±6.5%; Fig. 4.22A). STD observed immediately after induction of LTD was not significantly reduced in PST deficient mice (60.1±4.8%) as compared to wild-types and was 51.6±2.5%, 72.0±4.1%, and 55.2±6.3% in C57BL/6J, 129/Ola and C57BL/6J×129/Ola mice, respectively (P>0.1).

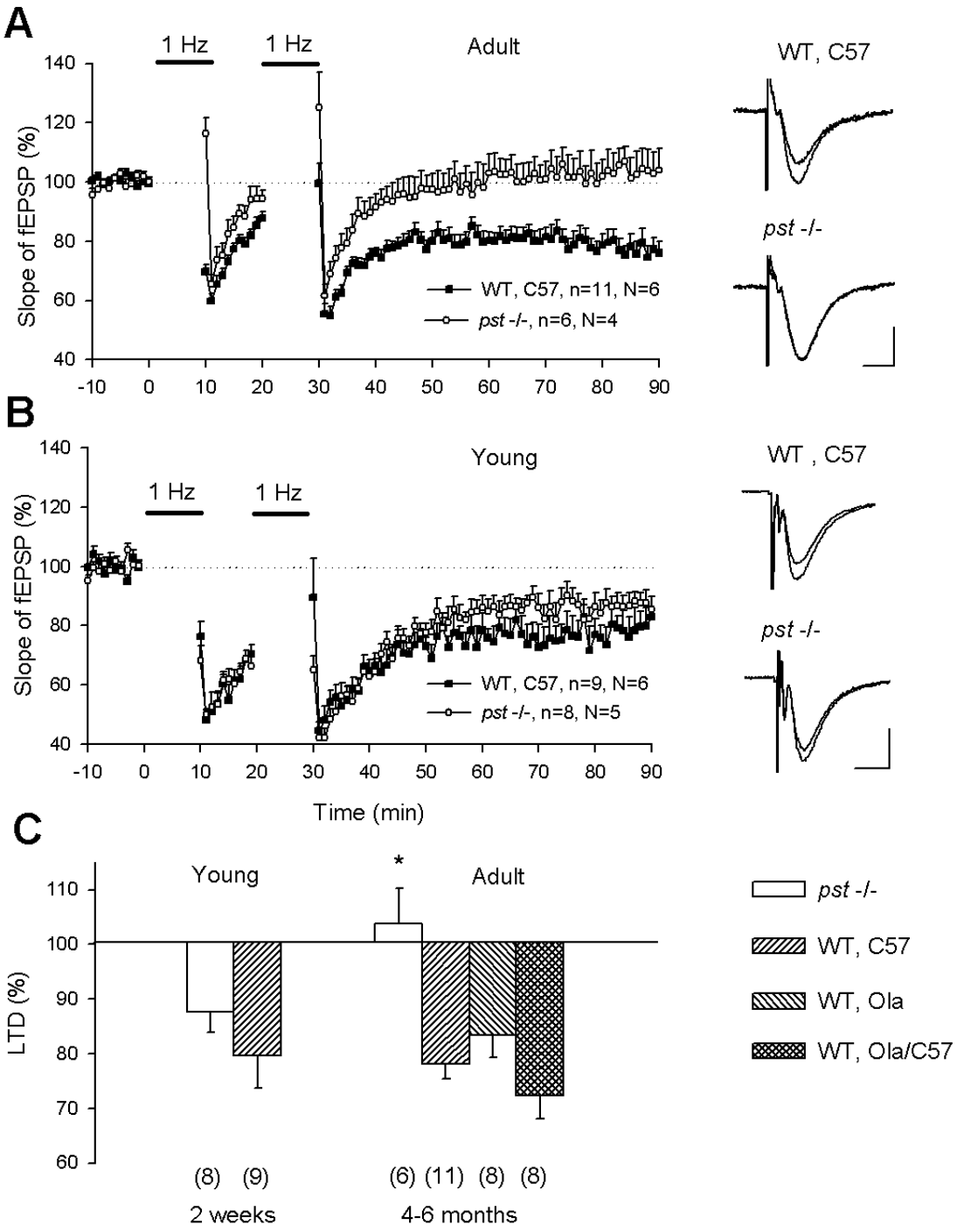
LTD evoked in slices from 14- to 19-d-old C57BL/6J mice (79±6.0%; 9 slices from 6 animals) was not significantly different from that observed in wild-type adult mice (P>0.1; Fig. 4.22B,C). LTD evoked in slices from 13- to 18-d-old mutant mice (87.1±3.7%; 8 slices from 5 animals) was significantly higher than that in adult mutants (P<0.05) and not different from LTD recorded in young wild-type mice (P>0.2). STD appeared normal in slices from young PST deficient mice (43.8±3.9%), as compare to age-matched wild-types (46.1±5.6%). Thus, there is an age-specific impairment of both LTP and LTD in adult but not young mutant mice.

Figure 4.22. LTD in the CA1 region is impaired in adult but normal in young PST deficient mice

A: LTD in slices from 4- to 6-month-old mice. Two trains of LFS (*1 Hz*, indicated by horizontal bars) of Schaffer collaterals reliably decreased the slopes of fEPSPs in slices from wild-type mice (*WT, C57*). In slices from PST deficient mice (*pst* -/-), the slope returned to the baseline. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals. The panels on the right show fEPSPs in wild-type and *pst* -/- mice before and 60 min after induction of LTD. Calibration: 10 msec, 250 μV.

B: LTD in slices from 13- to 19-day-old mice wild-type (*WT, C57*) and PST deficient mice (*pst* -/-). LTD in mutant mice appeared normal (P>0.5, unpaired *t* test). All abbreviations as in **A.**

C: Developmental changes in LTD in the CA1 region of *pst* -/- mice. In mutants, there is an age-dependent impairment of LTD, measured as the mean slope of fEPSPs recorded 50–60 min after induction of LTD. The levels of LTD are similar in wild-type mice of different genetic backgrounds and ages. The numbers in parentheses indicate the number of tested slices. * P<0.01, unpaired *t* test, significantly different from C57BL/6J wild-type mice.


A
1 Hz
1 Hz
Adult
WT, C57
pst -/-
Slope of fEPSP (%)
WT, C57, n=11, N=6
pst -/-, n=6, N=4
B
Young
WT , C57
pst -/-
WT, C57, n=9, N=6
pst -/-, n=8, N=5
Time (min)
C
Young
Adult
*
LTD (%)
pst -/-
WT, C57
WT, Ola
WT, Ola/C57
(8) (9)
(6) (11) (8) (8)
2 weeks
4-6 months

4.2.4. Long-term potentiation in the CA3 region

The fast kinetic of fEPSPs, high PPF ratio, potentiation during 0.33 Hz stimulation and downregulation of synaptic transmission by agonist of type II mGluRs L-CCG1 were taken as criteria to search for responses in CA3 mossy fiber synapses (Cremer et al., 1998; Maccaferri et al., 1998; Yeckel et al., 1999). LFS (0.33 Hz) potentiated fEPSPs to ~250% in C57BL/6J and PST deficient mice (Fig. 4.23A). L-CCG1 diminished the amplitude of fEPSPs in C57BL/6J and *pst* -/- mice by 80% (Fig. 4.23B). The NMDA receptor antagonist AP-5 did not affect the amplitude of selected fEPSPs in either C57BL/6J or in PST mutant mice (Fig. 4.23C). HFS performed in the presence of AP5 induced a strong increase in fEPSP amplitude (Fig. 5.23C). Maximal potentiation during the first 2 min after HFS was ~700%, and mean potentiation measured 50-60 min after induction of LTP was ~180%, resembling reported profiles of LTP in CA3 (Maccaferri et al., 1998; Yeckel et al., 1999). There was no difference between C57BL/6J and *pst* -/- mice in short- or long-term potentiation ($P>0.5$).

To examine the possibility that mossy fibers in mutants still express low amounts of PSA that could be sufficient to maintain LTP, we recorded mossy fiber LTP in slices from mutant mice incubated with endo-N. The effectiveness of endo-N treatment was verified by the loss of PSA immunoreactivity. Treatment of slices with endo-N did not reduce mossy fiber LTP (227.2±22.6%) or PTP (749.7±153.3%, 5 slices from 4 mice, $P>0.2$; Fig. 4.23D).

To exclude the possibility that the impairment in LTP in CA1 but not in CA3 was attributable to differences in slice preparation, we recorded LTP from CA1 and CA3 in slices prepared identically from the same 6-month-old mutant mouse according to the "CA3 protocol". Under these conditions, LTP could not be induced in CA1 (mean slopes of fEPSPs collected in two slices 50-60 min after TBS were 91.1 and 103.0% of the baseline), but was normal in CA3 (176.9%). Normal LTP was induced in CA1 in slices from wild-type mice prepared according to the "CA3 protocol" (151.6±14.6%; 4 slices from 3 animals).

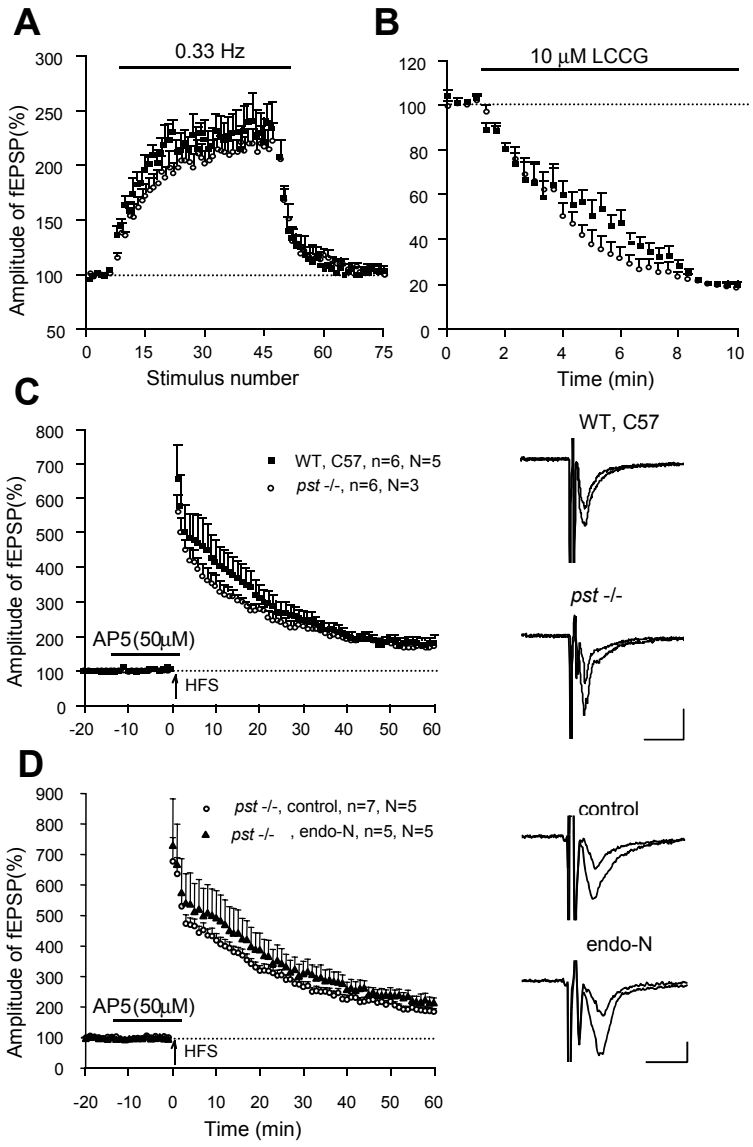


Figure 4.23. Normal LTP in the CA3 region of PST deficient mice

A: Stimulation of mossy fibers with a frequency of 0.33 Hz increased the amplitudes of fEPSPs in acute slices from both wild-type (*WT, C57*) and PST knockout mice (*pst* -/-). Data in **A-C** are from five wild-type and three *pst* -/- mice. Here and below, the graphs represent normalized mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals.

B: Application of the type II metabotropic glutamate receptor agonist L-CCGI (10 μM) reduced the amplitude of fEPSPs in slices from both wild-type and *pst* -/- mice.

C: HFS (indicated by arrow) of mossy fibers evoked a similar increase in slopes of fEPSPs in slices from wild-type and *pst* -/- mice. Insets show averaged fEPSPs recorded before and 60 min after induction of LTP in wild-type and *pst* -/- mice. Calibration: 10 msec, 100 μV.

D: HFS of mossy fibers evoked similar LTP in slices treated (*pst -/-, endo-N*) or non-treated (*pst -/-, control*) with enzyme. Recordings were performed in slices from five *pst* -/- mice. All abbreviations as in **C.**

4.3. ACTIVITY-DEPENDENT DISINHIBITION IN MICE DEFICIENT FOR RECOGNITION MOLECULES

A popular hypothesis for the increased epileptogenicity of hippocampal formation postulates an imbalance between excitation and inhibition. At the structural level, human and experimental epilepsy in animals has been shown to be associated with the formation of recurrent connections between mossy fibers and granule cell proximal dendrites (for reviews, see McNamara, 1994; Parent and Lowenstein, 1997). Although aberrant axonal reorganization has been extensively studied, its molecular basis remains largely unknown. Published data demonstrated that epileptic seizures could cause up-regulation of cell adhesion and extracellular matrix molecules, suggesting a functional role of these molecules in seizure-induced synaptic reorganization.

As an in vitro correlate of epileptic activity, we analyzed the potential of hippocampal slices to develop bursts discharges, which are characterized by the appearance of multiple population spikes (polyspikes) in response to repetitive stimulation. These burst discharges are based on the activity-dependent disinhibition of excitatory synaptic transmission (Thompson and Gahwiler, 1989) and on the activation of NMDA receptors (Dingledine et al., 1986; Masukawa et al., 1991). Taking an advantage of having animals deficient in TN-R, TN-C and PSA, we examined them in terms of activity-dependent disinhibition.

The area and numbers of secondary spikes turned out to be significantly higher ($P<0.05$, ANOVA with repeated measurements and chi-square test, correspondingly) in 12 slices from 5 TN-R mutants as compared to wild-type controls (12 slices from 5 animals; Fig. 4.24). The difference between genotypes was more obvious in the steady-state phase of polyspiking activity, showing a marginal or statistically significant difference after 10^{th} pulse of repetitive stimulation of Schaffer collaterls ($P<0.1$ and $P<0.05$ respectively, U-test). Application of $GABA_A$ receptor antagonist picrotoxin (50 μM) induces an increase in the area of polyspikes approximately to 70% in *tn-R* +/+ mice (14 slices from 3 animals). The polyspiking activity was less increased at the beginning of stimulation in TN-R deficient mice after blockade of $GABA_A$ inhibition (14 slices from 3 animals; Fig. 4.25), in agreement with reduced levels of inhibition found in *tn-R* -/-

mice (Saghatelyan et al., 2001). Nevertheless, an augmentation in the activity-dependent disinhibition profile in *tn-R* -/- mice remains to be significantly higher in comparison to wild-types ($P<0.02$; ANOVA with repeated measurements), mostly in the steady-state phase of polyspiking.

No difference in the numbers of secondary spikes was observed in 14 slices from 4 wild-type mice and in 14 slices from 4 TN-C mutants ($P>0.5$, chi-square test). The activity-dependent disinhibition tended to be lower in slices from TN-C deficient mice as compare to wild-type littermates, but the difference was not statistically significant ($P>0.1$, ANOVA; Fig. 4.26). A marginal ($P<0.1$) and statistically significant ($P<0.05$, U-test) decrease in the area of secondary polyspikes was seen only during initial phase of stimulation, between 6^{th} and 9^{th} pulses of stimulation. The polyspiking activity reached steady-state level after 20^{th} pulse and was about 40% in *tn-C* +/+ and about 20% in *tn-C* -/- mice.

The activity-dependent modulation of inhibition was also investigated in acute hippocampal slices incubated with chondroitinase ABC to digest CSs. Repetitive stimulation of Schaffer collaterals at 1 Hz produced the population spikes with similar time course and amplitude in sham- and chondroitinase ABC-treated slices (Fig. 4.27). The maximal polyspiking activity was observed after 20 pulses of stimulation and was about 80 % from initial level in 9 sham- or chondroitinase ABC- slices from 4 C57BL/6J mice, showing that CSs are not involved in the activity-dependent disinhibition processes.

Whether PSA carbohydrate is implicated in the activity-dependent disinhibition was analyzed in adult PST deficient mice. The initial rise (up to 10^{th} pulse) in the area of polyspike was quite similar in *pst* +/+ and *pst* -/- mice. Following stimulation pulses induced a marginal reduction of the polyspiking activity in mutants approximately to 30 % (17 slices from 4 animals, $P<0.1$, U-test). The activity-dependent disinhibition profile was not significantly lower in slices from PST deficient mice as compare to wild-type littermates (16 slices from 4 animals, $P>0.2$, ANOVA; Fig. 4.28).

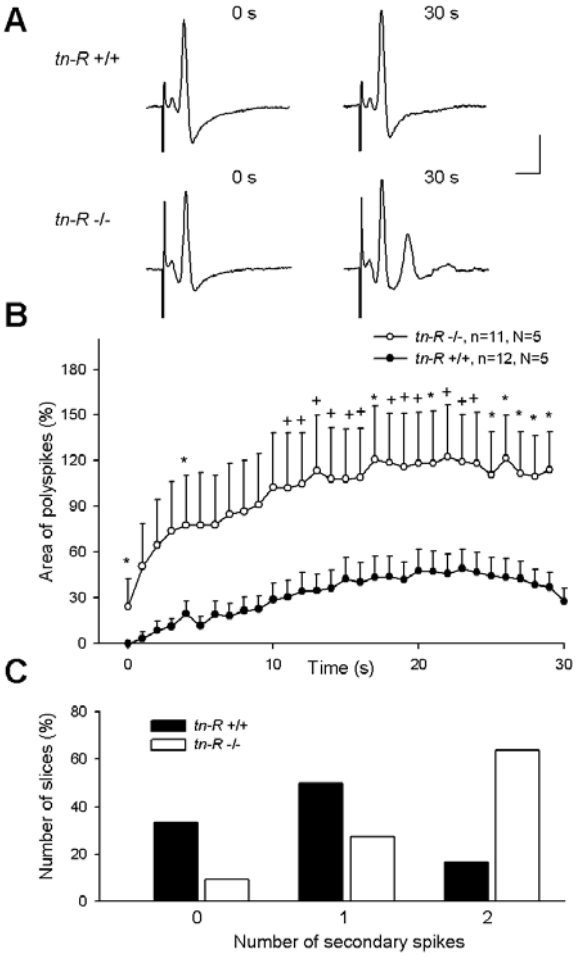


Figure 4.24. The area and number of population spikes is increased in TN-R deficient mice

A: Example of field potentials evoked by stimulation of the Schaffer collaterals and recorded in the *stratum pyramidale* of the CA1 region. With the repetitive stimulation (1Hz for 30 sec), slices from TN-R mutants exhibit a stronger increase in polyspiking, when compared to wild-type littermates. Calibration: 10 msec, 1 mV.

B: Time course of the appearance of polyspiking during repetitive stimulation (area of secondary spikes expressed as a percentage of the area of the first population spike) in slices from TN-R deficient mice (*tn-R* -/-) and wild-types (*tn-R* +/+). The activity-dependent disinhibition profile is significantly higher in *tn-R* -/- ($P<0.05$; ANOVA) as compared to wild-type mice. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals. * $P<0.05$, U-test, significantly different from wild-type mice. + $P<0.1$ U-test, marginally different from wild-type mice.

C: Cumulative plot representing percentage of slices with no, one or two population spikes after 30 sec of stimulation in *tn-R* +/+ and *tn-R* -/- mice. The numbers of secondary spikes were increased in slices from TN-R mutant compared to wild-type mice (Fisher's exact P-value $P<0.05$ chi-square test).

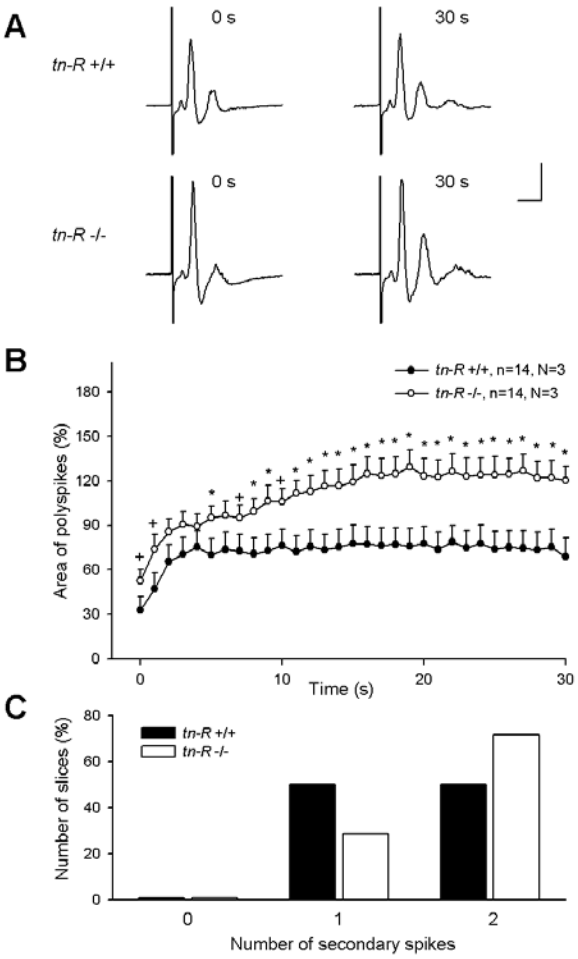


Figure 4.25. The area and number of population spikes remain to be increased in TN-R mutant after the $GABA_A$ receptors blockade

A: Example of field potentials evoked by stimulation of the Schaffer collaterals and recorded in the *stratum pyramidale* of the CA1 region in the presence of 50 μA picrotoxin. Pharmacological disinhibition results in increased polyspiking activity both in *tn-R*+/+ and *tn-R* -/- mice (time point 0 s). During repetitive stimulation the slices from *tn-R* -/- mice exhibit population spikes with a higher amplitude as compare to wild-type mice (time point 30 s). Calibration: 10 msec, 1 mV.

B: Time course of the appearance of polyspiking during repetitive stimulation (area of secondary spikes expressed as a percentage of the area of the first population spike) in slices from TN-R deficient mice (*tn-R* -/-) and wild-type mice (*tn-R* +/+) in the presence of picrotoxin. The activity-dependent disinhibition profile is significantly higher in *tn-R* -/- ($P<0.02$; ANOVA), as compared to wild-type mice. Note that the area of population spikes was increased in *tn-R* +/+ mice during pharmacological disinhibition of CA1 region. Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals. * $P<0.05$, U-test, significantly different from wild-type mice. + $P<0.1$ U-test, marginally different from wild-type mice.

C: Cumulative plot representing percentage of slices with no, one or two population spikes after 30 sec of stimulation in *tn-R* +/+ and *tn-R* -/- mice.

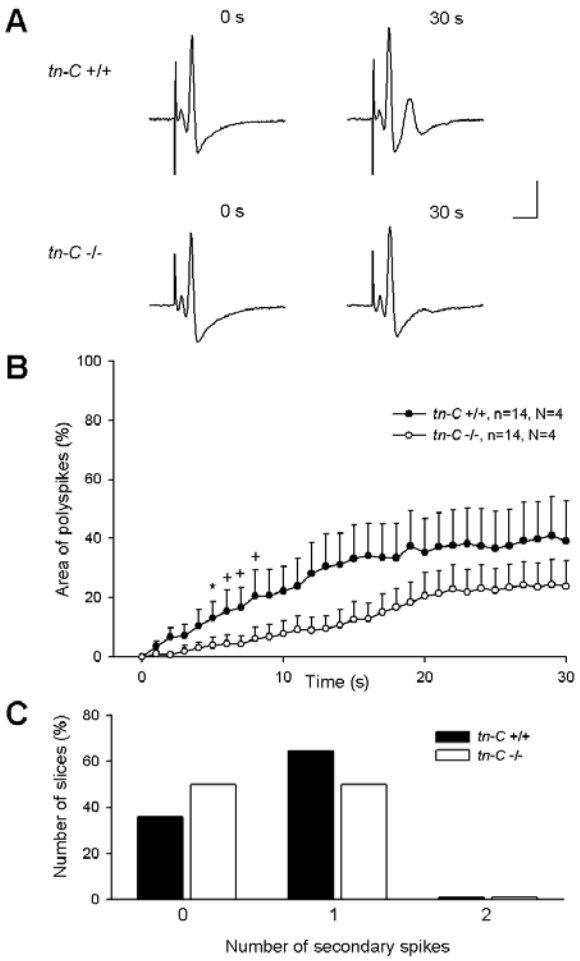


Figure 4.26. Activity-dependent disinhibition profile in TN-C deficient mice

A: Example of field potentials evoked by stimulation of the Schaffer collaterals and recorded in the *stratum pyramidale* of the CA1 region. During repetitive stimulation (1 Hz for 30 sec), slices from *tn-C* -/- mice exhibit a smaller population spike in comparison to *tn-C* +/+ mice. Calibration: 10 msec, 1 mV.

B: Time course of the appearance of polyspiking during repetitive stimulation (area of secondary spikes expressed as a percentage of the area of the first population spike) in slices from TN-C deficient mice (*tn-C -/-*) and wild-type mice (*tn-C +/+*). The activity-dependent disinhibition profile is tended to be lower in *tn-C* -/-, but this difference was not statistically significant (P>0.1; ANOVA). Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals. * P<0.05, U-test, significantly different from wild-type mice. + P<0.1 U-test, marginally different from wild-type mice.

C: Cumulative plot representing percentage of slices with no, one or two population spikes after 30 sec of stimulation in *tn-C* +/+ and *tn-C* -/- mice. There was no genotype-specific difference in the number of secondary spikes (Fisher's exact P-value P>0.1, chi-square test).

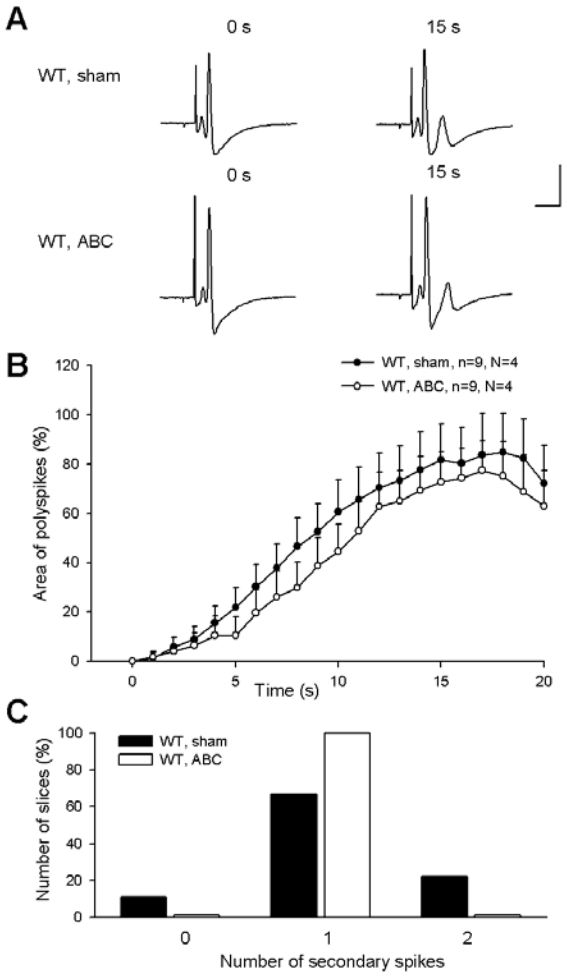


Figure 4.27. Treatment with chondroitinase ABC does not affect the polyspiking activity

A: Example of field potentials evoked by stimulation of the Schaffer collaterals and recorded in the *stratum pyramidale* of the CA1 region. With the repetitive stimulation both sham- and chondroitinase ABC-treated slices from C57BL/6J wild-type mice exhibit a population spike. Calibration: 10 msec, 1 mV.

B: Time course of the appearance of polyspiking during repetitive stimulation (area of secondary spikes expressed as a percentage of the area of the first population spike) was not different between sham-treated (*WT, C57, sham*) and in slices digested with chondroitinase ABC (*WT, C57, ABC*). Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals.

C: Cumulative plot representing percentage of slices with no, one or two population spikes after 20 sec of stimulation in sham- and chondroitinase-treated slices.

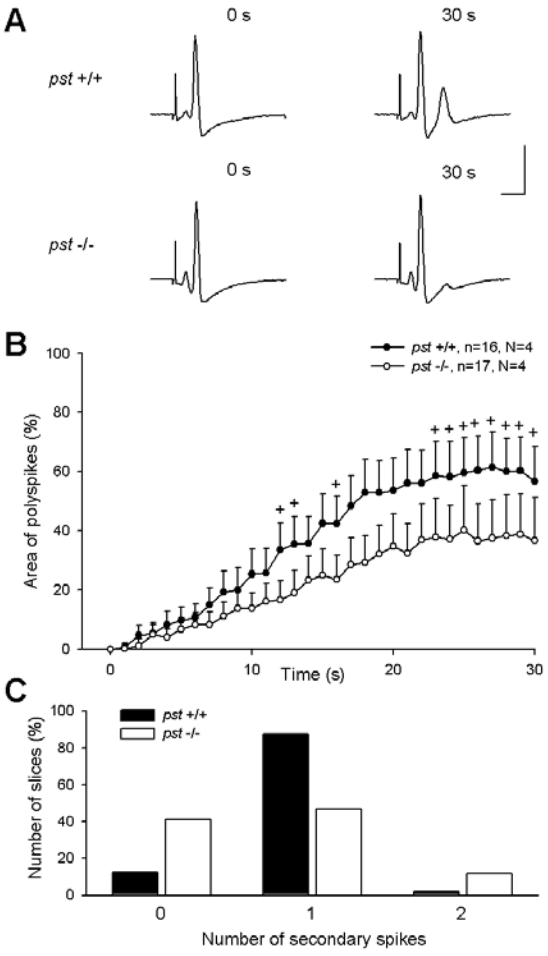


Figure 4.28. Normal polyspiking activity in PST deficient mice

A: Example of field potentials evoked by stimulation of the Schaffer collaterals and recorded in the *stratum pyramidale* of the CA1 region. With the repetitive stimulation (1Hz for 30 sec), slices from *pst* -/- mice exhibit a smaller population spike in comparison to *pst* +/+ mice. Calibration: 10 msec, 1 mV.

B: Time course of the appearance of polyspiking during repetitive stimulation (area of secondary spikes expressed as a percentage of the area of the first population spike) in slices from PST deficient (*pst* -/-) and wild-type (*pst* +/+) mice. There were no genotype-specific difference in the activity-dependent disinhibition profile in *pst* -/- mice and their littermates (P>0.2, ANOVA), but in some points in the steady-state phase a marginal difference between genotype was observed (+ P<0.1 U-test, different from wild-type mice). Data represent mean + SEM, *n* provides the number of tested slices, *N* provides the number of tested animals.

C: Cumulative plot representing percentage of slices with no, one or two population spikes after 30 sec of stimulation in *pst* +/+ and *pst* -/- mice.

5. DISCUSSION

This study demonstrates the involvement of ECM molecules, such as CSPGs, TN-R, and TN-C, and the cell adhesion molecule PSA-NCAM in hippocampal synaptic transmission and plasticity. Comparison of the effects of enzymatic treatment with chondroitinase ABC and the phenotypes of TN-C, TN-R and PST deficient mutants (for a summary of results, see Table 1) reveals a number of differences in the involvement of these molecules in CA1 subfield-specific synaptic plasticity. We have shown that the treatment with chondroitinase ABC, and TN-C and PST deficiency do not interfere with basal excitatory synaptic transmission and short-term plasticity, but reduce TBS-induced LTP in the CA1 region of the hippocampus. In contrast, increased levels of synaptic transmission, as well as impairment in both STP and LTP were seen in TN-R mutants. Reduction in LFS-induced STD and LTD was demonstrated in TN-C deficient mice, whereas LTD was normal, despite decreased STD, in TN-R mutant mice. On the other hand, removal of CSs and PSA blocks LTD but does not affect STD. Polyspiking activity was differently affected in slices from TN-R and TN-C deficient mice, suggesting an involvement of tenascin gene family molecules in an activity-dependent disinhibition process. Slices deficient in CSs and PSA were normal in terms of polyspiking. Synaptic plasticity in the CA3 region was not affected by TN-R, TN-C and PST deficiency.

5.1. Deficiency in TN-R and CSs impairs synaptic plasticity via different mechanisms

Since TN-R is a carrier of CSs (Probstmeier et al., 2000) that also binds to and colocalizes with CSPGs in PNs (Aspberg et al., 1997; Milev et al., 1998b; Weber et al., 1999), and in view of the known functionally important interactions between CSPGs and TN-R (Xiao et al., 1997a), a comparison of data on synaptic plasticity in a CS deficient cellular environment and in TN-R deficient mice is of particular interest.

Deficiency in CSs evoked effects different from those seen in TN-R mutants (Table 1): chondroitinase ABC treatment did not affect basal synaptic transmission in slices from wild-type control littermates, whereas it was increased in TN-R deficient mice. Digestion of CSs did not affect the increased level of synaptic transmission seen in TN-

Table 1. Summary of experiments on synaptic plasticity in the CA1 and CA3 region of the hippocampus in mice lacking extracellular matrix or cell adhesion molecules.

Parameter	Chondroitin -ase ABC treatment	*tn-R* -/-	Chondroitin -ase ABC treatment of *tn-R* -/-	*tn-C* -/-	*pst* -/-
CA1 region					
Basal excitatory synaptic transmission	normal	**increased**	**increased**	normal	normal
Paired-pulse facilitation	normal	normal	normal	normal	normal
TBS-induced short-term potentiation	normal	**reduced**	**reduced**	normal	normal
TBS-induced long-term potentiation	**reduced**	**reduced**	**reduced**	**reduced**	**reduced**
NMDA receptor-mediated transmission	normal	normal	ND	normal	ND
Long-term potentiation under blockade of $GABA_A$ receptors	normal	normal	ND	**reduced**	ND
Pairing-induced long-term potentiation (0 mV)	ND	normal	ND	ND	ND
Pairing-induced long-term potentiation (-10 mV)	ND	**reduced**	ND	ND	ND
Short-term depression	normal	**reduced**	ND	reduced	normal
Long-term depression	**reduced**	normal	ND	**reduced**	**reduced**
Polyspiking activity	normal	**increased**	ND	normal/ reduced	normal/ reduced
CA3 region					
Frequency facilitation	ND	normal	ND	normal	normal
Post-tetanic potentiation	ND	normal	ND	normal	normal
Long-term potentiation	ND	normal	ND	normal	normal

"Normal", "increased" or "reduced" refers to values that are not different or significantly different from slices of wild-type mice. ND, not determined.

R mutants. On the other hand, NMDA receptor-mediated transmission appeared normal both after chondroitinase ABC treatment and in TN-R mutants. Therefore, this parameter was not analyzed in enzymatically treated slices from TN-R knockout mice. Paired-pulse facilitation was normal both in TN-R deficient mice and after removal of CSs. Potentiation immediately following TBS (STP) as well as STD were impaired in the TN-R mutants, but were not significantly different between chondroitinase ABC- and sham-treated slices from wild-type animals. Since LTD was not affected in TN-R deficient mice, but was almost completely impaired after removal of CSs, our observations suggest that TN-R is not involved in at least some CS-dependent forms of long-term plasticity in the hippocampus.

However, TBS-induced LTP was similarly reduced in TN-R deficient mice and after chondroitinase ABC treatment in both wild-types and TN-R mutants. If CS-related mechanisms are not dependent on TN-R, one could expect a reduction of LTP levels in enzymatically treated slices from TN-R deficient mice as compared to sham-treated slices from TN-R deficient mice or enzymatically treated slices from wild-type mice. This was not seen in the present experiments. Furthermore, TBS-induced LTP could be rescued in chondroitinase ABC treated slices from wild-type mice and in TN-R mutants during pharmacological disinhibition of CA1 region, suggesting that CSPGs and TN-R could share downstream signaling mechanisms related to the expression of LTP.

5.2. Putative mechanisms activated by the removal of chondroitin sulfates

Reduction in LTP and LTD after chondroitinase ABC treatment demonstrates the importance of CSs in long-term plasticity in the CA region of hippocampus. Multiple mechanisms could be involved in these effects. Different forms of CSs were found in the adult mammalian CNS in association with PNs surrounding inhibitory interneurons, particularly in the hippocampus and cerebral cortex (Bertolotto et al., 1991; Hartig et al., 1994; Bruckner et al., 1994; Wintergerst et al., 1996; Haunso et al., 1999). Possibly, removal of CS chains from PN structures could affect the physiological properties of interneurons, shifting the hyperpolarizing action of GABA on the principal cells via feedback or feedforward inhibition and thus reduced LTP. Detailed investigation of

GABAergic transmission in the hippocampus of CSPGs deficient mutants would be necessary to verify this assumption.

CS chains of CSPGs could promote or inhibit outgrowth of hippocampal neurons, depending on the CSs structures and expression pattern of CS receptors (Faissner et al., 1994; Nadanaka et al., 1998; Clement et al., 1998). CSs are known to be important for the regulation of intracellular Ca^{2+} concentrations via activation of non-voltage-gated Ca^{2+}-channels (Snow et al., 1994). Modification of ECM evoked by chondroitinase ABC treatment could change the geometric parameters of extracellular space or association/dissociation of ECM with diffusible factors released during TBS and LFS, thus modifying the spillover of these factors from the synaptic cleft. The spillover of glutamate could activate NMDA, kainate and metabotropic glutamate receptors in non-stimulated synapses (Min et al., 1998; Kullmann et al., 1999; Rusakov et al., 1999). Analysis of Ca^{2+} signaling, transmitter spillover and activity of different neurotransmitter receptors after chondroitinase ABC treatment and in mutants deficient in CSPGs will be helpful to test these.

CSPGs can bind to various extracellular matrix components, growth factors, and interact with several cell surface molecules. Digestion of CSs could affect the distribution and reduce the binding of such partners of CSPGs as HB-GAM, FGF-2, or the transmembrane cell adhesion molecules NCAM and L1. The interaction of the neurocan with its cell-surface glycosyltransferase (GalNAcPTase) receptor is not CSs dependent, but this binding has a significant effect, coordinately inhibiting both N-cadherin- and β1-integrin-mediated adhesion and neurite outgrowth (Li et al., 2000). Since all of these molecules (HB-GAM, FGF-2, NCAM, L1, integrins and cadherins) have been implicated in hippocampal synaptic plasticity (Ishiyama et al., 1991; Lauri et al., 1998; Amet 2001; Benson et al., 2000), cleavage of CS chains from proteoglycan core structures may affect signal transduction cascades induced by listed molecules. Interestingly, removal of PSA carried by NCAM also leads to an impairment of both LTP and LTD in CA1 (Becker et al., 1996; Muller et al., 1996) similar to that observed in our experiments after removal of CSs. In both instances, a decrease in LTP after digestion of CS or PSA appeared to be not due to modulation of NMDA receptor-mediated activity. Thus, it could be of interest to analyze whether the distribution of

PSA-NCAM or intracellular signaling mediated by PSA-NCAM is disturbed after digestion of CSs.

Interactions of RPTPζ/β with PSD-95/SAP90 and sodium channels (Kawachi et al., 1999; Raticliffe et al., 2000) could also modify synaptic formation and plasticity by regulating RPTPζ/β activity important for modification of the tyrosine phosphorylation levels of proteins at the synapses.

The first investigations of CSPGs knockout mice have recently been performed. Mice deficient in the three isoforms of RPTPζ/β showed no obvious abnormality in anatomical structure of the nervous system, neurite outgrowth, node formation and conduction velocity was not altered in these mutants (Harroch et al., 2000). Neurocan knockout mice are viable and fertile, exhibit no gross anatomical brain defects (Zhou et al., 2001). In these mutants no significant differences in input-output curve and pair-pulse facilitation ratio were detectable. Our results supporting these findings, suggesting that CSs, including those carried by neurocan, do not interfere with basal synaptic transmission. Neurocan deficiency results in a mild decrease in late LTP in the CA1 region of hipocampus (Zhou et al., 2001). Potentiation appeared to decline more rapidly in knockout mice than in wild-types during application of the $GABA_A$ antagonist picrotoxin, but there was no genotype-specific difference in early LTP, as has also been demonstrated in our experiments.

Thus, the mechanisms by which CSs could effect synaptic plasticity may depend on particular CSPGs and analysis of mutants deficient in such molecules will be instrumental to elucidate the cascade of events triggered by CSs.

5.3. CSPGs in activity-dependent disinhibition

Since changes in the expression of phosphacan and neurocan (Kurazono et al., 2001) and synthesis of CSs (Naffan-Mazzacoratti et al., 1999) have been demonstrated in Ihara's epileptic and pilocarpine treated rats, the activity-dependent modulation of inhibition was investigated in acute hippocampal slices incubated with chondroitinase ABC. Removal of CSs does not change polyspiking activity in slices from wild-type animals, showing that at the time period of 30 sec CS does not interfere with activity-dependent disinhibiton. We would suggest that CSPGs are essential for progression of

epileptic seizure, when hippocampal excitatory neurons become pathologically active and when formation of recurrent connections between mossy fibers and granule cell proximal dendrites occurs. Recent publications support this suggestion. The tPA/plasmin extracellular proteolytic system regulates seizure-induced hippocampal mossy fiber outgrowth through CSPGs as a substrate. It has been shown that tPA/plasmin mediated processing of phosphacan is critical for terminating appropriately the extension of mossy fibers at the supragranular/molecular boundary (Wu et al., 2000). The synthesis of heparin sulfate (HS) and CS in the hippocampus and cortex of pilocarpine treated rats showed marked changes in the time-course during *status epilepticus*. The results demonstrated a decreased synthesis of HS during the acute phase and an increased synthesis of CS during the silent period in the cortex and hippocampus (Naffan-Mazzacoratti et al., 1999).

Further morphological, electrophysiological and behavior analysis of mice deficient in CSPGs in experimental models of epilepsy, as well as detailed investigations of changes in ECM composition during pathogenesis in human hippocampus would be of interest.

5.4. Synaptic efficacy in TN-R deficient mice

Our data showing enhanced excitatory synaptic transmission and reduced LTP in TN-R deficient mice are in agreement with previous experiments (Saghatelyan et al., 2001). In the present study, we additionally demonstrate normal levels of LTD and modified threshold for induction of LTP/LTD in TN-R mutants.

Increased basal synaptic efficacy in TN-R deficient mice correlates with the reduced amplitude of unitary perisomatic inhibitory postsynaptic currents recorded from CA1 pyramidal cells in slices from TN-R mutants, when compared to wild-type controls (Saghatelyan et al., 2001). The reduction in perisomatic inhibition in mutants can be explained by the fact that TN-R is a carrier of the HNK-1 carbohydrate in CA1 and that functional block of HNK-1 is known to reduce $GABA_A$-mediated perisomatic inhibitory currents in CA1 pyramidal cells (Saghatelyan et al., 2000). Thus, a deficit of HNK-1 in TN-R mutants could lead to the reduced perisomatic inhibition that would increase synaptic strength of Schaffer collateral-CA1 synapses. In agreement with these findings, morphological analysis of TN-R deficient mice revealed a significant increase in the

number of perforated synapses in the *startum radiatum* of CA1 region, in comparison to wild-type animals (A. Nikonenko, unpublished observations).

It is widely accepted that the threshold for synaptic plasticity is not a fixed value, but can be dynamically adjusted according to the recent history of synaptic activity (that is, synapses are metaplastic) (for reviews, see Abraham and Bear, 1996; Abraham and Tate, 1997). The reduction in perisomatic disinhibition could promote LTP in CA1 during early development, resulting in elevated basal excitatory synaptic transmission found in TN-R deficient mice. Impairment or abolishment in LTP in the synapses that have been previously potentiated would be predicted in these mutants because of metaplastic processes. Indeed, "weak" protocols like a pairing of presynaptic LFS with depolarization of pyramidal cells to –10 mV, or TBS of Schaffer collateras, resulting in LTP of similar magnitudes in slices from wild-type mice, induced significantly reduced potentiation in TN-R knockout mice. This impairment in LTP is not age-dependent, since reduction to the same values was noted in adult (2-6-month-old) and young (2-week-old) mutants. In contrast to impairment in LTP at the Schaffer collaterals, mossy fiber LTP was normal in TN-R deficient mice, showing region-specificity of the role of TN-R in synaptic plasticity.

Alteration in synaptic morphology has been postulated as the mechanism behind changes in synaptic efficacy, including LTP (reviewed by Bailey and Kandel, 1993; Muller, 1997; Geinisman, 2000; Luscher et al., 2000). In general, increase in the spine number, in the average spine area and width of PSD, as well as clustering of vesicles closer to the release site and accelerated insertion of glutamate receptors have been reported to occur in LTP in a time scale beginning already 15 min from induction of LTP (reviewed by Bailey and Kandel, 1993; Muller, 1997; Geinisman, 2000; Luscher et al., 2000). Since certain structural and functional modifications have been facilitated by reduced inhibition *in vivo* in TN-R deficient mice, there could be no room for further modifications of these types. Therefore, in slices from TN-R mutants a "weak" induction protocol could not further induce changes, normally accompanying LTP. This interpretation is in a perfect agreement with our recent electronmicroscopic data, showing the doubled number of perforated asymmetric (i.e. putative excitatory) synapses in the *stratum radiatum* of untreated TN-R deficient mice, as compared to

wild-type controls (A. Nikonenko, unpublished observations). Usually, such increase is found after induction of LTP *in vitro*, but in the TN-R mutants the process of "recruitment" of synapses to form perforations is facilitated under basal conditions, thus occluding their further modifications in response to weak LTP inducive protocols.

On the other hand, "strong" protocols for LTP induction, like pairing of presynaptic stimulation with depolarization of pyramidal cells to 0 mV, or TBS applied during pharmacological disinhibition of hippocampus (Wigstrom and Gustafsson, 1983; Mott and Lewis, 1991; Ngezahayo et al., 2000) induced a strong potentiation in TN-R deficient slices to the levels not different from those seen in wild-types. We assume that under these conditions additional mechanisms of synaptic plasticity are activated, for instance formation of new spines in the vicinity of the activated spines or formation of multiple bouton synapses, both in wild-type and TN-R knockout mice. In this regard, morphological investigations of structural changes in TN-R deficient mice after induction of LTP would shed light on these mechanisms. However, such analysis is complicated by the difficulty to clearly distinguish synapses stimulated during induction of LTP.

5.5. Activity-dependent disinhibition in TN-R deficient mice

The area and numbers of secondary spikes turned out to be significantly higher in TN-R mutants as compared to wild-type controls. These results are in agreement with a previous study showing reduced perisomatic inhibition and increased levels of excitatory synaptic transmission in the CA1 region of hippocampus in TN-R deficient mice (Saghatelyan et al., 2001). Furthermore, pharmacological disinhibition does not enhance increased polyspiking activity in TN-R deficient mice. Thus, with the development of burst discharge no further changes in activity-dependent modulation of inhibition could be obtained in these mutants. Interestingly, HFS- and forskolin-induced LTP has been shown to be impaired at the perforant path-dentate gyrus and mossy fiber synapses in hippocampus of patients with TLE and in rats after experimentally induced epilepsy (Beck et al., 2000; Goussakov et al., 2000). In addition, increase in the number of perforated synapses was found in the hippocampal dentate gyrus in kindled rats and in humans with TLE (Geinisman et al., 1990; Zhang and Houser, 1999). Similar to that

found in experimental and human epilepsy, reduced LTP and doubled number of perforated synapses was observed in the CA1 region of TN-R deficient mice.

5.6. Selective deficits in synaptic plasticity as a result of lacking TN-C

In light of published data showing the activity-dependent expression of TN-C (Nakic et al., 1996, 1998), we decided to study this phenomenon in TN-C knockout mice. Despite the normal hippocampal histoarchitecture and a wild-type-like performance of TN-C deficient mice in the water maze, an examination of several forms of synaptic plasticity in acute slices of TN-C mutant mice showed an impairment of TBS-induced LTP, reduced LFS-induced STD, and an abolished LTD and at Schaffer collateral-CA1 synapses. This reduction was not due to abnormal NMDA receptor-mediated transmission in the mutants. TBS-induced LTP could be not rescued by application of the $GABA_A$ receptor antagonist picrotoxin, indicating that mechanisms distinct from GABAergic inhibition mediate most, if not all, reduction of LTP in TN-C deficient mice.

In contrast to the abnormalities seen in the CA1 subfield, PTP and LTP were normal in the CA3 region of the hippocampus. Pharmacological interference with the NMDA receptor and mGluRs at mossy fiber-CA3 synapses was also indistinguishable between TN-C deficient mutants and wild-type mice.

The combined observations suggest that the lack of TN-C causes subtle differences in synaptic plasticity, but not basal synaptic activity specifically in the CA1 region of the hippocampus. These observations are interesting in view of the findings that altered synaptic activity changes expression of TN-C in the hippocampus (Nakic et al., 1996, 1998). It is therefore conceivable that a feedback-loop exists between synaptic plasticity, which depends on TN-C expression, and the upregulation of TN-C after induction of increased synaptic activity by chemical and electrical stimulation, leading to an overall enhanced plasticity that could be maintained over several days in the presence of increased TN-C expression (Nakic et al., 1996, 1998). Whether these alterations in synaptic efficacy are due to subtle alterations during development of synapses or to the acute action of TN-C on normally developed synapses remains to be elucidated by using conditionally deficient TN-C mutants. Interestingly, it has been

recently found that injection of TN-C fragments (FN-III domains 6-8 but not domains 3-5) into the CA1 region of acute hippocampal slices from wild-type mice reduced LTP, thus arguing in favor of a direct involvement of TN-C in synaptic plasticity (M. Sun, unpublished observations).

5.7. Impairment of L-type VDCCs at Schaffer collateral-CA1 synapses in TN-C deficient mice

Both LTP and LTD at Schaffer collateral-CA1 synapses have been shown to consist of NMDA receptor-specific and VDCC-specific components *in vitro* and *in vivo* (Bolshakov and Siegelbaum, 1994; Huber et al., 1995; Christie et al., 1997; Morgan and Teyler, 1999, 2001). These two components appear to activate different signal transduction pathways since inhibition of serine-threonine kinases selectively blocks NMDA receptor-dependent LTP, whereas inhibitors of tyrosine kinases block VDCC-dependent LTP (Cavus and Teyler, 1996). Experiments with nifedipine, an antagonist of L-type VDCCs, allowed insights into the mechanisms underlying the impairment of hippocampal plasticity in TN-C deficient mice. TBS-induced LTP in the CA1 region has been examined using a stimulation protocol very similar to that reported to activate the L-type VDCCs (Morgan and Teyler, 2001). Consistent with that study, bath applied nifedipine reduced TBS-induced LTP in wild type mice (B. Salmen, unpublished observations). Strikingly, in these experiments LTP was reduced to levels comparable to those of TBS-induced LTP in TN-C deficient littermates without prior blocking of L-type VDCCs, suggesting that mainly an L-type VDCC-specific component of LTP at Schaffer collateral-CA1 synapses is diminished in the TN-C deficient mutants.

Chemically induced LTP with the K^+ channel blocker TEA in the CA1 subfield is another form of synaptic plasticity that is accepted to be dependent on the activation of L-type VDCCs (Huang and Malenka, 1993; Song et al., 2001). Reduced TEA-induced LTP in TN-C deficient animals (B. Salmen, unpublished observations) further supports the view that L-type VDCCs or, more indirectly, L-type VDCC-mediated signalling events are impaired. The involvement of L-type VDCCs in LTD at Schaffer collateral-CA1 synapses is well documented (Bolshakov and Siegelbaum, 1994; Christie et al., 1997). It is noteworthy in this respect that LTD is abolished in TN-C deficient mice,

underscoring the idea that TN-C dependency of hippocampal plasticity is mediated by L-type VDCCs. On the other hand, LTP induced by short HFS, i.e. under rather "weak" induction conditions, resulted in an LTP not involving activation of VDCCs. In agreement with this notion, no difference in short HFS-induced LTP in the CA1 region was found between genotypes (B. Salmen, unpublished observations). A similar dependency on the temporal pattern of stimulation has been found by other investigators studying TrkB signalling in hippocampal plasticity (Kang et al., 1997).

Investigating LTP in the CA3 region completed our analysis of hippocampal synaptic plasticity. We used an induction protocol that has been shown to induce LTP in the CA3 region without the activation of L-type VDCCs (Kapur et al., 1998). In another study it has been shown that P-type VDCCs are of importance in synaptic transmission in the CA3 region, but both induction and expression of LTP in this region can occur in the absence of P/N-type VDCCs (Castillo et al., 1994). These observations further support the view that deficits in synaptic plasticity in TN-C deficient mice are only observed under conditions involving significant activation of L-type VDCCs.

TN-C may, on the one hand, modulate L-type VDCC-dependent synaptic plasticity in an indirect manner by interaction with its cellular receptors, namely with different types of integrins (Jones and Jones, 2000). On the other hand, the impairment of L-type VDCC-dependent plasticity in TN-C deficient mice may result from a direct interaction of TN-C with these ion channels. TN-C is known to interact with voltage-dependent Na^+ channels (Srinivasan et al., 1998). It is interesting in this context that the α subunits of the evolutionarily linked voltage-dependent Ca^{2+} and Na^+ channels show a similar molecular topology (Barchi, 1991; Anderson and Greenberg, 2001). We therefore favor the possibility that TN-C might directly affect L-type VDCCs.

Functional significance of our *in vitro* data is supported by recent behavioral experiments. Although TN-C deficient mice show normal learning and relearning in the water maze (F. Morellini, unpublished observations), they have impaired long-term memory in step-down paradigm (T. Strekalova, unpublished observations). Furthermore, since several studies have shown an implication of L-type VDCCs in age-associated neurodegeneration and learning impairments (Deyo et al., 1989; Sandin et

al., 1990; Thibault and Landfield, 1996; Thibault et al., 2001), it will be important to test spatial learning and memory of aged TN-C deficient mice.

5.8. Polyspiking activity in TN-C deficient mice

The activity-dependent disinhibition tended to be lower in TN-C mutants, although a significant difference in the area of secondary polyspike was observed only for initial period of stimulation, suggesting involvement of TN-C glycoprotein in early steps of activity-dependent modulation of inhibition. We suggest that TN-C-mediated reduction of polyspiking activity could be explained by impaired functions of L-type VDCCs expressed by CA1 pyramidal cells in TN-C deficient mice. In two animal models of epilepsy the enhancement of Ca^{2+} currents in CA1 pyramidal cells has been observed (Vreugdenhil and Wadman, 1994; Faas et al., 1996). Moreover, L-type channel immunoreactivity appeared to be selectively enhanced in reactive astrocytes in kainate-treated animals (Westenbroek et al., 1998). TN-C secreted by astrocytes shows a dramatic increase and redistribution in the sclerotic human hippocampus with temporal lobe epilepsy (Scheffler et al., 1997). As TN-C has been associated with the regulation of axon guidance and outgrowth (for reviews, see Faissner, 1997; Jones and Jones, 2000), its induction in molecular layer and neuropil of various hippocampal subfields may provide a signal for axonal reorganization of sprouting neurites. Further morphological, electrophysiological and behavior analysis of TN-C deficient mice in experimental models of epilepsy would provide insights in to its involvement in generation of epileptic seizures.

5.9. Age-dependent decline of PSA-NCAM expression in PST deficient mice

Mice homozygous for a null mutation in the polysialyltransferase PST gene revealed normal development of gross anatomical features (Eckhardt et al., 2000). During postnatal development, a progressive loss of PSA was observed in all brain regions examined, especially in the CA1 and CA3-CA4 regions of hippocampal formation. In this brain region PSA is expressed in the dentate gyrus as well as in the CA4, CA3, and CA1 subfields in wild-type mice. In PST mutants PSA expression is high in young animals, but drops dramatically during later postnatal stages. The strong reduction of

PSA in the hilus region of the hippocampus and in the CA3 is in agreement with data from Hildebrandt et al. (1998), showing that at postnatal day 21 STX is only expressed in the inner layer of the dentate granule cells in rat hippocampus, whereas PST remains expressed throughout the hippocampus. Thus, STX, possibly in concert with PST, is responsible for polysialylation during development of the nervous system, but only the latter one generates PSA in the adult animal. However, morphologically the entire structure appears normal, suggesting that the striking lamination defect in NCAM-deficient mice (Cremer et al., 1997; Seki and Rutishauser, 1998) is attributable to the lack of PSA during development and that this phenotype reflects more the absence of PSA generated by STX (Table 2). In this case, mice lacking both PST and STX, which will soon be available, are expected to exhibit the same deficiencies as NCAM-deficient or endo-N treated mice. However, the loss of PSA from the hippocampus without changes in the organization of the structure allowed us to address the involvement of this carbohydrate in synaptic plasticity independent of the NCAM glycoprotein.

5.10. The role of PSA-NCAM in synaptic plasticity

NCAM deficiency results in impaired LTP in CA1 of the hippocampus in organotypic slice cultures and in acute slices (Muller et al., 1996; Dityatev et al., 1999; A. Artola, unpublished observations; but see Holst et al., 1998). Furthermore, examination of endo-N-treated hippocampal slice cultures or acute slice preparations yielded similar results (Becker et al., 1996; Muller et al., 1996). Endo-N treatment was also found to cause a block of LTD in CA1 (Muller et al., 1996). We show here that the absence of PST, which results in reduced PSA expression in CA1 in adult mice, also impairs LTP and LTD at Schaffer collateral-CA1 synapses, thus providing genetic evidence for the essential role of PSA produced by PST in synaptic plasticity at these sites (Table 2). Basal synaptic activity and STP, in contrast, were normal in the mutant, suggesting that the basic synaptic machinery is not affected by the mutation. Normal STP in Schaffer collateral synapses in the presence of endo-N was also found after high-frequency stimulation of acute hippocampal slices (Becker et al., 1996), but not when potentiation was induced in organotypic cultures (Muller et al., 1996).

Table 2. Roles of NCAM lacking PSA, PSA, and polysialyltransferases in neural development and synaptic plasticity

Function	NCAM mutants	Endo-N treatment	PST mutants	Putative molecular basis
Lamination of mossy fibers	impaired	impaired	normal	PSA (STX)
LTP and LTD in CA1	impaired	impaired	impaired	PSA (PST)
LTP in CA3	impaired	impaired[a]	normal[b]	NCAM glycoprotein without PSA

"Normal" or "impaired" refers to values that are not different or significantly different from slices of wild-type mice. *a* only in adult mice; *b* in *pst* -/- mice.

Synaptic plasticity in CA3 was normal in PST deficient mice. This observation is different from the results showing impaired LTP in the CA3 region in NCAM mutants where PSA is virtually absent (Cremer et al., 1998). There are two explanations for impaired long-term plasticity in CA3 of NCAM but not of PST knockout mice. One is that the NCAM glycoprotein but not its PSA moiety may play an essential role in synaptic plasticity. It is noteworthy in this context that mossy fiber synapses do not express detectable levels of PSA in adult rat and mice (Seki and Rutishauser, 1998; Seki and Arai, 1999). Whereas PSA is detectable in spine synapses in the CA3/CA4 and CA1 regions of the hippocampus, but not detectable in giant mossy fiber terminals, NCAM protein is detectable in both types of structures (Schuster et al., 2001). Alternatively, impaired CA3 LTP could be an indirect consequence of the altered distribution of mossy fiber terminals in NCAM deficient mice, which is not observed when PST is absent. A developmental defect is unlikely to account for the deficit in CA1 LTP and LTD in PST mutants, because STX appears to compensate for PST deficiency at earlier stages and we observed reduced LTP, LTD, and PSA levels only in the adult hippocampus.

Our findings of impaired NMDA receptor-dependent synaptic plasticity in CA1 and normal NMDA receptor-independent synaptic plasticity in CA3 suggest that PSA in

synaptic membranes is an important determinant of some but not all forms of synaptic plasticity in the hippocampus. In light microscopic studies of the CA1 region of wild-type mice, PSA has been suggested to be present on neurons and glia (Fox et al., 1995; Muller et al., 1996), but careful electronmicroscopic analysis remains to be done. Recently, electronmicroscopic analysis of the CA3 region and dentate gyrus of rats showed that strong differences exist in the localization of PSA between synapses in these two regions (Schuster et al., 2001). Synaptic membrane of mossy fiber synapses did not show detectable level of PSA immunoreactivity, although PSA was detectable on axonal membranes of fasciculating mossy fibers. In combination with our electrophysiological experiments these data suggest that PSA expression on preterminal axons is not necessary for expression of normal LTP at the mossy fiber synapses. In contrast to mossy fiber synapses, 32 % of spine synapses in the outer third of the molecular layer of dentate gyrus were immunopositive for PSA presynaptically and postsynaptically (Schuster et al., 2001). Further investigations of LTP in the dentate gyrus of PST mutants would complete our analysis of the role of PSA-NCAM in hippocampal synaptic plasticity.

Our findings of impaired synaptic plasticity in PSA deficient mice are underscored by data from behavioral experiments. NCAM deficient and endo-N treated mice show deficits in hippocampus-dependent spatial learning in the water maze. NCAM mutants also have impaired emotional memory in the contextual fear conditioning test (Cremer et al., 1994; Stork et al., 2000).

5.11. Putative mechanisms underlying a change in synaptic activating by PSA-NCAM

The exact mechanisms underlying an increase in synaptic efficacy during LTP (expression of LTP) are not fully understood, but it appears that they involve changes in the number and functional properties of AMPA-type glutamate receptors (Barria et al., 1997; Benke et al., 1998; Shi et al., 1999). In view of these results, it is noteworthy that colominic acid, a homopolymer of sialic acid, can prolong AMPA receptor channel open time and increase its bursting activity (Suppiramaniam et al., 1999). Enzymatic

removal of sialic acid from hippocampal membrane fractions modified binding of AMPA to AMPA receptors (Hoffman et al., 1997).

Furthermore, there is an activity-dependent rapid shift of PSA-NCAM to the cell surface in cultured neurons, endocrine cells, and in hippocampal slice cultures (Kiss et al., 1994; Muller et al., 1996). Muller et al. (1996) proposed that neural activity leads to increased exocytosis of PSA-NCAM at the hippocampal synapses creating an "anti-adhesive" environment as a prerequisite for structural changes. Block of perforated synapse formation by endo-N treatment supports an involvement of PSA in structural changes at the synapse (Toni et al., 1998). If PSA is differentially distributed in different locations of the synaptic membranes, this could also change diffusion of soluble factors including neurotrophins (Muller et al., 2000) and neurotransmitters into and out of the synaptic cleft by altering its microenvironment. Demonstration of a direct modulation of reconstituted AMPA receptors by PSA strongly suggests that in addition to promoting structural changes at the synapse, an activity-dependent increase of PSA-NCAM on postsynaptic sites could directly enhance AMPA receptor activity and therefore increase synaptic strength. Since L-type VDCCs are coactivated during TBS and are involved in impairment of LTP in TN-C knockout mice, it would be interesting to examine VDCC-mediated forms of synaptic plasticity in mutants deficient in NCAM and PSA.

5.12. PSA-NCAM in activity-dependent disinhibition

The activity-dependent disinhibition profile has been analyzed in adult PST deficient mice. A marginal reduction in the steady-state phase of the polyspiking activity was observed in the mutants, suggesting that PSA is not the main component implicated in activity-dependent modulation of inhibition in a short-time window. Our findings, however, do not exclude the possibility that PSA-NCAM is involved in long-term structural remodeling during epileptogenesis. This is plausible in light of observations, showing that the expression of NCAM-180 and PSA-NCAM is activity-dependent and upregulated in human epileptic brains or after seizures induced by kainic acid in rats (Le Gal La Salle et al., 1992; Mikkonen et al., 1998; Schuster et al., 1998). Since the postsynaptic expression of PSA-NCAM promotes formation of synapses (Dityatev et

al., 2000), PSA synthesized by PST can be a factor involved in formation of ectopic synapses in epileptic brains, an event which occurs later in epileptogenesis. In support of this hypothesis it has been found that increased PSA-NCAM immunoreactivity is correlated with the density of mossy fiber sprouting in the hippocampus of patients with temporal lobe epilepsy (Mikkonen et al., 1998). Additionally, cleavage of adhesive connections (for instance, in fascicles of mossy fibers) could be an early step in the formation of new synaptic configurations during epileptogenesis. The intense stimulation of NMDA receptors, also observed after kainic acid induced seizures in rats, has been shown to result in the extracellular proteolysis of NCAM (Hoffman et al., 1998; Endo et al., 1999). On the other hand, NCAM downregulates Kir3 inwardly rectifying potassium currents that could modify excitability of neurons (M. Delling, unpublished observations). In order to directly address the question of wether NCAM and PSA-NCAM are indeed necessary for induction and/or progression of epilepsy, it would be of interest to examine mice deficient in NCAM and PSA in terms of axonal sprouting, induction of hyperexcitability and seizures associated with epileptogenesis.

6. SUMMARY

The present work revealed the important role of extracellular matrix components such as condroitine sulfates (CSs) borne by chondroitin sulfate proteoglycans (CSPGs), tenascin-R (TN-R), tenascin-C (TN-C) and polysyalylated neural cell adhesion molecules (PSA-NCAM) in the modulation of synaptic efficacy of the mouse hippocampus. Different forms of hippocampal synaptic plasticity were investigated in slices treated with chondroitinase ABC, as well as in slices from TN-R, TN-C and polysialyltransferase (PST) deficient mice. Deficiency in these molecules differentially modulated several forms of synaptic plasticity, depending on the hippocampal region, developmental stage, temporal pattern and strength of stimulation used to induce synaptic plasticity, suggesting that different mechanisms are involved.

Despite the functional interaction and colocalization of CSPGs and TN-R our data do not support the possibility of synergetic effects produced by removal of CSs and TN-R on synaptic transmission and plasticity in the CA1 region of hippocampus. CSPGs and TN-R could, however, share some signaling mechanisms related to the impaired expression of theta-burst stimulation (TBS)-induced long-term potentiation (LTP) in the CA1 subfields, the being only similarily found phenotype.

TN-R and TN-C glycoproteins, two members of one gene family are also differently involved in CA1 plasticity. On the one hand, our data showed enhanced excitatory synaptic transmission and modified threshold for induction of LTP/LTD in TN-R deficient mice, correlating with an increased number of perforated synapses in this mutant. On the other hand, TN-C appeared to affect L-type voltage-dependent calcium channel (VDCC)-mediated currents/signaling.

Analysis of mice lacking PST, one of the polysialyltransferases responsible for addition of PSA to NCAM in adult brain, allowed us to distinguish between PSA- and NCAM-dependent phenomena, on the one hand, and between developmental and acute functions, on the other. Loss of PSA in the presence of NCAM protein but in the absence of obvious histological changes allowed us to directly investigate the role of

PSA in synaptic plasticity. Schaffer collateral-CA1 synapses, which express PSA in wild-types, showed impaired LTP and long-term depression (LTD) in adult mutants. This impairment was age-dependent, following the time course of developmental disappearance of PSA. Contrary to NCAM mutant mice, LTP in PST mutants was undisturbed at mossy fiber–CA3 synapses, which do not express PSA in wild-type mice. The results demonstrate an essential role for PST in synaptic plasticity in hippocampal CA1 synapses, whereas PSA produced by different polysialyltransferase or polysialyltransferases at early stages of differentiation regulates correct lamination of mossy fibers. We suggest that NCAM but not PSA is likely to be important for LTP in the hippocampal CA3 region.

Polyspiking activity was differentially affected by deficiency in CSs, TN-R, TN-C and PSA. Treatment of wild-type slices with chondroitinase ABC did not change polyspiking activity, whereas TN-R deficient mice exhibited a significant increase in the area and numbers of secondary polyspikes. TN-C mutants demonstrated a slight reduction in the initial phase of activity-dependent disinhibition profiles. A marginal reduction in the steady-state phase of the polyspiking activity was observed in PST knockout mice. We suggest that recognition molecules are likely to be involved in regulation of excitability via multiple mechanisms, which could represent endogeneous processes underlying some aspects of epilepsy.

Thus, the combined observations represent an important advance in elucidating the molecular mechanisms involved in modulation of synaptic functions by extracellular matrix glycoproteins and their associated carbohydrates.

6. ZUSAMMENFASSUNG

Die vorliegende Arbeit konnte die Bedeutung folgender Bestandteile der extrazellulären Matrix für die Modulation synaptischer Plastizität im Hippocampus der Maus zeigen: Chondroitinsulfate (CS), den Zuckerresten der Chondroitinsulfatproteoglykane (CSPG), Tenascin-R (TN-R), Tenascin-C (TN-C) und polysyalisiertes neurales Zelladhäsionsmolekül (PSA-NCAM). Unterschiedliche Formen synaptischer Plastizität wurden an akuten Schnitten des Hippocampus untersucht, die entweder mit Chondroitinase ABC behandelt waren, was die Zuckerreste der Proteoglykane entfernt, oder von TN-R, TN-C oder Polysialyltransferase (PST) defizienten Mäusen stammten. Abhängig von der Region innerhalb des Hippocampus, dem Entwicklungsstadium und der unterschiedlichen Stärke und Frequenz der Stimulation führte das Fehlen der einzelnen Moleküle zu einer Modulation unterschiedlicher Formen von synaptischer Plastizität. Dies deutet auf unterschiedliche und spezifische Mechanismen, durch die die oben aufgeführten Moleküle jeweils bestimmte Formen von synaptischer Plastizität beeinflussen.

Trotz bekannter funktioneller Interaktion und Colokalisierung von CSPG und TN-R, wird durch das Fehlen von CS und TN-R in unseren Untersuchungen kein synergistischer Effekt auf synaptische Weiterleitung und Plastizität in der CA1 Region des Hippocampus deutlich. Dennoch könnten sich CSPG und TN-R gemeinsame Mechanismen der Signaltransduktion teilen, da das Fehlen beider Moleküle zu einer verringerten Ausbildung der durch Theta-Burst Stimulation (TBS) induzierten Lang-Zeit-Potenzierung (LTP) führte.

Im Vergleich mit TN-R wirkt auch TN-C, ein Mitglied derselben Genfamilie, unterschiedlich in der Plastizität der CA1 Region des Hippocampus. Die TN-R defizienten Mäuse zeigen eine Erhöhung der exzitatorischen synaptischen Transmission und eine modifizierte Schwelle zur Induktion von LTP/LTD. Diese Daten korrelieren mit einer erhöhten Anzahl perforierter Synapsen. TN-C dagegen beeinflußt die durch L-typ spannungsabhängiger Calciumkanäle (VDCC) vermittelte synaptische Potenzierung.

Die Analyse der Mäuse, die in einer der Polysialyltransferasen defizient sind, die für die Anheftung von PSA an NCAM im adulten Gehirn verantwortlich sind, machten es möglich, zwischen PSA- und NCAM - abhängigen Phänomenen zu unterscheiden. Durch das Fehlen von PSA in Anwesenheit des NCAM Protein und ohne offensichtliche histologische Veränderungen konnte direkt die Rolle von PSA in synaptischer Plastizität untersucht werden. Die PSA exprimierenden Schafferkollaterale-CA1 Synapsen zeigten ein reduziertes LTP und Lang-Zeit-Depression (LTD) in adulten Mutanten. Diese Veränderung war altersabhängig und korrelierte mit dem Auftreten von PSA. Im Unterschied zur NCAM defizienten Maus war in der PST Mutante LTP an den Moosfaser-CA3 Synapsen, die im Wildtyp kein PSA exprimieren, nicht verändert. Diese Ergebnisse zeigen, daß PST in synaptischer Plastizität in hippocampalen CA1 Synapsen eine wichtige Rolle spielt. Während PSA in früheren Entwicklungssstadien -dann gebildet von anderen Polysialyltransferasen- für eine korrekte Laminierung der Moosfasern entscheident ist, nehmen wir an, daß für LTP in der CA3 Region des Hippocampus NCAM und nicht PSA wichtig ist.

Das Auftreten von Polyspikes wurde durch die Abwesenheit von CS, TN-R, TN-C und PSA in unterschiedlicher Weise beeinflusst. Durch die Entfernung von CS durch Chondroitinase ABC in Wildtypschnitten wurde es nicht verändert. TN-R defiziente Mäuse zeigten dagegen eine signifikante Erhöhung der Fläche und Häufigkeit des zweiten Polyspikes. TN-C Mutanten zeigten eine leichte Reduktion in der initialen Phase der aktivitätsabhängigen Disinhibierung. Eine minimale Reduktion in der Gleichgewichtsphase der Polyspiking Aktivität konnte in PST knock-out Mäusen beobachtet werden. Wir nehmen an, daß Zellerkennungsmoleküle an der Regulierung von Erregbarkeit durch mehrere Mechanismen wirken. Diese Mechanismen könnten bei der Entstehung und Ausbreitung epileptischer Anfälle eine Rolle spielen.

Die zusammengefaßten Beobachtungen sind ein wichtiger Fortschritt bei der Aufklärung der molekularen Mechanismen die an der Modulation von synaptischen Funktionen durch extrazelluläre Matrixmoleküle und derern assoziierter Kohlenhydrate beteiligt sind.

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ACKNOWLEDGEMENTS

This study has been performed in the Institute for Biosynthis of Neuronal Structures of the Centre for Molecular Neurobiology (ZMNH) at the University of Hamburg. I would like to warmly thank Prof. Melitta Schachner for providing facilities for this research, fruitful discussions and for support and guidence during these years.

I am especially grateful to Dr. Alexander Dityatev for excellent supervision, for providing a big source of ideas and for the efforts, patience, and time spent supporting me in science and in life.

I also wish to thank the present and former colleagues in the lab for their help during this work. I thank Dr. Armen Saghatelyan, who helped me a lot with the first steps in this project. Thanks to Benedikt Salmen, Sun Mu and Martin Hammond for a productive and cooperative teamwork. I am grateful to Sandra Schmidt for breeding of TN-R deficient mice, and Achim Dahlmann for their genotyping. Many thanks to Dr. Matthias Evers for providing TN-C mutants and fruitful colaboration. I also thank Dr. Alexander Nikonenko for sharing data of his morphological investigations of TN-R knockout mice. I want to thank Dr. Markus Delling for the organizational help with my Ph.D. thesis. Thanks to Mirjam, Martin, Benedikt, Markus and Matthias for help with translation and corrections of this manuscript. Many thanks to all members of "Synaptic plasticity" club for interesting seminars and helpful discussions. I would express my special thanks to Galina Dityateva and Tatyana Kharkovets, who kindly supported me during this study. Tatyana Strekalova, Vladimir Sytnik, Irina Leschins'ka, Elena Strekalova, Oleg Senkov - thanks to all of you for your friendship which have made everyday work really enjoyable.

I would like to cordially thank all my teachers in Lyceum of Jovti Vody and Dniepropetrovsk State University for inspiring me to the scientific work. I am grateful to my family and my friends in Ukraine who supported me at the thousands kilometer distance all this time. My special thanks to my husband Vladimir for his trust in me, patience and love.

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