DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
**297**

NATALIA LOBANOVSKAYA

# The role of PSA-NCAM in the survival of retinal ganglion cells

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
**297**

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
**297**

**NATALIA LOBANOVSKAYA**

# The role of PSA-NCAM in the survival of retinal ganglion cells

UNIVERSITY OF TARTU
Press

Department of Pharmacology, Institute of Biomedicine and Translational Medicine
University of Tartu, Tartu, Estonia

The dissertation is accepted for commencement of the degree of Doctor of Philosophy in Medicine on December 18th, 2019 by the Council of the Faculty of Medicine, University of Tartu, Estonia

Supervisor: Alexander Zharkovsky, MD, PhD, Professor,
Department of Pharmacology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

Reviewers: Kuldar Kaljurand, PhD, Head of Clinic,
Associate professor in Ophthalmology,
Department of Ophthalmology, University of Tartu, Estonia

Mario Plaas, PhD, Head of the Laboratory Animal Center,
Laboratory Animal Center, University of Tartu, Estonia

Opponent: Dan Lindholm, MD, PhD, Professor,
Department of Biochemistry and Developmental Biology,
Institute of Biomedicine, University of Helsinki, Finland

Commencement: March 6th, 2020

Publication of this dissertation is granted by the University of Tartu.

ISSN 1024-395X
ISBN 978-9949-03-269-3 (print)
ISBN 978-9949-03-270-9 (pdf)



University of Tartu Press
www.tyk.ee

*to Dr. YA Bellyy*

# CONTENTS

# LIST OF ORIGNAL PUBLICATIONS

**I.** **Lobanovskaya N**, Zharkovsky A, Jaako K, Jürgenson M, Aonurm-Helm, Zharkovsky A. PSA modification of NCAM supports the survival of injured retinal ganglion cells in adulthood. Brain Res 2015;1625:9–17.

**II.** **Lobanovskaya N**, Jürgenson M, Aonurm-Helm A, Zharkovsky A. Alterations in the polysialylated neural cell adhesion molecule and retinal ganglion cell density in mice with diabetic retinopathy. Int J Ophthalmol 2018;11:1608–1615.

**III.** **Lobanovskaya N**, Zharkovsky A. A role of PSA-NCAM in the survival of retinal ganglion cells (RGCs) after kainic acid damage. Neurotoxicology 2019;72:101–106.

Contribution of the author:

I. The author participated in the study design, performed all experiments, data analysis and wrote the draft version of the manuscript.

II. The author participated in the study design, induction of diabetes in the animals, prepared the histological slices, performed immunohistochemical, western blot analyses, data analysis and wrote the draft of the manuscript.

III. The author participated in the study design, performed all experiments, data analysis and wrote the draft of the manuscript.

# ABBREVIATIONS

| | |
|---|---|
| CAMs | cell adhesion molecules |
| NCAM | neural cell adhesion molecule |
| PSA | polysialic acid |
| ST8SiaII (STX) | polysialyltransferase II |
| ST8SiaIV (PST) | polysialyltransferase IV |
| FGFR1 | fibroblast growth factor receptor 1 |
| PI3K | phosphatidylinositol 3-kinases |
| Akt | serine/threonine-specific protein kinase (protein kinase B) |
| MAPK (ERK) | mitogen-activated protein kinases (extracellular signal-regulated kinases) |
| BDNF | brain-derived neurotrophic factor |
| TrkB | tyrosine receptor kinase B |
| GDNF | glial cell line-derived neurotrophic factor |
| GFR-alpha | glial factor receptor-alpha |
| Fyn | tyrosine specific phospho-transferase |
| FAK | focal adhesion kinase |
| NMDA | N-methyl-D-aspartate receptor |
| SGZ | subgranular zone |
| SVZ | subventricular zone |
| RMS | rostral migratory stream |
| CA3 | cornu Ammonis region 3 |
| Endo-N | endoneuraminidase-N |
| ONL | outer nuclear layer |
| GCL | ganglion cell layer |
| INL | inner nuclear layer |
| RGC | retinal ganglion cell |
| NFL | nerve fiber layer |
| GCL | ganglion cell layer |
| GFAP | glial fibrillary acidic protein |
| DR | diabetic retinopathy |
| KA | kainic acid |
| MMP-9 | matrix metalloproteinas-9 |
| WT | wild type |
| AMPA | α-amino-3-hydroxy-5-methyl-4-iso-xazolepropionic acid receptor |
| MMP-9i | matrix metalloproteinase-9 inhibitor |
| STZ | streptozotocin |

# INTRODUCTION

Retinal ganglion cells (RGCs) undergo degeneration in many human diseases, such as glaucoma, diabetic retinopathy, optic nerve atrophy, some inherited diseases (dominant optic atrophy, Leber hereditary optic neuropathy). There is no effective treatment for progressive vision loss due to RGC degeneration. Polysialylated neural cell adhesion molecule (PSA-NCAM) is expressed abundantly in the retina in close proximity to the RGCs. PSA is attached to NCAM by two specific polysialyltransferases, II and IV (ST8SiaII, ST8SiaIV). It is thought that PSA-NCAM affects not only adhesive properties between cells, but also plays a role in the intracellular signaling, which influences cell survival, proliferation, differentiation, migration, axon outgrowth and pathfinding, and synaptic plasticity. The functions of PSA-NCAM in the retina in the adult are not clearly understood. One of the putative roles of PSA-NCAM in the retina is in the maintenance of RGC survival.

The aims of this study were to investigate in detail (a) the survival of RGCs in mice deficient for NCAM and ST8SiaII or ST8SiaIV; (b) the protective roles of NCAM/PSA-NCAM during excitotoxic retinal damage induced by kainic acid (KA); (c) the protective roles of PSA-NCAM in the mouse model of diabetic retinopathy (DR).

Our study demonstrates that the viability and survival of RGCs is dependent on the presence of PSA-NCAM expressed by astroglial and Müller cells. Excitotoxic retinal damage or diabetic retinopathy induces a decrease in the levels of PSA-NCAM and thereby promotes RGC degeneration. The reduction of PSA-NCAM levels in these models is mediated by matrix metalloproteinase-9 (MMP-9), which induces shedding of the extracellular domain of PSA-NCAM. Our study demonstrates that PSA-NCAM and MMP-9 might be new pharmacological targets to combat retinal degeneration.

# 1. REVIEW OF LITERATURE

## 1.1. Cell adhesion molecules

Cell adhesion molecules (CAMs) are proteins associated with the cell membrane. They mediate adhesion of cells to one other or to the components of the extracellular matrix through homo- or heterophilic binding. Cell adhesion is crucial for histogenesis, morphogenesis as well as for tissue maintenance and functioning in embryogenesis and in adulthood. CAMs are divided into four major superfamilies: the immunoglobulin cell adhesion molecules, the cadherins, the integrins and the selectins. Neural cell adhesion molecule (NCAM) belongs to the immunoglobulin superfamily of CAMs and was first described as a synaptic membrane glycoprotein named D2 by Jørgensen and Bock in 1974 (Jørgenson and Bock, 1974). Hirn and colleagues described it as BSP-2 protein (Hirn et al., 1981). Thiery and collegues identified and described it from the retinal and brain tissues as neural cell adhesion molecule (Thiery et al., 1977; Cunningham et al., 1987). NCAM appears early in embryogenesis and is continued to be expressed in adulthood (Chuong and Edelman, 1984). It was recognized that NCAM or its polysialylated form PSA-NCAM induces intracellular signaling pathways that influence neural activity, the formation of neuronal connections and also neuroprotection (Ditlevsin et al., 2007).

## 1.2. Neural cell adhesion molecule (NCAM)

### 1.2.1. Structure of NCAM

NCAM is a cell-surface glycoprotein. Alternative splicing of messenger RNA from a single *NCAM1* gene yields three major isoforms of NCAM, named due to their molecular weight in kilodaltons (kDa), namely NCAM-180, NCAM-140 and NCAM-120 (Cunningham et al., 1987; Owens at al., 1987). Two largest, NCAM-180 and NCAM-140, are integral membrane polypeptides with cytoplasmic domains. NCAM-120 is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (Owens et al., 1987). The extracellular parts of the three main proteins are identical and they include five immunoglobulin-like (Ig) domains followed by two fibronectin type III (FNIII) modules (Fig. 1). The cytoplasmic domain of NCAM-180 differs from NCAM-140 by an additional sequence of 261 amino acids (Murray et al., 1986; Owens et al., 1987). NCAM can exist in a secreted form due to expression of a stop-exon located between the $12^{th}$ and $13^{th}$ exons, which terminates the cording sequence, producing a truncated extracellular part of NCAM (Bock et al., 1987; Gower et al., 1988). Variation is also achieved by the expression of a variable alternatively spliced exon (VASE), located between the $7^{th}$ and $8^{th}$ exons, which downregulates neurite outgrowth induced by NCAM (Doherty et al., 1992). Expression of the VASE exon increases during development and is thought to play a role in promotion of tissue stability.

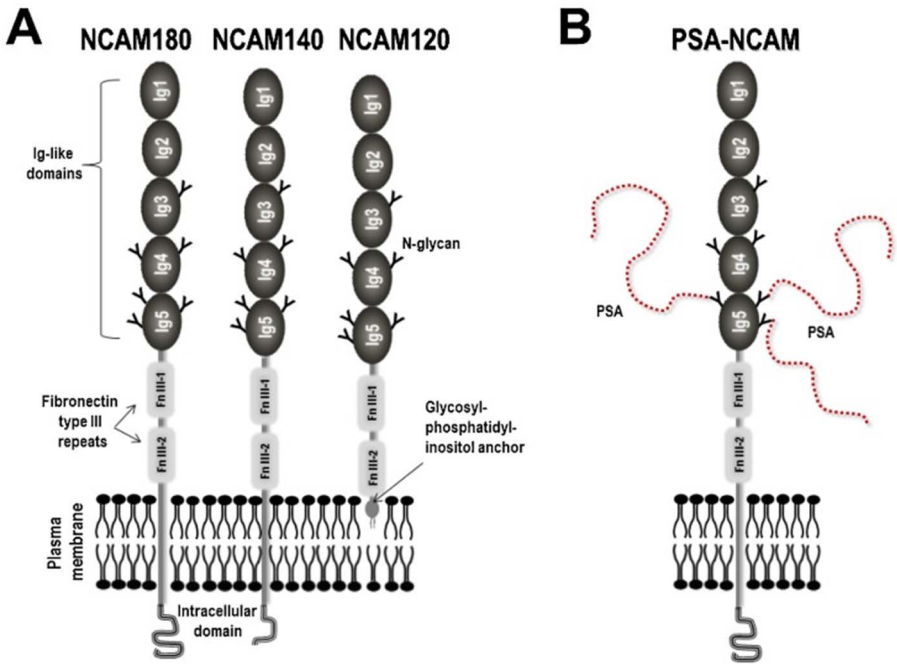


**Figure 1.** NCAM and PSA-NCAM molecules. (A) Isoforms of NCAM: NCAM-180, NCAM-140, NCAM-120. All isoforms consist of five Ig-like domains and two fibronectin type III modules. NCAM-180 and NCAM-140 have transmembrane module and cytoplasmic domain with different molecular weight. NCAM-120 is attached to the cell membrane by glycosylphosphatidylinositol (GPI) anchor. (B) Polysialylated form of NCAM, where polysialic acid is attached to NCAM at the fifth Ig-like domain of NCAM (Aonurm-Helm et al., 2016).

### 1.2.2. Posttranslational modification of NCAM

Major posttranslational modification of NCAM with polysialic acid (PSA) plays an important role in NCAM function. PSA is a long, linear, negatively charged homopolymer of alfa-2,8-linked sialic acid, comprising more than 50 residues. Three isoforms of NCAM can occur in sialylated form (Rougon et al., 1982). PSA has an enormous degree of hydration, thus, being polysialylated NCAM, occupiers much a higher volume and reduces contacts between cell membranes (Hoffman et al., 1982; Johnson et al., 2005). NCAM is the predominant carrier of PSA in vertebrates (Hoffman et al., 1982). Polysialylation significantly modifies the functions of the NCAM protein. PSA content is strongly regulated during embryonic and postnatal development and this process is protein-specific. A limited number of other proteins, apart from NCAM, have been found to be polysialylated in mammals, namely: the alfa subunit of the voltage-dependent sodium channels (Zuber et al., 1992), CD36 scavenger receptors in human and mouse milk (Yabe et al., 2003), and the polysialyltransferases, which autopolysialylate themselves (Close et al., 2003). There are

six known polysialylation sites in the NCAM protein. The majority of PSA is attached to the fifth and sixth polysialylation sites located on the Ig5 domain of NCAM (Nelson et al., 1995). It was also demonstrated that the adjacent Ig4 and FNIII1 domains are necessary for NCAM distinction and subsequent polysialylation (Nelson et al., 1997, Close et al., 2003). PSA is attached to NCAM in the Golgi complex by two specific polysialyltransferases: ST8SiaII (STX) and ST8SiaIV (PST). Both STX and PST are highly expressed during development, though STX transcript levels are higher. Soon after birth, STX is decreased dramatically; however, PST is declined only moderately (Phillips et al., 1997; Bruses et al., 2001). It was shown that STX and PST work cooperatively: the addition of PSA at the sixth N-glycosylation site on the Ig5 domain by PST facilitates the synthesis of PSA at the fifth N-glycosylation site in the Ig5 by STX, and vice versa (Angata et al., 1998). Thus, PST highly prefers the sixth N-glycosylation site, but STX strongly prefers the fifth. It was further demonstrated that STX and PST together polysialilate NCAM more effectively, than STX or PST alone (Angata et al., 1998). PSA chains synthesized by PST are longer than those synthesized by STX.

## 1.3. Homophilic interactions of NCAM

It has been shown that NCAMs can attach to each other, an example of homophilic binding. Binding between NCAM proteins expressed on the surface of one cell membrane is called homophilic cis-interactions. Binding between NCAM proteins located on adjacent membrane surfaces is called homophilic trans-interactions (Chuong and Edelman, 1984). There are a lot of controversial mechanisms in the literature concerning homophilic NCAM binding. NCAMs, located on the cell membrane, are arranged in cis-dimeric forms: the first two Ig-like modules of one NCAM form parallel cis-bindings with two Ig-repeats of another NCAM (Kasper et al., 2000; Soroka et al., 2003). However, in the present of adjacent NCAM-expressing cells, cis-dimers are arranged in anti-parallel manner where bindings occur between the second and third Ig-modules. Anti-parallel NCAM-dimers interact in trans- and form the one-dimensional “flat zipper” conformation, where the second and third Ig domains of the two opposing NCAMs bind two each other, or the one-dimensional “compact zipper” conformation, where the first three Ig-like domains of the two adjusting NCAMs interact with each other. Combinations of the flat and compact zippers form a strong two-dimensional zipper (Soroka et al.,2003; Kiselyov et al., 2003). Thus, NCAM molecules interact in *trans* and form the tight two-dimensional zippers, which leave no space for PSA-moiety and favor adhesion between cells. PSA molecules attached to NCAM occupy a large volume and, therefore, polysialylated NCAM can only form the weak one-dimensional zippers, which favor signaling via heterophilic interactions of PSA-NCAM (Kiselyov et al., 2005).

## 1.4. Heterophilic interactions of PSA-NCAM

### 1.4.1. Interaction with fibroblast growth factor receptor 1 (FGFR1)

It is thought that expression of PSA on NCAM firstly reduces the adhesive properties of NCAM and secondly that PSA positively regulates interaction with other proteins and modulates signaling processes. It has been suggested that NCAM/PSA-NCAM can play a role as a receptor, independently of its adhesive properties. Using a surface plasmon resonance analysis and nuclear magnetic resonance, a direct interaction between FNIII 1 and 2 modules of NCAM and Ig modules 2 and 3 of FGFR1 were shown (Kiselov et al., 2003). Interactions with FGFR1 take place only when NCAM presents in a polysialylated form. Trans-homophilic association between PSA-NCAM leads to the formation of one-dimensional zippers and permits the interactions between NCAM and FGFR1. When NCAM is not polysialylated, NCAM forms the two-dimensional tight zipper conformation, without space for interaction with other molecules (Kiselov, et al., 2005). Thereby, FGFR1 cannot be activated when NCAM is unpolysialylated. Activation of FGFR1 mediated by heterophilic PSA-NCAM binding results in dimerization and phosphorylation of FGFR1 that leads to activation of phosphatidylinositol 3-kinases (PI3K) and phosphorylation and activation of serine/threonine-specific protein kinase (Akt), also known as protein kinase B (PKB) (Neiiendam et al., 2004). Akt has a number of downstream effects which play a key role in multiple cellular processes, such as apoptosis, cell proliferation, differentiation, migration. The Ras-Raf-MEK-MAPK (ERK) pathway is also initiated by the activation of FGFR1 (Neiiendam et al., 2004). Mitogen-activated protein kinases (MAPK) are also known as extracellular signal-regulated kinases (ERK). MAPK regulate the activities of several transcription factors. Indeed, activation of FGFR1 and subsequent activation of PI3K-Akt and Ras-MAPK downstream cell signaling pathways resulted in neuronal cell survival and neurite outgrowth (Neiiendam et al., 2004).

### 1.4.2. Interaction with tyrosine receptor kinase B (TrkB) receptor

PSA-NCAM plays a role in brain-derived neurotrophic factor (BDNF) signaling, due to promotion of phosphorylation of the TrkB receptor (Vutskis et al., 2001). PSA may delay the diffusion of BDNF away from the cell membrane, thereby facilitating the activation of TrkB (Muller et al., 2000). A direct interaction between PSA-NCAM and the TrkB receptor is also possible, leading to a clustering and more efficient signaling of the TrkB receptor (Cassens et al., 2010). TrkB receptor phosphorylation leads to activation of Ras-MAPK and PI3K-Akt signaling cascades, which increases neuronal survival (Cheng et al., 2002; Hetman et al., 1999).

### 1.4.3. Interaction with glial cell line-derived neurotrophic factor (GDNF) and glial factor receptor-alpha (GFR-alpha)

PSA-NCAM can interact with GDNF and with its receptor GFR-alpha leading to phosphorylation of receptor tyrosine kinase Ret, and activation of downstream cascades including tyrosine specific phosphor-transferase (Fyn), focal adhesion kinase (FAK), MAPK, PI3K pathways, eventually increasing cell viability (Paratcha, et al., 2003; Jing et al., 1996; Worby et al., 1996). GDNF has been demonstrated to be a survival factor for neuronal cells (Linn et al., 1993; Trupp et al., 1996).

### 1.4.4. NCAM-signaling via non-receptor tyrosine kinase

NCAM induces phosphorylation of non-receptor tyrosine kinase Fyn and subsequent phosphorylation of another non-receptor tyrosine kinase, FAK (Chattopadhyaya et al., 2013). Activated Fyn and FAK trigger downstream cascades, which finally activate MAPK and PI3K-Akt that are involved in cell survival, migration, division (Carry et al., 1999; Neiiendam et al., 2004). Other pathways triggered by FAK and also by activation of FGFR1 involve regulation of activity of phospholipase C ($PLC_y$), p53, that influences cell apoptosis (Franke et al., 1997; Neiiendam et al., 2004).

### 1.4.5. Interaction with N-methyl-D-aspartate (NMDA) receptor

It was shown previously that PSA-NCAM interacts with NMDA. NMDA is a type of ionotropic glutamate receptor found abundantly in nerve cells. The NMDA receptor is assembled as a tetramer, consisting of different combinations of seven subunits: GluN1, GluN2(A-D), GluN3(A-B). Overactivation of the receptor induces excessive influx of $Ca^{2+}$ into the cell and subsequent excitotoxicity. It was demonstrated that PSA-NCAM inhibited NMDA receptors in hippocampus and deficits in NCAM/PSA-NCAM increased GluN1 and GluN2B-mediated $Ca^{2+}$ influx in CA1 pyramidal cells in hippocampal slices (Kochlamazashvili et al., 2010). PSA prevented the activation of GluN1 and GluN2B subunits and decreased glutamate-induced cell death in primary hippocampal culture (Hammond et al., 2006).

## 1.5. NCAM/PSA-NCAM expression in the brain

During embryogenesis and early postnatal development, PSA-NCAM is expressed throughout the entire nervous system, though its expression gradually shifts to the NCAM protein at different rates in different regions of the adult nervous system (Rougon et al., 1982; Hoffman et al. 1983; Schlosshauer et al., 1984; Seki and Arai, 1991; Rutishauser et al., 1996). The relative amount of

each of the three isoforms of NCAM varies between different brain regions. All three isoforms of NCAM polypeptides have been found in extracts of adult forebrain and cerebellum; however, cerebral cortex contains only NCAM-140 and NCAM-120 (Rougon et al., 1982). Interestingly, PSA-NCAM protein was largely found in the adult brain in the regions with ongoing plasticity and neurogenesis, such as the hippocampal subgranular zone (SGZ), located in the dentate gyrus between the granule cell layer and the hilus, the subventricular zone in the walls of the lateral ventricular (SVZ), and the rostral migratory stream (RMS) of the olfactory system. PSA-NCAM is expressed by transit amplifying cells (D1 cells) in the SGZ, and by committed neuroblasts (D2, D3 cells in SGZ, and A-cells in SVZ). Neurogenesis in the hippocampus in adulthood gives rise to new granule cells in the dentate gyrus, the axons of which comprise the mossy fiber tract that connect the dentate gyrus with the cornu Ammonis region 3 (CA3), one of the hippocampal subfields. Interneurons in the olfactory bulb are generated in the SVZ in the adult and they migrate through the RMS to reach their final location (Altman et al., 1969; Luskin et al., 1993). PSA-NCAM protein is a characteristic feature of neurogenic niches in the adult, although PSA-NCAM is not expressed by stem cells nor in SGZ or SVZ (Gascon et al., 2010). PSA-NCAM is found also in neurons of piriform cortex layer II, which receives olfactory fibers from the olfactory bulb, in the cerebral cortex in L cells, in amygdala (Seki and Arai, 1993; Kempermann et al., 2015; Job et al., 2015; Varea et all, 2005; Varea et al., 2011). Thereby, PSA-NCAM expression is maintained in the adult brain mostly in the sites with ongoing plasticity (Gascon et al., 2010).

## 1.6. NCAM/PSA-NCAM functions in the brain

PSA-NCAM regulates cell migration, differentiation, neurite outgrowth, synapse formation, neuronal survival during development (Angata et al., 2007, Seki and Rutishauser, 1998). Genetic or enzymatic removal of PSA causes divergent innervation and ectopic synaptogenesis in hippocampus (Seki and Rutishauser, 1998). Mice deficient for all isoforms of NCAM demonstrate a significant reduction in olfactory bulb size and accumulation of migrating precursors along the RMS (Cremer et al., 1994).

PSA-NCAM guides directed and efficient migration of neuroblasts in neurogenic niches, plays a role in axonal growth and fasciculation, synaptic integration of newborn neurons in the adult (Rousselot et al., 1995; Hu et al., 1996; Cremer et al., 1994; Cremer et al., 1997). PSA-NCAM is abruptly downregulated soon after newborn neurons reach their final location (Rousselot et al., 1995). PSA-NCAM supports the survival of newly generated neurons in the SVZ and RMS (Gascon et al., 2007) and of neurons in vitro (Vutskis et al., 2006). PSA-NCAM can also reduce glutamate-induced death of hippocampal neurons in vitro (Hammond et al., 2006). Moreover, PSA-NCAM is involved in regeneration of neuronal fibers (Muller et al., 1994).

The hippocampus has been thought a critical zone for learning and memory formation. NCAM/PSA-NCAM is required for structural remodeling of synaptic connections during long-term memory formation in the synapses of the hippocampal mossy fibers (Muller et al., 1996). An increase in NCAM polysialylation in hippocampal dentate granule cells and in neurons of the entorhinal cortex was observed as a response to learning tasks (Murphy et al., 1996; O'Connell et al., 1997). Removal of PSA with injections into the hippocampus of endoneuraminidase-N (Endo-N), an enzyme which cleaves alpha-2,8-linked polysialic acid, significantly impaired spatial learning in rats (Becker et al., 1996). The functions of NCAM are believed to shift from promotion of plasticity during development (PSA-NCAM) to mediating structural stability in adulthood (NCAM) (Rutishauser and Landmesser, 1996). However, PSA-NCAM remains expressed in regions of ongoing plasticity and neurogenesis in the adult brain (rev. by Seki, Arai, 1993).

## 1.7. PSA-NCAM in the retina

### 1.7.1. Cellular organization of the retina

Retinal progenitor cells arise from the anterior portion of the neural tube. They give rise to all of the neurons and Müller glia cells in the retina, and are themselves distributed in three nuclear layers interconnected by two plexiform layers (Fig. 2). Photoreceptors (rods and cones) form the outer (most external) nuclear layer (ONL). Photoreceptors convert the entering photons into a chemical signal. RGCs constitute the innermost nuclear layer (GCL) and their axons form the optic nerve and tract. Cell bodies of bipolar, Müller cells and other neurons form the inner nuclear layer (INL), located between the GCL and ONL. Bipolar cells transfer the visual signal from the photoreceptors to the retinal ganglion cells (RGCs). Astrocyte progenitor cells originate from the neuroepithelium of the optic stalk and migrate into the retina through the optic nerve head (Tao et al., 2014). Müller cells stretch throughout the entire thickness of the retina and arrange in a regular fashion. Astrocytes are confined to the nerve fiber layer (NFL) and to the ganglion cell layer (GCL) and their distribution correlates with the thickness of the NFL. It has been shown previously that the bodies of the RGCs are enveloped by close-fitting sheaths formed by the membrane of Müller cells (Stone et al., 1995). The intraretinal part of the RGC axons is also surrounded by Müller cells and astrocyte processes (Stone et al., 1995). Each Müller cell comprises a core of a column, which is the smallest unit for processing of visual information. Every unit includes, in addition to the Müller cell, one cone and a variable number of rods, three bipolar cells, and one or two ganglion cells. Müller cells support the survival of neurons, remove glutamate from the inner retinal layers, play an important role in the regulation of the volume of extracellular space, guide the light to the photoreceptors. In mammals, retinal gliosis is a nonspecific response of glia, including Müller

cells, astrocytes, microglial cells, to different pathogenic processes. The up-regulation of intermediate filament proteins, such as glial fibrillary acidic protein (GFAP), is a mark of gliosis. Reactive Müller cells and astrocytes protect neuronal survival by secreting neurotrophic factors including basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), GDNF ( Zhu et al., 2012). On the other hand, reactive gliosis induces retinal damage due to formation of a glial scar and tractional retinal detachment. Activated glial cells also release matrix metalloproteinases (Limb et al., 2002). Interestingly, in non-mammalian vertebrates, injury-induced Müller cells have been demonstrated to be able to dedifferentiate, re-enter to a progenitor state, asymmetrically proliferate and generate new retinal neurons, including RGCs for retinal repair (Bernardos et al., 2007).

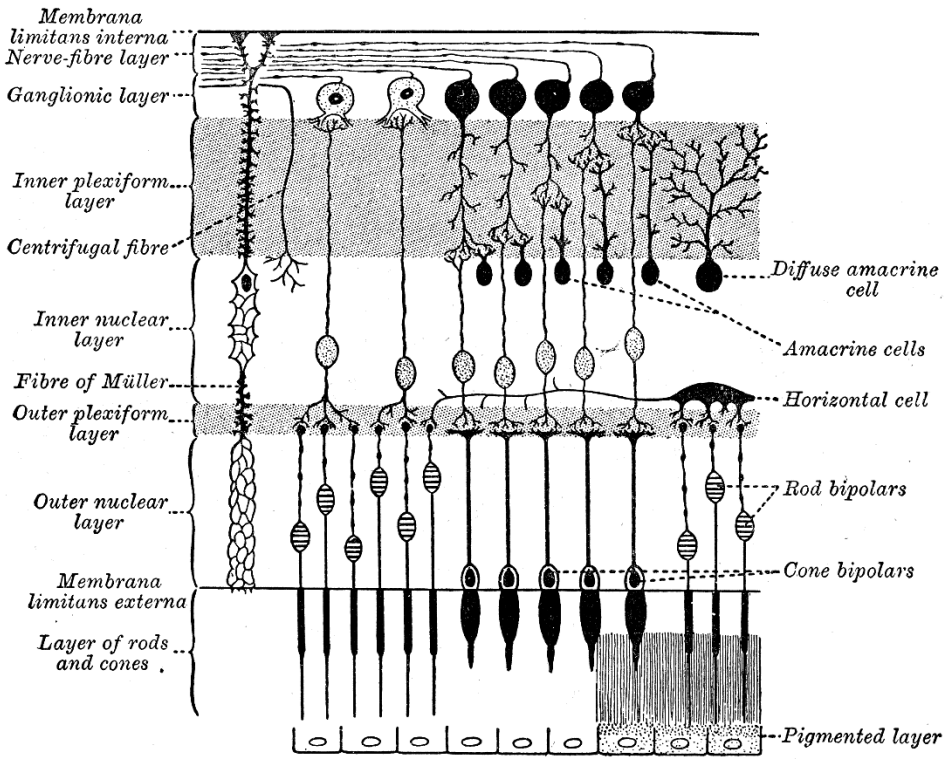


**Figure 2.** Schematic drawing of the retina layers by Henry Vandyke Carter (Gray H. Anatomy: Descriptive and Surgical. England, 1858).

### 1.7.2. Expression of PSA-NCAM in the retina

All three isoforms of NCAM are expressed during development and adulthood in mammalian retina (Sawaguchi et al., 1999, Bartsch et al., 1989). During embryogenesis, NCAM/PSA-NCAM is expressed abundantly in all retinal layers, by all cell types, including soma and growth cones of RGC axons and their contact sites with endfeet of Müller cells (Silver and Rutishauser, 1984; Bartsch, 1989). PSA-NCAM is found also in the environment surrounding RGCs and their axons in the optic tract and tectum (Yin et al., 1995). In the adult vertebrate retina, NCAM is found primarily in the plexiform layers and nerve fiber layer, with less immunoreactivity observed in the inner and outer nuclear layers, and no detectable histochemical signal of NCAM in the photoreceptor layer. (Fliesler et al. 1990). In early postnatal ages, highly sialylated NCAM continues to be expressed by neuroblasts, young postmitotic neurons, including RGC bodies and axons, and Müller cells and astrocytes (Bartsch et al., 1989). Starting from the third postnatal week, highly polysialylated NCAM disappears from RGC bodies and processes and continues to be expressed only by Müller cells and astrocytes in adulthood, although PSA-NCAM is not expressed on the parts of Müller cells, contacting the basal lamina in the outer retina. Immunohistochemistry at both the light and electron microscopic levels reveals, weakly sialylated NCAM in all cell types in the retina and optic nerve in adulthood; however, PSA-NCAM is not expressed in the outer segments of photoreceptors (Bartsch et al., 1989). Thus, poorly polysialylated NCAM is expressed by RGCs in the mature retina, whereas highly polysialylated NCAM is expressed by Müller cells and astrocytes in adulthood and remains in close proximity to the RGCs (Bartsch et al., 1989).

### 1.7.3. PSA-NCAM functions in the retina

During development, PSA-NCAM is involved in RGC axon pathfinding in the retina: regulates RGC axonal projection to the visual centers (superior colliculi/ optic tectum) and plays an important role in RGC axon fasciculation (Monnier et al., 2001; Yin et al., 1995; Thanos et al., 1984; Murphy et al., 2007). NCAM/ PSA-NCAM facilitates RGC axonal growth along the endfeet of Müller cells (Stier and Schlosshauer et al., 1995; Silver and Rutishauser, 1984). Inhibition of NCAM/PSA-NCAM in mammals disrupts RGC growth cones, slow their speed down and finally stops them in midretina. PSA-NCAM is also involved in RGC survival during development. In vitro, neonatal RGCs express PSA-NCAM, and removal of PSA-NCAM by Endo-N induces approximately 42% loss of these cultured cells (Murphy et al., 2009). At all developmental stages and in adulthood, NCAM is present at contacts between all cell types in the retina; thus, it seems that NCAM is important for stabilization (Bartsch et al., 1989). Functions of PSA-NCAM in the adult retina however, remains unclear. It has been proposed that PSA-NCAM is involved in RGC survival in adulthood. PSA-

NCAM is highly expressed by adult regenerating RGC axons following axotomy in vitro (Bates et al., 1999). Cleavage of PSA-NCAM in the adult retina in vivo induced 25% loss of RGC at 14 days (Murphy et al., 2009). The reduction in RGCs density was significantly greater following optic nerve transection in retinas where PSA had been removed (Murphy et al., 2009). Optic nerve injury upregulates significantly PSA-NCAM levels in the retina and in the contralateral superior colliculus (SC), which is the target tissue for RGC axons. PSA-NCAM was upregulated mainly on astrocytes in SC; however, some intact RGC axons (less than 5%) projecting from the ipsilateral eye did express PSA-NCAM (Murphy et al., 2007; Murphy et al., 2009). PSA-NCAM was not co-localized with RGC axons in the uninjured SC. Thus, PSA-NCAM could be involved in RGC axonal remodeling after injury. On the other hand, at 28 days after aspiration of SC, RGC densities decreased by approximately 15% in the contralateral eye of NCAM-/- mice, compared with the ipsilateral eye. In contrast, there was no a difference in RGC density after SC damage between contralateral and ipsilateral retinas in WT mice. Moreover, the SC of NCAM-/- mice expressed significantly higher levels of BDNF compared with WT littermates. It seems that in adult NCAM-/- animals, the SC promotes RGC survival. (Murphy et al., 2009). It could be that BDNF expression is adaptively increased in SC of NCAM-/- mice in order to compensate for the lack of PSA-NCAM. Therefore, it seems that PSA-NCAM may increase survival of uninjured and injured RGCs, although the mechanism underlying PSA-NCAM protection of RGCs is unclear. PSA-NCAM signaling through FGFR1, TrkB receptor, NMDA receptor, GFR-alpha may play a role in the maintenance of RGCs after injury. Previously, significant expression of FGFR1 was found on RGCs and Muller cells (Catalani et al., 2009). It was also shown that FGFR1 and FGFs have an important role in the survival and regeneration of RGCs (Blanco et al., 2008). TrkB receptor is expressed on RGCs and intraocular injections of BDNF increases survival of RGCs following axotomy (Peinado-Ramon et al., 1996; Mansour-Robaey et al., 1994; Jelsma et al., 1993). Early studies demonstrated that PSA-NCAM interacts with GluN1 and GluN2B subunits and decreases glutamate-induced cell death in vitro (Hammond et al., 2006). GluN2B subunit plays a critical role in RGC degeneration induced by glutamate excitotoxicity (Bai et al., 2013). NCAM knockout mice demonstrated altered NMDA-induced $Ca^{2+}$ dynamics in RGCs accompanied by a greater loss of RGCs (Murphy et al., 2012). Thus, PSA-NCAM may influence the survival of RGCs due the regulatory activity of NMDA receptors on RGCs. PSA-NCAM/NCAM interact with GDNF and its receptor GFR-alpha. It was shown that GDNF promotes survival of RGCs after optic nerve transection (Koeberle, 1998). GDNF can also increase survival of RGCs via glial cells because GFR-alpha exists in soluble form (Jing et al., 1996). GDNF-GFR-a-Ret complex may diffuse from glial cells via extracellular space and activate a signal transduction cascade in RGCs thereby enhancing cell survival. Thus, PSA-NCAM may regulate signaling through GDNF/GFR-alpha and affect survival of RGCs. However, considering the diverse roles of PSA-NCAM in adult brain, the

function of PSA-NCAM in the retina in adult might be wider than only a protection of RGCs.

### 1.7.4. The roles of PSA-NCAM in the retinal pathologies

The roles of PSA-NCAM in RGC survival in different retinal diseases has not been extensively studied. Increased levels of PSA-NCAM in astrocytes and Müller cells of optic nerve has been observed in mice with experimental glaucoma (Puranen et al., 2014). Excessive light can induce retinal degeneration experimentally and may contribute to the development and progression of human age-related degeneration (Grimm et al., 2013; Luke et al., 2016b). PSA is upregulated in mouse retina following light damage, and retinal cell death occurred earlier after enzymatic removal of PSA from the retina (Luke et al., 2016a). Optic nerve transection is used as a model of optic nerve traumatic damage or atrophy. It was demonstrated that injury of optic nerve in mice increases PSA-NCAM levels significantly in retina (Murphy et al., 2007). A 27% greater reduction in RGC density was observed following optic nerve transection in the retinas of mice where PSA was removed by intravitreal injection of Endo-N compared with control. (Murphy et al., 2009). Thus, pathological conditions induce an increase in PSA-NCAM in the retina and optic nerve. On the other hand, removal of PSA-NCAM from the retina exacerbates degeneration of RGGs. The role of PSA-NCAM in diabetic retinopathy (DR) has not been studied thus far.

## 1.8. Summary of the literature review

NCAM belongs to the immunoglobulin superfamily of CAMs. Polysialylated form of NCAM influences several intracellular signaling pathways and is involved in the regulation of neuroplasticity, such as neurite outgrowth, synapto-, neuritogenesis and neuroprotection in the brain. PSA-NCAM is abundantly expressed in retina close to RGCs. The roles of PSA-NCAM in retina remain unknown. Previous studies have demonstrated changes in the levels of PSA-NCAM in retina and optic nerve during several pathological processes accompanied by RGC degeneration thus suggesting a protective role of PSA-NCAM for RGCs. Studying PSA-NCAM formation, interactions and functions in retina in physiological and pathological conditions can provide new insights into the roles of PSA-NCAM that may have potential for improving and restoring vision. Furthermore, knowledge on the roles of PSA-NCAM is essential for development of new therapies aimed at increasing RGC survival in eye diseases, such as glaucoma, diabetic retinopathy and optic nerve atrophy.

# 2. AIMS OF THE STUDY

1. To evaluate PSA-NCAM expression in the retina of mice with deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes and after intravitreal administration of endoneuraminidase-N (Endo-N), which cleaves PSA chains from NCAM, to wild type animals.
2. To explore the impact of retinal PSA-NCAM in the survival of RGCs in transgenic animals lacking *ST8SiaII, ST8SiaIV* or *NCAM* genes and following intravitreal administration of Endo-N to wild type mice.
3. To evaluate the roles of PSA-NCAM in RGC degeneration following intravitreal administration of kainic acid (KA).
4. To evaluate the mechanisms underlying the reduction in PSA-NCAM and RGC degeneration in KA-induced excitotoxicity, with a focus on metallo-proteinase-9 (MMP-9).
5. To explore the protective roles of PSA-NCAM in the survival of RGCs in streptozotocin-induced diabetic retinopathy in mice.

# 3. MATERIALS AND METHODS

## 3.1. Animals

Experiments were performed in accordance with the guidelines established in the Principles of Laboratory Animal Care (Directive 2010/63/EU). All experiments conformed to regional guidelines on the ethical use of animals. Ethical committee license nr. 77 (12.02.2016). NCAM-/- mice and NCAM+/+ mice used for experiments were obtained by crossing C57BL6-Ncam$^{tm1Cgn+\backslash-}$heterozygous mice that were purchased from Jackson Laboratories, USA. The generation of ST8SiaII and ST8SiaIV knockout mice has been described previously (Eckhardt et al., 2000; Angata et al., 2004). The mouse *ST8SiaII* and *ST8SiaIV* genes were isolated from a mouse129/SvJ genomic DNA library and used to create a targeting vector (Angata et al., 2004, Angata at al., 2007). ST8SiaII and ST8SiaIV knockout mice were then back-crossed to C57BL/6J background for at least six generations. Male and female F5 generation knockout mice (ST8SiaII-/-, ST8SiaIV-/-, NCAM-/-) mice and their wild-type littermates were used at age 5–6 months (Lobanovskaya et al., 2015). Diabetes was induced in male Swiss Webster mice at 2–2.5 months of age by intraperitoneal injection of 90 mg/kg streptozotocin twice (STZ, Sigma-Aldrich, St Louis, MO, USA). Mice were euthanized at two months after the onset of diabetes (Lobanovskaya et al., 2018). Three-to-four-month-old C57BL/6NTac male mice were used for experiments (Lobanovskaya et al., 2019).

## 3.2. Intravitreal injections

Intravitreal injections were performed using a Hamilton syringe (Agilent Manual Syringe, 5; Agilent Technologies, Australia). The needle was inserted through the sclera and retina into the vitreous chamber of the eye. Attention was taken to prevent lens injury as it may promote survival and regeneration after puncture (Pernet Di Polo, 2006). Mice were anesthetized with 1.5–2% isoflurane inhalation for intravitreal administrations. Intravitreal injection rises intraocular pressure for some period of time that can lead to RGC impairment. In our experiments each intravitreal injection was performed in a final volume of 2 µl. Thus, equal rising of intraocular pressure was induced in all groups of comparison. The dose of KA for intravitreal administration was 5 nmol and 10 nmol. To inhibit MMP-9 activity, a specific MMP-9 inhibitor (2-(N-Benzyl-4-methoxyphenylsulfonamido)-5-((diethylamino)methyl)-N-hydroxy-3-methylbenzamide; Abcam, USA) was chosen for intravitreal injections. This compound inhibits MMP-9 in low concentrations (IC50=5 nM), whereas its inhibitory activity for MMP-1 and for MMP-13 is higher (IC50=113 nM and 1050 nM, respectively). Endo-N, provided by Professor Rita Gerardin-Schahn, is an enzyme that selectively cleaves PSA chains from the extracellular domain of NCAM (Gerardy-Schahn et al., 1995). The dose of Endo-N for intravitreal administration was selected on the

basis of a series of preliminary experiments. The effectiveness of Endo-N to remove PSA from NCAM was tested by using both Western blotting and immunohistochemistry at 24 hours following enzyme administration. It was found that 6.5 U of Endo-N was able to complete remove PSA from NCAM in the retina (Lobanovskaya et al., 2015, 2019) and this concentration was used for intravitreal injections. Lower doses provided only partial efficacy.

## 3.3. RGC immunostaining and cell density analyses

Mice were anesthetized deeply with chloral hydrate (300 mg/kg, i/p) and perfused transcardially using 0.9% saline and then with 4% paraformaldehyde in phosphate buffered saline (PBS, 0.1 M, pH=7.4). Retinas were extracted, post-fixed for 20 min, and wholemounted on gelatinized slides (Lobanovskaya et al., 2015). For RGC immunohistochemical staining, goat anti-Brn3a (C-20) antibody (1:100, Santa Cruz Biotechnology, Germany) was used. The secondary antibody was biotinylated anti-goat (1:200; DAKO, Denmark). Immunoreactivity of Brn3a-positive cells was visualized using the peroxidase method (Vectastatin ABC kit and Peroxidase substrate kit DAB, Vector Laboratories, USA) (Lobanovskaya et al., 2015). The density of Brn3a-positive cells was analyzed using a stereology system comprising an Olympus BX-51 microscope and the Visiopharm Integrator System (Version 3.6.5.0, Denmark) (Lobanovskaya et al., 2015).

## 3.4. Immunoblotting analysis

Tissue preparation, supernatant resolution and transfer of proteins onto membranes was performed as described previously (Lobanovskaya et al., 2015). Membranes were blocked with 0.1% (w/w) Tween-20/TBS containing 0.5% (w/w) non-fat dried milk powder or with Odyssey Blocking Buffer (PBS) (LI-COR Biotechnology, USA). For protein detection, the following primary antibodies were used: mouse anti-PSA-NCAM (1:1000; IgM, clone 2–2B, AbCys, France or 1:1000; IgM, clone 2–2B, MAB5324, Millipore, USA), rabbit anti-ST8SiaII (1:400; Proteintech, USA), rabbit anti-ST8SiaIV (1:250; Thermo Scientific, USA), mouse anti-active MMP-9 (4A3) (1:1000; Novus, USA), mouse anti-GFAP (1:1000; IgG, clone 2-A-5, Chemicon, UK), rabbit anti-β-actin (1:5000; Sigma, St. Louis, USA or LI-COR, USA) or mouse anti-β-actin (1:5000; Sigma, St. Louis, USA). Membranes were then washed and incubated with secondary antibodies: anti-mouse HRP (1:400; Thermo Scientific, USA) or anti-rabbit HRP (1:2000; Pierce, USA) followed by incubation with ECL detection reagent (Amersham, ECL, UK); biotinylated goat anti-mouse IgM (1:2000; Vector Laboratories, Inc. USA) followed by IR Dye 680 LT Streptavidin; goat anti-mouse IR Dye 680 LT, IgM (for PSA-NCAM detection), goat

anti-mouse IR Dye 680 LT, goat anti-mouse IR Dye 800 CW, goat anti-rabbit IR-Dye 680 LT, goat anti-rabbit IR-Dye 800 CW (1:10000; LI-COR, USA).

The membranes were analyzed by exposure to autoradiography X-ray film (Amersham, hyperfilm, UK) or using the Odyssey CLx Infrared Imaging System (USA). Optical densities ratios were then calculated and used for analysis (Lobanovskaya et al., 2015; 2018; 2019).

## 3.5. Immunohistochemistry

Sections for immunohistochemical analyses were prepared as described previously (Lobanovskaya et al., 2015). Sections were incubated with the following primary antibodies: mouse anti-PSA-NCAM (1:1000; IgM, clone: 2–2B, AbCys, France or 1:1000; IgM, clone 2–2B, MAB5324, Millipore, USA), goat anti-Brn3a (C-20) (1:100, Santa Cruz Biotechnology, Germany), rabbit anti-GFAP (1:800; 1mg/ml, MAB 3402, Chemicon, USA), rabbit anti-GFAP (1:1000; Dako, Denmark), goat anti-MMP-9 (1:500; Abcam, USA) or mouse anti-active MMP-9 (4A3) (1:800; Novus, USA). After washes, sections were incubated with an appropriate secondary antibody: DyLight 594 goat anti-mouse IgG+IgM (H+L) (1:300; Thermo Scientific, USA), Alexa Fluor 488 goat anti-rabbit IgG (H+L) (1:300–1:500; Invitrogen Molecular probes, USA), Streptavidin, Alexa Fluor 488 conjugate (1:1000; Invitrogen Molecular probes , USA), biotinylated anti-mouse (IgM), anti-rabbit, anti-goat (1:200; Vector Laboratories, Inc., USA), Alexa Fluor 594 goat anti mouse IgM (1:500; Invitrogen Molecular probes, USA).

Fluorescent signals were detected using a confocal microscope (LSM 510 Zeiss, Germany) and further analyzed for the co-localization of PSA-NCAM signal with Brn3a or GFAP. DP55561-10, 561 nm and Argon/2 458, 477, 488, 514 nm lasers were used. Immunoreactivities were visualized using a peroxidase method (Vectastatin ABC kit and Peroxidase substrate kit DAB, Vector Laboratories, USA) and observed using Olympus BX-51 microscope (Lobanovskaya et al., 2015; 2018; 2019).

## 3.6. Data analysis

All data are expressed as mean±SEM. One-way ANOVA, two-way ANOVA or two-way ANOVA with repeated measures were used for statistical analysis. Post-hoc comparisons were made using Bonferroni or Tukey's tests. Two group comparisons were performed using either Mann-Whitney (non-parametric) or Student's t-test (parametric), where appropriate. A p value of less than 0.05 ($p<0.05$) was considered to indicate a statistically significant difference.

# 4. RESULTS

## 4.1. PSA-NCAM expression in the retina of mice with deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes and after administration of Endo-N

It was shown previously a significant expression of PSA-NCAM in the retina of wild type (WT) mice (Murphy et al., 2009). PSA-NCAM protein have not yet been studied it the retinas of knockout ST8SiaII or ST8SiaIV mice. We assumed that these transgenic animals might have a lack of PSA-NCAM during development or in adulthood, which could influence the viability of RGCs in mature mice. Western blot analysis and immunohistochemistry were employed to examine the levels and distribution of PSA-NCAM in the retina. Unexpectedly, western blotting demonstrated that PSA-NCAM is expressed in the same degree in the retinas of adult WT and in knockout ST8SiaII-/- or ST8SiaIV-/- animals (Fig. 3). These data were in sharp contrast to the PSA-NCAM expression pattern in ST8SiaIV-/- brain where a substantial reduction of PSA-NCAM levels was observed (Fig. 3) (Lobanovskaya et al., 2015).

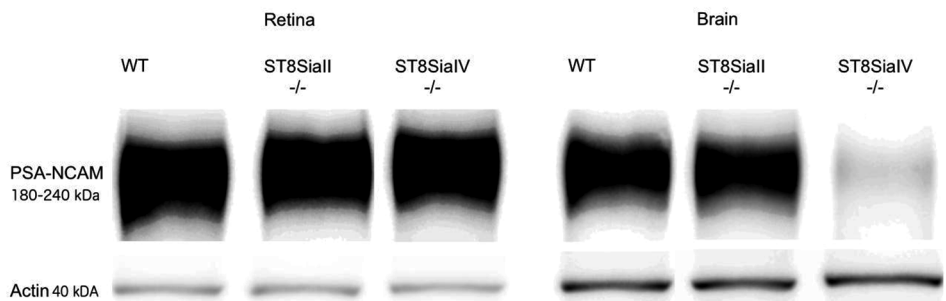


**Figure 3.** Western blots demonstrating PSA-NCAM protein levels in the adult retina and in the mature brain of WT, ST8SiaII-/-, ST8SiaIV-/- mice (Lobanovskaya et al., 2015).

Immunohistochemistry revealed that the distribution of immunopositive signal of PSA-NCAM in the retinal sections of WT, *ST8SiaII-/-* and *ST8SiaIV-/-* mice was the same (Fig. 4). As predicted, immunohistochemical analysis did not detect PSA-NCAM protein in the retinal sections of NCAM-/- knockout mice (Lobanovskaya et al., 2015) (Fig.4).

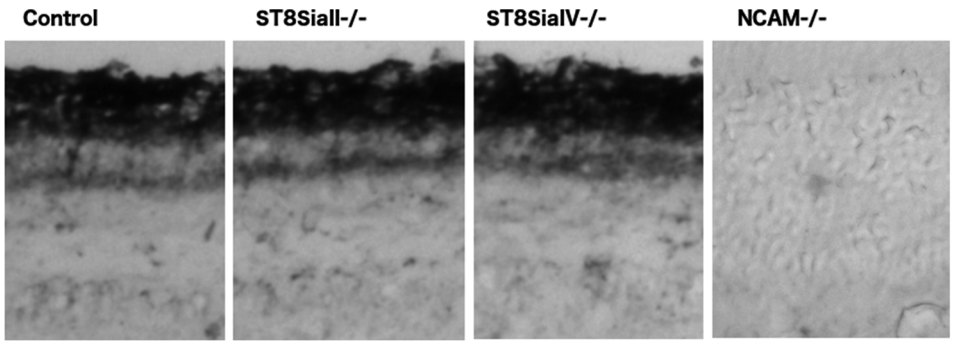


**Figure 4.** Representative microphotographs of PSA-NCAM expression in the retinal sections from WT, NCAM-/-, ST8SiaII-/- or ST8SiaIV-/- mice. Magnification x400 (Lobanovskaya et al., 2015).

In all genotypes, PSA-NCAM was expressed predominantly in the inner part of the retina, largely concentrated near to RGCs although it was not co-localized with RGCs (Fig. 5). Further analysis demonstrated that PSA-NCAM co-localized with GFAP, in the inner part of the retina, where astrocytes and the endfeet of Müller cells are located (Fig. 5) (Lobanovskaya et al., 2015). It seems that the major source of PSA-NCAM in the adult retina is astrocytes and Müller cells surrounding RGCs.

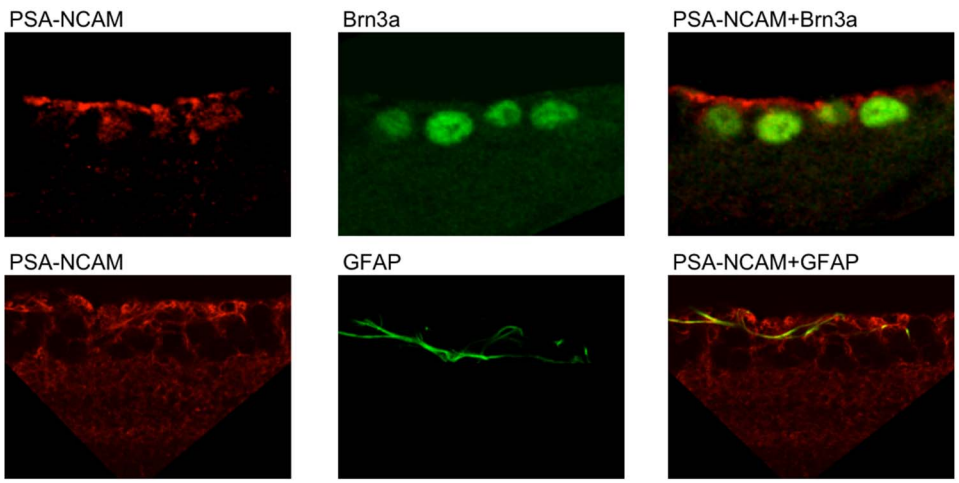


**Figure 5.** Confocal images of sections double-labelled for PSA-NCAM (red) and Brn3a (green, upper panel), a marker for RGCs, or GFAP (green, bottom panel), a marker for astrocytes. Magnification x400 (Lobanovskaya et al., 2015).

Thus, as PSA-NCAM levels in the retina of adult ST8SiaIV-/- animals was unchanged, we assumed that ST8SiaII compensates for the absence of ST8SiaIV in adult transgenic ST8SiaIV-/- mice. To prove this hypothesis, we measured the levels of sialyltransferases ST8SiaII and ST8SiaIV in the retinas of transgenic mice. Western blotting revealed that both sialyltransferases are expressed in the retina of WT mice. However, the level of ST8SiaII protein was much higher in ST8SiaIV transgenic mice in comparison with controls. The level of ST8SiaIV was not changed in the retinas of ST8SiaII-/- mice (Lobanovskaya et al., 2015). Next, we were interested whether newborn (P1) mice with a deficiency in ST8SiaII or ST8SiaIV protein had altered levels of PSA-NCAM expression in the retina. Western blotting demonstrated high PSA-NCAM signal in the retinas of newborn (P1) WT animals, but PSA-NCAM signal was barely visible in the retinas of knockout ST8SiaII-/- animals, and approximately 2-fold lower in the retinas of ST8SiaIV-/- mice compared with WT animals (Lobanovskaya et al., 2015). These results were expected, as ST8SiaII is not expressed during embryogenesis in ST8SiaII-/- transgenic mice. Apparently, sialyltransferases work cooperatively during retina development; however, ST8SiaIV has less compensatory ability in newborn (P1) animals. As PSA-NCAM protein was not reduced in adult transgenic ST8SiaII-/- or ST8SiaIV-/- mice, Endo-N was used to eliminate PSA from NCAM in the retina. Endo-N is an enzyme that selectively cleavages PSA chains on the NCAM molecule (Gerardy-Scahn et al., 1995). Western blotting showed that intravitreal injection of Endo-N induced a complete long-lasting reduction in the levels of PSA-NCAM at 2, 14 and 28 days following administration (Lobanovskaya et al., 2015).

## 4.2. RGC density in the retina of mice with deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes and after administration of Endo-N

Next, we were interested to know whether constitutive deficiency in NCAM and sialyltransferases or Endo-N treatment influenced the survival of RGCs. To answer this question, we counted Brn3a-immunopositive cells in wholemounted retinas of WT, transgenic ST8SiaII-/-, ST8SiaIV-/- or NCAM-/- mice or Endo-N-treated mice. Brn3a is a marker for RGCs (Nadal-Nikolas et al., 2009). The density of RGCs was significantly lower in ST8SiaII-/- mice compared with their WT littermates, whereas density in ST8SiaIV-/- animals was normal. Interestingly, in NCAM-/- mice the density of RGCs was significantly higher compared with WT littermates (Table 1). A two-way ANOVA demonstrated a significant effect of genotype ($F_{3,63}$=20.98; $p<0.0001$) (Lobanovskaya et al., 2015). Thus, the significant decrease in PSA during development in ST8SiaII-/- mice may influence the survival of RGCs. Previously, in vitro removal of PSA by Endo-N was demonstrated increased death of neonatal RGCs (Murphy et al., 2009). The

higher density of RGCs in NCAM-/- animals compared with control might be due to the ability of NCAM to inhibit the proliferation of progenitor cells (Amoureux et al., 2000). In order to examine whether removal of PSA from the adult retina by Endo-N administration affects the survival of RGCs at 2 and 14 days following Endo-N treatment, we calculated RGC density in wholemounted retinas. Endo-N-induced reduction in polysialylated NCAM did not affect the viability of RGCs in the mature retina (Table 1) (Lobanovskaya et al., 2015). Thus, removal of PSA does not affect the survival of RGCs in uninjured retinas in adulthood.

**Table 1.** Effect of intravitreal injection of KA on RGC densities in adult wild-type (WT), ST8SiaII-/-, ST8SiaIV-/- or NCAM-/- mice.

| Group | Cell density, cells/mm$^2$ | | % reduction |
|---|---|---|---|
| | Control | KA | |
| WT | 3064 ±73 (13) | 1170 ± 76 (13) | 61 |
| NCAM -/- | 3703 ± 121*** (6) | 1379 ± 51 (6) | 62 |
| ST8SiaII -/- | 2772 ± 47* (9) | 1009 ± 82 (9) | 63 |
| ST8SiaIV -/- | 2944 ± 97 (9) | 1172 ± 97 (6) | 60 |

Data are expressed as the mean±SEM. *p<0.05, ***p<0.0001 (two-way ANOVA, followed by Bonferroni post-hoc test).
Abbreviations: KA – kainic acid, RGCs – retinal ganglion cells.

## 4.3. A constitutive deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes does not affect KA-induced loss of RGCs in the adult retina

Death of RGCs is a hallmark of different retinal diseases, such as glaucoma, retinal and optic nerve ischemia and diabetic retinopathy. Overactivation of glutamate receptors is thought to play a key role in RGC damage in these diseases (Dreyer et al., 1998; Osborn et al., 1999). Glutamate ionotropic receptors in mammals are divided into three groups: α-amino-3-hydroxy-5-methyl-4-iso-xazolepropionic acid (AMPA), kainate and NMDA receptors, all of which are widely expressed in the retina. (Hampson and Manalo, 1998; Santiago et al., 2008; Puro et al., 1996). KA is a neurotoxin that induces overactivation of all types of glutamate receptors with a predominant action at AMPA/kainate receptors. To examine whether deficiency in sialyltransferases or NCAM genes influences the survival of RGCs after KA-induced injury, intravitreal injections

of 5 nmol KA were performed in WT, ST8SiaII-/-, ST8SiaIV-/- or NCAM-/- mice. At 24 h following administration of 5 nmol KA, a reduction in the density of RGCs by approximately 60% was observed in all groups of animals. A two-way ANOVA demonstrated a significant effect of KA ($F_{1,63}$=1029; $p<0.0001$) (Lobanovskaya et al., 2015). It seems that a constitutive deficiency in sialyl-transferases or NCAM does not affect the toxicity of KA (Table 1).

## 4.4. Endo-N-induced reduction in PSA-NCAM enhances the loss of RGCs after KA-administration in wild type mice

To assess whether a reduction of PSA-NCAM in retina followed by Endo-N treatment affects KA-induced toxicity, we performed intravitreal injections of KA on the 2nd and 14th day following Endo-N treatment. We found that in Endo-N-treated animals, KA was significantly more toxic on the 14th day (82% reduction in RGC density; $^{***}p<0.001$) but not on the 2nd day following Endo-N exposure compared with control animals (62% reduction in RGC density) (two-way ANOVA followed by post-hoc Bonferroni tests) (Lobanovskaya et al., 2015). We can conclude that the enzymatic reduction in PSA by Endo-N enhances the toxic effects of KA on RGCs. It seems that PSA-NCAM plays a role in the survival of injured RGCs, though the mechanisms by which PSA-NCAM contributes to the viability of neurons remain unclear.

## 4.5. KA-induced reduction of PSA-NCAM in wild type mice

Next, we were interested to know whether intravitreal injection of KA itself interferes with PSA-NCAM levels in the retina. The experiments demonstrated that intravitreal administration of KA (10 nmol) induced a loss of RGCs that was accompanied by a decrease in PSA-NCAM (Fig. 8, bottom panel). Immunohistochemistry in retinal sections showed a pronounced loss of PSA from the inner layers of the retina where RGCs are located (Fig. 8, upper panel). GFAP up-regulation was also noted after KA treatment, as a nonspecific reaction to the retinal injury (Fig. 6) (Lobanovskaya et al., 2019).

GFAP is a marker for glial cells including Müller cells and astrocytes in the retina. After KA treatment, we observed increased GFAP immunoreactivity in the NFL and GCL due to activation of astrocytes or Müller cells. GFAP-positive immunostaining, following KA injection, appeared also in the IPL, INL, OPL, ONL in the soma and along the inner and outer stem process of Müller cells (Fig. 6) (Lobanovskaya et al., 2019). Thus, a quick response of the retina to the injury manifested in up-regulation of GFAP, which is a protective reaction of glial cells, and in the shedding of preexisting PSA-NCAM.

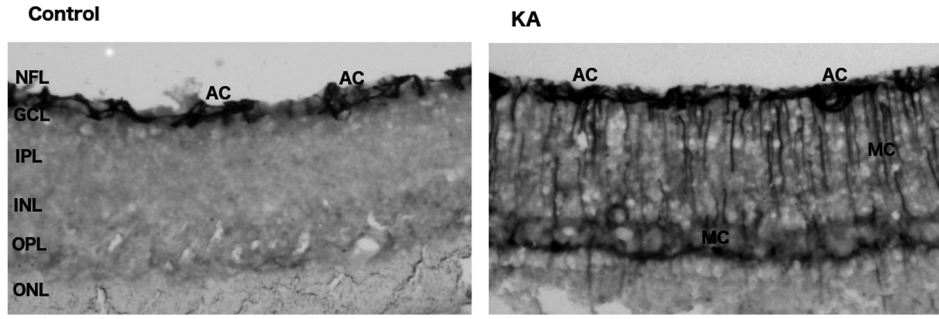


**Figure 6.** Representative microphotographs of GFAP-immunoreactive astroglia and Müller cells in retinal sections following intravitreal injection of vehicle (control) or KA (10 nmol). Magnification ×200. NFL – Nerve fiber layer; GCL – Ganglion cell layer; IPL – Inner plexiform layer; INL – Inner nuclear layer; OPL – Outer plexiform layer; ONL – Outer nuclear layer. AC – astrocytes, MC – Müller cells, KA – kainic acid (Lobanovskaya et al., 2019).

## 4.6. Mechanisms of the KA-induced reduction of PSA-NCAM

Our next question was to elucidate the mechanisms by which KA induces the loss of PSA-NCAM. Previous studies indicated that matrix metalloproteinases (MMPs) are involved in KA-induced RGC apoptosis (Zhang et al., 2004a). Previous studies from our laboratory showed that MMP-9 induces NCAM/PSA-NCAM cleavage in neural cell lines (Jaako et al., 2016). Therefore, we proposed that KA administration might upregulate MMP-9, which may then lead to the observed reduction of PSA-NCAM. Western blotting revealed greatly increased expression of the active form of MMP-9 in the retina after KA administration (Fig. 7). Immunohistochemistry in retinal sections demonstrated that active MMP-9 protein after KA treatment localized primarily to the inner parts of the retina where the majority of PSA-NCAM is expressed (Fig. 7) (Lobanovskaya et al., 2015).

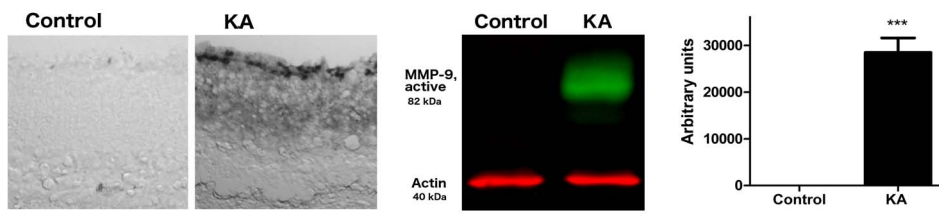


**Figure 7.** Effect of intravitreal KA (10 nmol) administration on active MMP-9 protein levels. Left panel: immunohistochemistry of active MMP-9 in retinal sections after vehicle (control) or KA administration. Middle panel: western blot of active MMP-9 protein in lysates from control or KA-treated retinas. Right panel: quantitative analysis of the MMP-9 protein. The results are expressed as mean ± SEM. ***$p < 0.0001$ (Student's t-test), n = 6 animals per group (Lobanovskaya et al., 2019).

Next, our task was to investigate whether MMP-9 is involved in PSA-NCAM shedding in the retina after KA administration. To do that, we selected a specific inhibitor of MMP-9 (MMP-9i). It was found that simultaneous injection of KA and MMP-9i decreased both RGC degeneration and PSA-NCAM loss from the retina (Table 2), (Fig. 8). The administration of MMP-9i alone did not affect PSA-NCAM levels in the retina (Fig. 8) (Lobanovskaya et al., 2019).

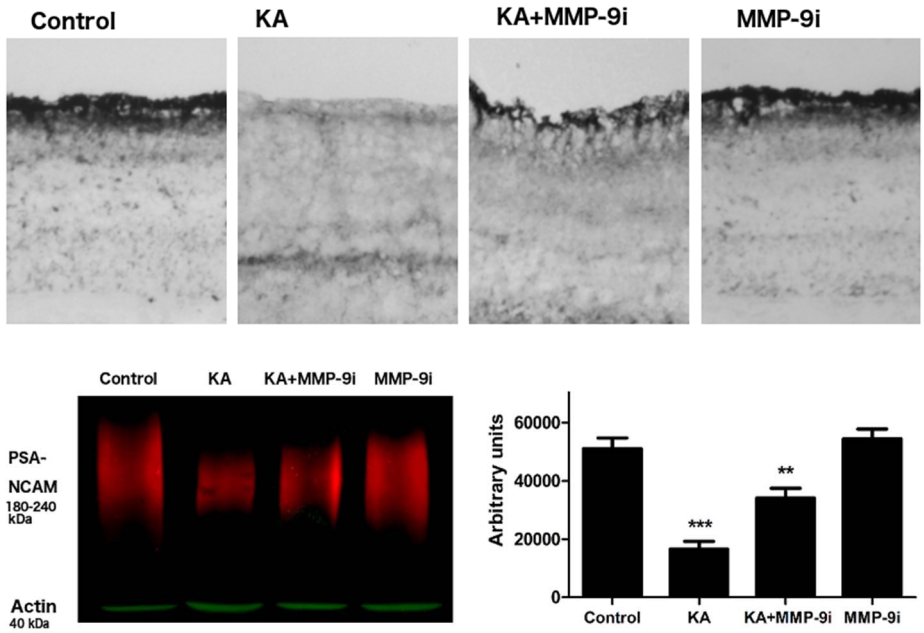


**Figure 8**. The effect of MMP-9 inhibitor on KA-induced reduction in PSA-NCAM levels. Upper panel: representative microphotographs of immunohistochemistry of PSA-NCAM protein in retinal sections after vehicle (control), KA, KA+MMP-9i or MMP-9i treatments, respectively (from left to right). Bottom panel (left): western blot of PSA-NCAM protein in lysates from vehicle (control), KA, KA + MMP- 9i or MMP-9i-treated retinas. Bottom panel (right): quantitative analysis of PSA-NCAM protein in western blot. Western blotting demonstrated approximately 70% reduction in PSA-NCAM signal in the retinas treated with KA compared with control. The results are expressed as mean ± SEM. ***$p < 0.0001$ (Tukey's test). Intravitreal administration of KA + MMP-9i induced approximately 30% reduction in PSA-NCAM signal compared with control. The results are expressed as mean ± SEM. **$p < 0.001$ (Tukey's test), n = 6 animals per group (Lobanovskaya et al., 2019).

To clarify whether the protective effect of MMP-9i on RGCs is due to decreased shedding of PSA-NCAM, PSA was removed from the retina using Endo-N administration. Animals received co-injections of Endo-N, MMP-9i and KA. We observed that removal of PSA from the retina decreased the

protective effect of MMP-9i to the RGCs by approximately 20% (Table 2) (Lobanovskaya et al., 2015). As Endo-N itself does not influence the survival of RGCs, we concluded that the presence of PSA-NCAM at least partly rescues RGCs after KA administration.

**Table 2**. The effects of MMP-9 inhibition and its combination with Endoneuraminidase-N (Endo-N) on KA-induced RGC death. The data are expressed as the mean ± SEM. ***p < 0.0001 (Tukey's Multiple Comparison Test). In brackets: the number of retinas studied.

| **Group** | **RGC density (cell/mm$^2$), n=12** | **RGC reduction in comparison to control (%)** | **Group comparisons** |
|---|---|---|---|
| Control (Vehicle) | 2533 (12) | | |
| KA | 505,5 (11) *** | 80 | KA vs Control |
| KA+MMP-9i | 1327 (12) *** | 48 | KA +MMP-9i vs KA |
| Endo-N+KA+MMP-9i | 815 (12) *** | 68 | Endo-N +KA+MMP-9i vs KA+MMP-9i |

Abbreviations: KA – kainic acid, RGC – retinal ganglion cell, Endo-N – Endoneuraminidase-N, MMP-9 – matrix metalloproteinase-9, MMP-9i – matrix metalloproteinase-9 inhibitor.

## 4.7. Survival of RGCs and alterations in the levels of PSA-NCAM in diabetic retinopathy (DR)

DR is a common complication of diabetes. DR can lead to vision deterioration and loss due to retinal neurodegeneration. The influence of diabetes on the retina has been studied widely in rodent models (Olivares et al., 2017). Streptozotocin (STZ)-mediated induction of diabetes is one of the most frequently used chemical models of diabetes in animals (Tonade et al., 2017). STZ is toxic to insulin-producing pancreatic beta cells due to its similarity in structure to glucose, and it can induce Type 1 diabetes (Aileen, 2012). Our task was to explore whether STZ-induced diabetes affected PSA-NCAM levels in the retina and whether it was associated with RGC loss. At two months after the onset of diabetes, we performed immunohistochemistry for Brn3a (marker of RGCs) in wholemounted retinas of control and diabetic animals. A reduction in the density of RGCs in diabetic mice of approximately 19% in comparison with controls was observed (Lobanovskaya et al., 2018). To assess PSA-NCAM protein levels in the diabetic retinas, western blotting was performed. We did not find any difference in the total levels of PSA-NCAM protein in the diabetic retinas versus control. However, immunohistochemistry detected pronounced changes in the distribution of PSA-NCAM in diabetic retinas (Fig. 9). In control retinas, PSA-NCAM was primarily expressed in the inner part of the retina, i.e.,

in the NFL, GCL, with some also present in IPL. In contrast, DR induced a strong reduction in PSA-NCAM immunopositive signal (approximately 2.2-fold) in the NFL and GCL with enhanced PSA-NCAM immunoreactivity in the IPL and INL (approximately 1.3-fold) and especially in the OPL and ONL (about 2.3-fold), where the processes and soma of Müller cells extend (Fig. 9) (Lobanovskaya et al., 2018).

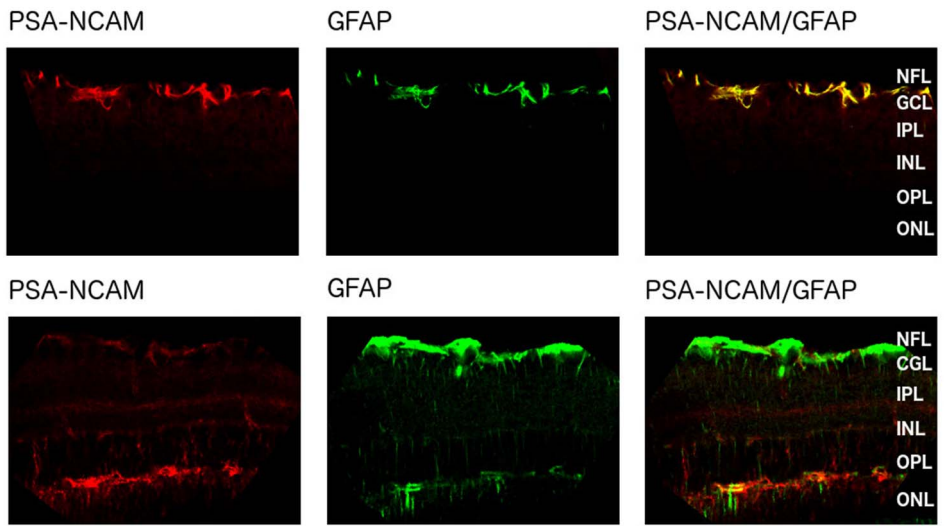


**Figure 9.** Confocal images of sections double-labelled for PSA-NCAM (red) and GFAP (green) of control (upper panel) and diabetic mice (bottom panel). Magnification x400. NFL: Nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; INL: Inner nuclear layer; OPL: Outer plexiform layer; ONL: Outer nuclear layer (Lobanovskaya et al., 2018).

Western blotting also demonstrated increased levels of GFAP in diabetic retinas by (approximately 2.0-fold compared with control retinas). Upregulation of GFAP in the retinas of diabetic animals was nonspecific and resembled the pattern observed after KA injury. In control retinas, sections double-labelled for PSA-NCAM and GFAP demonstrated strong co-localization of these markers in NFL and GCL. However, in diabetic retinas, both markers were found abundantly in the OPL and ONL (Fig. 9) (Lobanovskaya et al., 2018). We also found increased levels of MMP-9 protein (nearly 1.9-fold) in the NFL and GCL of diabetic retinas compared with controls. In IPL, INL, OPL, and ONL of diabetic retinas, MMP-9 levels were increased to a much lower extent (Lobanovskaya et al., 2018). We propose that MMP-9 may induce PSA-NCAM shedding in the retina of diabetic mice and that this reduction could contribute to the observed loss of RGCs.

# 5. DISCUSSION

## 5.1. PSA-NCAM expression in the retina of WT, ST8SiaII-/-, ST8SiaIV-/-, NCAM-/- mice

The present study demonstrates pronounced expression of PSA-NCAM in the retinas of WT animals with predominant localization of the protein in the NFL, GCL, IPL. In the other layers of the retina, minimal PSA-NCAM signal was found. Co-localization of PSA-NCAM with GFAP marker revealed that only Müller cells and astrocytes express PSA-NCAM (Lobanovskaya et al., 2015). Our results are consistent with previous studies that demonstrated pronounced expression of PSA-NCAM in the mammalian adult retina, primarily by Müller and astroglial cells (Bartsch et al., 1989; Murphy et al., 2009). In contrast, RGCs do not express PSA-NCAM or at least the level of expression is too low to be revealed by the methods employed in this study (Lobanovskaya et al., 2015). Interestingly, it was shown earlier, using immunohistochemistry at the light and electron microscopic levels, that slightly sialylated NCAM is expressed by all cell types in the retina and optic nerve in adulthood with the exception of the outer segments of photoreceptor cells (Bartsch et al., 1989). However, PSA-NCAM is certainly localized closely to RGCs, as observed in the double-labelled images for PSA-NCAM and Brn3a. Moreover, it was previously found that the membrane of Müller cells and astrocytes is in close apposition to the soma and processes of RGCs (Stone et al., 1995). Next, we explored the protective role of PSA-NCAM for RGCs in adulthood. We used transgenic mice with a constitutive deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes. As sialyltransferase II is predominant during embryogenesis and sialyltransferase IV functions primarily in adulthood, we assumed a reduction in retinal PSA-NCAM in ST8SiaII-/- or ST8SiaIV-/- transgenic animals in the corresponding periods of life. Surprisingly, our data showed that the levels and distribution pattern of PSA-NCAM in adult retina was not affected by a deficiency of either ST8SiaII or ST8SiaIV. These data contrasted data from brain tissue of ST8SiaIV-/- mice, where a remarkable reduction in PSA-NCAM levels was observed (Lobanovskaya et al., 2015). Knockout ST8SiaIV animals showed a strong reduction in PSA levels in most brain regions: olfactory bulb, hippocampus, neocortex, hypothalamus, medulla oblongata in comparison with WT at 5–6 weeks of age. However, ST8SiaIV-/- mice did not show any obvious changes in brain (Eckhart et al, 2000). However, it was demonstrated that sialyltransferase IV is important for synaptic plasticity in hippocampal CA1 synapses and cognitive performance (Eckhardt et al., 2000; Angata et al., 1998). In ST8SiaII -/- mice, a reduction in PSA levels in hippocampus, olfactory bulb and cerebral cortex was also observed, which resulted in defasciculation of mossy fibers and altered synaptogenesis in the hippocampus (Angata et al., 1998). In contrast to adult retinas, PSA-NCAM immunological signal in the retinas of ST8SiaII-/- newborn (P1) mice was virtually absent, and in ST8SiaIV-/- mice

PSA-NCAM levels were 2-fold two lower than controls. These data clearly demonstrate that a deficiency in ST8SiaII or ST8SiaIV can be fully compensated for during retinal maturation. Previous studies revealed that in the brain of newborn (P1) ST8SiaII-/- animals, 45% of NCAM was non-polysialylated; however, in ST8SiaIV-/- mice, only 6% of NCAM was without PSA (Galushka et al., 2006). In the same study, ST8SiaII and ST8SiaIV mRNA levels were similar to control. Thus, ST8SiaII is more powerful when compensating for NCAM polysialylation in the brain of newborn (P1) ST8SiaIV-/- animals due to its higher kinetic properties (Galushka et al., 2006). Thus, these previous data are in a line with our results and demonstrate that the compensatory ability of ST8SiaII is higher in the retina of newborn (P1) mice. It has been suggested that ST8SiaII and ST8SiaIV mice have different involvement in PSA formation and the control of neurological functions with some overlap. As expected, animals deficient for the NCAM gene did not show any PSA-NCAM-related signal in the retina (Lobanovskaya et al., 2015).

## 5.2. RGC density in mice with a deficiency in *ST8SiaII*, *ST8SiaIV* or *NCAM* genes and after administration of Endo-N

Our next experiments demonstrated that constitutive deficiency in the *ST8SiaII* gene results in decreased RGC density in adult mice compared with control, whereas in ST8SiaIV-/- mice, the density of RGCs remained unchanged (Lobanovskaya et al., 2015). A decrease in PSA-NCAM during development and the early postnatal period in ST8SiaII knockout mice may influence the survival of RGCs. Recent studies demonstrated an increase in neonatal RGC death in vitro after enzymatic removal of PSA by neuraminidase (Murphy et al., 2009). Furthermore, the viability of RGCs is dependent upon the presence of trophic factors produced by their targets – tectal neurons – upon the establishment of their synaptic contacts (Vecino et al., 2004). It seems that PSA-NCAM ensures the proper formation of these synaptic contacts during development (Yin et al., 1995). Remarkably, in NCAM-/- mice, the density of RGCs was statistically increased compared with control (Lobanovskaya et al., 2015). These data are consistent with previous work (Murphy et al., 2007). It seems that NCAM, in unpolysialylated form, may influence the balance between neural progenitor cell proliferation and differentiation. Previous studies demonstrated that NCAM inhibited proliferation and stimulated neural differentiation of neonatal hippocampal and cerebellar progenitor cells (Amoureux et al., 2000). Thus, a deficiency in NCAM results in an increased production of progenitors without significant alteration to their survival. Previously, NCAM-/- mice were shown to have higher levels of BDNF in the superior colliculi (SC) (Murphy et al., 2007). It may be proposed that the survival of RGCs in adult NCAM-/- mice is increased due to enhanced or prolonged influences of trophic factors produced in SC (Murphy et al., 2007). To further clarify the roles of PSA-NCAM in the survival of RGCs, we enzymatically reduced PSA in the

retina using Endo-N. Intravitreal injection of Endo-N enzyme resulted in complete cleavage of PSA chains from the NCAM molecule at the 2nd, 14th and 28th postinjection days. Our results demonstrated that temporary reduction of PSA induced by Endo-N does not affect the survival of RGCs (Lobanovskaya et al., 2015). Our data are in contrast with previous studies where a reduction in the densities of RGCs was observed following PSA elimination by Endo-N (Murphy et al., 2009). The survival of RGCs may depend upon the age of animals also. We used animals at 5–6 months of age, whereas previously, 2–3 – month-old animals were used (Murphy et al., 2009).

## 5.3. Endo-N-induced reduction in PSA-NCAM enhances the loss of RGCs after KA-administration in the adult retina

The toxic effect of KA on the RGC viability was not changed in mice with constitutive deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes. In contrast, enzymatic cleavage of PSA-NCAM in WT mice by Endo-N following intravitreal injection of KA at 14th day, but not at 2nd decreases survival of RGCs compared with control mice (Lobanovskaya et al., 2015). These data are in a line with previous studies showing that the loss in RGCs induced by optic nerve transection was higher in retinas that had been pretreated with Endo-N (Murphy et al., 2009). The mechanisms underlying the prosurvival effect of PSA-NCAM remain to be elucidated. It is not clear why the adult retina is more sensitive to the toxic effect of KA after reducing PSA levels. It was demonstrated previously that PSA-NCAM is able to interact with FGFR1, TrkB receptor, and GFR-alpha to trigger downstream cascades affecting the viability of RGCs (Blanco et al., 2008; Koeberle, 1998; Peinado-Ramon et al., 1996; ). It was also shown that PSA-NCAM promotes survival of RGCs due to its modulatory effect on the NMDA receptor and $p75^{NTR}$ (Luke et al., 2016a; Murphy et al., 2012).

## 5.4. Mechanism of KA-induced reduction of PSA-NCAM and RGC degeneration

Our experiments demonstrated a remarkable reduction in PSA-NCAM levels at 24 hours after intravitreal KA in the inner part of the retina (NFL, GCL) (Lobanovskaya et al., 2019). The role of PSA-NCAM in RGC survival in this model of KA-induced excitotoxicity has not been studied thus far. Previous studies suggested that MMPs are involved in NCAM/PSA-NCAM cleavage in neurons (Hübsmann et al., 2005; Hinkle et al., 2006; Shichi et al., 2011; Brennmann et al., 2014). It was observed in our laboratory that MMP-9 is the major metalloproteinase inducing NCAM/PSA-NCAM cleavage in neuroblastoma cells (Jaako et al., 2016). Earlier studies also demonstrated that KA-induced excitotoxicity in the retina stimulated upregulation of MMP-9 (Zhang et al.,

2004a). Our results showed that the active form of MMP-9 was virtually absent in the retinas of control animals. In contrast, substantial levels of active MMP-9 were detected after KA treatment in the NFL and GCL, where PSA-NCAM is primarily expressed (Lobanovskaya et al., 2019). We assumed that MMP-9 was involved in retinal PSA-NCAM shedding after KA injections. Our next experiments revealed that a specific inhibitor of MMP-9, injected in the same time as KA, reduced PSA-NCAM cleavage, significantly, in the inner part of the retina. Moreover, the survival of RGCs in the KA+MMP-9 inhibitor group was also significantly increased compared with the KA alone group. Further experiments demonstrated that the removal of PSA from the retina by Endo-N partly decreases the protective effect of MMP-9 inhibitor on RGC survival after KA-induced excitotoxicity (Lobanovskaya et al., 2019). These results concur with previous data demonstrating that KA-mediated upregulation of MMP-9 increases RGC death partly due to degradation of laminin in the retina (Zhang et al., 2004a). Here we revealed that upregulation of KA-induced MMP-9 results in PSA-NCAM shedding and RGC degeneration. Inactivation of MMP-9 partly rescued RGC number, due to decreased PSA-NCAM cleavage (Lobanovskaya et al., 2019). MMP-9 levels are increased in the retina in various diseases, such as diabetic retinopathy, age-related macular degeneration, glaucoma (Kowluru et al., 2010; Kowluru et al., 2014; Singh and Tyagi, 2017). It was suggested that MMP-9 is one of the important factors in the progression of proliferative diabetic retinopathy (Kosano et al., 1999). MMPs belong to the family of zinc-dependent proteases. They have the capacity to degrade and remodel the extracellular matrix and affect a wide spectrum of substrates: cell surface receptors, proteinases, and signaling molecules (Birkedal-Hansen et al., 1993). Notably, MMP-9 inhibition only partially decreased PSA-NCAM shedding and RGC degeneration. Thus, MMP-9-independent mechanisms are also activated to promote PSA-NCAM cleavage and RGC death. The ADAMs family of metalloproteinases may be involved in NCAM/PSA-NCAM cleavage in the retina (Kalus et al., 2006). For example, it has been shown that plasmin can degrade PSA-NCAM and exacerbate RGC degeneration (Endo et al., 1998; Mali et al., 2005).

## 5.5. Survival of RGCs and alterations in the levels of PSA-NCAM in diabetic retinopathy (DR)

Considering that DR is a severe complication of diabetes and a leading cause of blindness in working-age adults in developed countries, it is critical to study the roles of PSA-NCAM in DR and its association with RGC survival. Here, we demonstrated a redistribution of PSA-NCAM in the retina of mice during the development of STZ-induced DR. In diabetic retinas, PSA-NCAM was markedly reduced in the NFL, GCL and was greatly increased in the OPL and ONL compared with control retinas (Lobanovskaya et al., 2019). The reduction in PSA-NCAM levels in the inner part of the retina was associated with RGC

degeneration in diabetic animals. Our study also demonstrated increased levels of MMP-9 primarily in the NFL and GCL. MMP-9 was slightly increased in the other layers of the retina (Lobanovskaya et al., 2019). We propose that a significant upregulation of MMP-9 expression may be involved in PSA-NCAM shedding in the inner part of diabetic retina, which exacerbated RGC neurodegeneration. MMP-9 is elevated due to retinal inflammation during DR. It has been shown that MMP-9 is involved in angiogenesis, that it promotes degradation of extracellular matrix and the break-down of the blood-retinal barrier (Kowluru et al., 2012). We also observed that DR leads to activation of retinal glial cells (Lobanovskaya et al., 2019). It seems that PSA-NCAM is expressed on the membrane of Müller cells and astrocytes in the inner and middle part of diabetic retinas. Activated Müller cells and astrocytes are the source of the MMP-9 in the retina (Limb, 2002; Zhang, 2004b). Moreover, gliosis induces the formation of a glial scar and tractional detachment of the retina. It was demonstrated previously that NCAM inhibited astrocyte proliferation in vitro and in vivo after damage (Krushel et al., 1995, 1998). It may be that reduced glial cell proliferation in the retina helps to create an environment more suited to neuronal survival. Thus, on the one hand, PSA-NCAM increases survival of RGCs after injury, but on the other, reduction of PSA from the middle part of the retina may reduce glial activity and the severity of DR. The influence of PSA-NCAM on glial cell proliferation in the retina has not yet been studied.

# CONCLUSIONS

1. PSA-NCAM is expressed in the same high levels and distributed similarly in the retinas of adult WT, ST8SiaII-/-, ST8SiaIV-/- knockout mice. We propose that sialyltransferases in the retina of transgenic mice are able to compensate for each other effectively.
2. Knockout ST8SiaII-/- mice have decreased density of RGCs compared with controls. In contrast, NCAM-/- mice demonstrate an increase in RGC density. Thus, the substantially lower levels of PSA during embryogenesis in newborn ST8SiaII-/- mice influence the survival of RGCs. Intravitreal administration of Endo-N to wild type mice does not influence the survival of RGCs.
3. A constitutive deficiency in *ST8SiaII, ST8SiaIV* or *NCAM* genes does not affect the toxic effects of KA on RGCs, which may be partly explained by the compensatory activation of the existing sialyltransferase. In contrast, Endo-N-induced reduction of PSA-NCAM in wild type mice increases toxicity of KA to RGCs suggesting that survival of RGCs is dependent upon the presence of PSA-NCAM.
4. KA-induced reduction in PSA-NCAM is mediated by the upregulation of MMP-9, which causes shedding of PSA-NCAM and thereby reduces the viability of RGCs.
5. Diabetic retinopathy is associated with a decrease in RGC density and a marked reduction in PSA-NCAM protein in the inner part of the retina, where RGCs are located. This is accompanied by greatly increased levels of MMP-9, in the same region part of the retina close to RGCs. We propose that MMP-9 is associated with PSA-NCAM shedding and RGC degeneration during diabetic retinopathy.

# REFERENCES


1. Aileen JFK. The use of animal models in diabetes research. Br J Pharmacol 2012;166:877–894.
2. Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 1969;137:433–457.
3. Amoureux MC, Cunningham BA, Edelman GM, Crossin KL. N-CAM binding inhibits the proliferation of hippocampal progenitor cells and promotes their differentiation to a neuronal phenotype. J Neurosci 2000;20:3631–3640.
4. Angata K, Huckaby V, Ranscht B, Terskikh A, Marth JD, Fukuda M. Polysialic acid-directed migration and differentiation of neural precursors are essential for mouse brain development. Mol Cell Biol 2007;27:6659–6668.
5. Angata K, Long JM, Bukalo O, Lee W, Dityatev A, Wynshaw-Boris A, Schachner M, Fukuda M, Marth JD. Sialyltransferase ST8Sia-II assembles a subset of polysialic acid that directs hippocampal axonal targeting and promotes fear behavior. J Biol Chem 2004;279:32603–32613.
6. Angata K, Suzuki M, Fukuda M. Differential and cooperative polysialylation of the neural cell adhesion molecule by two polysialyltransferases, PST and STX. J Biol Chem 1998;273:28524–28532.
7. Aonurm-Helm A, Jaako K, Jürgenson M, Zharkovsky A. Pharmacological approach for targeting dysfunctional brain plasticity: focus on neural cell adhesion molecule (NCAM). Pharmacol Res 2016;113:731–738.
8. Bai N, Aida T, Yanagisawa M, Katou S, Sakimura K, Mishina M, Tanaka K. NMDA receptor subunits have different roles in NMDA-induced neurotoxicity in the retina. Mol Brain 2013;6:34.
9. Bartsch U, Kirchhoff F, Schachner M. Immunohistological localization of the adhesion molecules L1, N-CAM, and MAG in the developing and adult optic nerve of mice. J Comp Neurol 1989;284:451–462.
10. Bates CA, Becker CG, Miotke JA, Meyer RL. Expression of polysialylated NCAM but not L1 or N-cadherin by regenerating adult mouse optic fibers in vitro. Exp Neurol 1999;155:128–139.
11. Becker CG, Artola A, Gerardy-Schahn R, Becker T, Welzi H, Schachner M. The Polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J Neurosci Res 1996; 45:143–152.
12. Bernardos RL, Barthel LK, Meyers JR, Raymond PA. Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. J Neurosci 2007;27:7028–7040.
13. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. Crit Rev Oral Biol 1993;4:197–250.
14. Blanko RE, Lopez-Roca A, Soto J, Blagburn JM. Basic fibroblast growth factor applied to the optic nerve after injury increases long-term cell survival in the frog retina. J Comp Neurol 2000;423:646–58.
15. Bock E, Edvarsen K, Gibson A, Linnemann D, Lyles JM, Nybroe O. Characterization of soluble forms of NCAM. FEBS Lett 1987;225:33–36.

16. Brennamann LH, Moss ML, Maness PF. EphrinA/EphA-induced ectodomain shedding of neural cell adhesion molecule regulates growth cone repulsion through ADAM10 metalloprotease. J Neurochem 2014;128:267–279.
17. Bruses JL, Rutishauser U. Roles, regulation, and mechanism of polysialic acid function during neural development. Biochimie 2001;83:635–643.
18. Carry LA, Guan JL. Focal adhesion kinase in integrin-mediated signaling. Front Biosci 1999;4:102–113.
19. Cassens C, Kleene R, Xiao MF, Friedrich C, Dityateva G, Schafer-Nielsen C, Schachner M. Binding of the receptor tyrosin kinase TrkB to the neural cell adhesion molecule (NCAM) regulates phosphorylation of NCAM and NCAM-dependent neurite outgrowth. J Biol Chem 2010;285:28959–28967.
20. Catalani E, Tomassini S, Dal Monte M, Bosco L, Casini G. Localization patterns of fibroblast growth factor 1 and its receptors FGFR1 and FGFR2 in postnatal mouse retina. Cell Tissue Res 2009;336:423–438.
21. Chattopadhyaya B, Baho E, Huang ZJ, Schachner M, Cristo G. Neural cell adhesion molecule-mediated Fyn activation promotes GABAergic synapse maturation in postnatal mouse cortex. J Neurosci 2013;33:5957–5968.
22. Cheng L, Sapieha P, Kittlerova P, Hauswirth WW, Di Polo A. TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. J Neurosci 2002;22:3077–3986.
23. Chuong CM, Edelman GM. Alterations in neural cell adhesion molecules during development of different regions of the nervous system. J Neurosci 1984;4:2354–2368.
24. Chuong CM, Edelman GM. Alterations in neural cell adhesion molecules during development of different regions of the nervous system. J Neurosci 1984;4:2354–68.
25. Close BE, Mendiratta SS, Geiger KM, Broom LJ, Ho LL, Colley KJ. The minimal structural domains required for neural cell adhesion molecule polysialylation by PST/ST8Sia IV and STX/ST8Sia II. J Biol Chem 2003;278:30796–30805.
26. Cremer H, Chazal G, Goridis C, Represa A. NCAM is essential for axonal growth and fasciculation in the hippocampus. Mol Cell Neurosci 1997;8:323–335.
27. Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown, R, Baldwin S, Kreamer P, Scheff S, et al. Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 1994;367:455–459.
28. Cunningham BA, Hemperly JJ, Murray BA, Prediger EA, Blackenbury R, Edelman GM. Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. Science 1987;236:799–806.
29. Ditlevsin DK, Kohler LB, Berezin V, Bock E. Cyclic guanosine monophosphate signaling pathway plays a role in neural cell adhesion molecule-mediated neurite outgrowth and survival. J Neurosci Res 2007;85:703–711.
30. Doherty P, Moolenaar CECK, Ashton, Michalides RJAM, Walsh FS. The VASE exon downregulates the neurite growth-promoting activity of NCAM-140. Nature 1992;356:791–793.
31. Dreyer EB. A proposed role for excitotoxicity in glaucoma. J Glaucoma 1998; 7:62–67.
32. Eckhardt M, Bukalo O, Chazal G, Wang L, Goridis C, Schachner M, Gerardy-Schahn R, Cremer H, Ditatev A. Mice deficient in the polysialyltransferase ST8SiaIV/PST-1 alow discrimination of the roles of neural cell adhesion molecule

protein and polysialic acid in neural development and synaptic plasticity. J Neurosci 2000;20:5234–5244.
33. Endo A, Nagai N, Urano T, Ihara H, Takada Y, Hashimoto K, Takada A. Proteolysis of highly polysialylated NCAM by the tissue plasminogen activator-plasmin system in rats. Neurosci Lett 1998;1:37–40.
34. Flieser SJ, Cole GJ, Adler AJ. Neural cell adhesion molecule (NCAM) in adult vertebrate retinas: tissue localization and evidence against its role in retina-pigment epithelium adhesion. Exp Eye Res 1990;50:475–482.
35. Franke TF, Kaplan DR, Cantley LC. Pi3K: downstream AKTion blocks apoptosis. Cell 1997;88:435–437.
36. Galuska S, Oltmann-Norden I, Geyer H, Weinhold B, Kuchelmeister K, Hilder-brandt H, Gerardy-Schahn R, Geyer R, Mühlenhoff M. Polysialic acid profiles of mice expressing variant allelic combinations of the polysialyltransferases ST8SiaII and ST8SiaIV. J Biol Chem 2006;42:31605–31615.
37. Gascon E, Vutskis L, Kiss JZ. The role of PSA-NCAM in adult neurogenesis. Adv Exp Med Biol 2010;663:127–136.
38. Gascon E, Vutskits L, Jenny B, Durbrc P, Kiss JZ. PSA-NCAM in postnatally generated immature neurons of the olphactory bulb: a crucial role in regulating p75 expression and cell survival. Development 2007;134:1181–1190.
39. Gerardy-Schahn R, Brennecke T, Mühlenhoff M, Eckhardt M, Ziesing S. Molecular cloning and functional expression of bacteriophage PK1E+encoded endoneuraminidase Endo NE. Mol Microbiol 1995;16:441–450.
40. Gower HJ, Barton CH, Elsom VL, Thompson J, Moore SE, Dickson G, Walsh FS. Alternative splicing generates a secreted form of N-CAM in muscle and brain. Cell 1988;55:955–964.
41. Gray H. Anatomy: Descriptive and Surgical. England, 1858.
42. Grimm C, Reme CE. Light damage as a model of retinal degeneration. Light damage as a model of retinal degeneration. Methods Mol Biol 2013;935:87–97.
43. Hammond MS, Sims C, Parameshwaran K, Suppiramaniam V, Schachner M, Dityatev A. Neural cell adhesion molecule-associated polysialic acid inhibits NR2B-containing N-methyl-D-aspartate receptors and prevents glutamate-induced cell death. J Biol Chem 2006;281:34859–34869.
44. Hampson DR, Manalo JL. The activation of glutamate receptors by kainic acid and domoic acid. Nat Toxins 1998;6:153–158.
45. Hetman M, Kanning K, Cavanaugh JE, Xia Z. Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal regulated kinase and phosphatidylinositol 3-kinase. J Biol Chem 1999;274:22569–22580.
46. Hinkle CL, Diestel S, Lieberman J, Maness PF. Metalloprotease-induced ecto-domain shedding of neural cell adhesion molecule (NCAM). J Neurobiol 2006; 66:1378–1395.
47. Hirn M, Pieres M, Deagostini-Bazin H, Hirsch M, Goridis C. Monoclonal antibody against cell surface glycoprotein of neurons. Brain Res 1981;214:433–439.
48. Hoffman S, Egelman GM. Kinetics of homophilic binding by embryonic and adult forms of neural cell adhesion molecule. Proc Natl Acad Sci U S A 1983;80:5762–5766.
49. Hoffman S, Sorkin BC, White PC, Brackenbury R, Mailhammer R, Rutishauser U, Cunningham BA, Edelman GM. Chemical characterization of a neural cell

adhesion molecule purified from embryonic brain membranes. J Biol Chem 1982;257:7720–7729.
50. Hu H, Tomasiewicz H, Magnuson T, Rutishauser U. The role of polysialic acid in migration of olfactory bulb interneurons precursors in the subventricular zone. Neuron 1996;16:735–743.
51. Hübsmann MV, Skladchikova G, Bock E, Berezin V. Neural cell adhesion molecule function is regulated by metalloproteinase-mediated ectodomain release. J Neurosci Res 2005;15:826–837.
52. Jaako K, Waniek A, Parik K, Klimaviciusa L, Aonurm Helm A, Noortoots A, Miner K, VanElzen R, Gerard M, Lambier AM, Robner S, Morawski M, Zharkovsky A. Prolyl endopeptidase is involved in the degradation of neural cell adhesion molecules in vitro. J Cell Sci 2016;129:3792–3802.
53. Jelsma TN, Friedman HH, Berkelaar M, Bray GM, Aguayo AJ. Different forms of the neurotrophin receptor trkB mRNA predominate in rat retina and optic nerve. J Neurobiol 1993;24:1207–1214.
54. Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M Tamir R, Antonio L, Cupples R, Louis JC, Hu S, Sltrock BW, Fox GM. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. Cell 1996;85:1113–1124.
55. Job MO, Cooke BM. PSA-NCAM in the posterodorsal medial amygdala is necessary for the pubertal emergence of attraction to female odors in male hamsters. Horm Behav 2015;75:91–99.
56. Johnson CP, Fujimoto I, Rutishauser U, Leckband DE. Direct evidence that neural cell adhesion molecule (NCAM) polysialylation increases intermembrane repulsion and abrogates adhesion. J Biol Chem 2005;280:137–145.
57. Jørgenson OS, Bock E. Brain specific synaptosomal membrane proteins demonstrated by crossed immunoelectrophoresis. J Neurochem 1974;23:879–880.
58. Kalus I, Bormann U, Mzoughi M, Schacher M, Kleene R. Proteolytic cleavage of the neural cell adhesion molecule by ADAM17/TACE is involved in neurite outgrowth. J Neurochem 2006;98:78–88.
59. Kasper C, Rasmussen H, Kastrup JS, Ikemizu S, Jones EY, Berezin V, Bock E, Larsen IK. Structural basis of cell-cell adhesion by NCAM. Nat Struct Biol 2000;7:389–393.
60. Kempermann G, Song H, Gage FH. Neurogenesis in adult hippocampus. Cold Spring Harb Perspect Biol 2015;7:a018812.
61. Kiselyov VV, Skladchikova G, Hinsby AM, Jensen PH, Kulahin N, Soroka V, Pedersen N, Tsetlin V, Poulsen FM, Berezin V, Bock E. Structural basis for a direct interaction between FGFR1 and NCAM and evidence for a regulatory role of ATP. Structure 2003;11:691–701.
62. Kiselyov VV, Soroka V, Berezin V, Bock E. Structural biology of NCAM homophilic binding and activation of FGFR. J Neurochem 2005;94:1169–1179.
63. Kochlamazashvili G, Senkov O, Grebenyuk S, Robinson K, Xiao MF, Stummeyer K, Gerardy-Schahn R, Engel AK, Feig L, Semyanov A, Suppiramaniam V, Schachner M, Dityatev A. Neural cell adhesion molecule-associated polysialic acid regulated synaptic plasticity and learning by restraining the signalling through GluN2B-containing NMDA receptors. J Neurosci 2010;30:4171–4183.
64. Koeberle PD, Ball AK. Effect of GDNF on retinal ganglion cell survival following axotomy. Vision Res 1998;38:1505–1515.

65. Kosano H, Okano T, Kataura Y, Noritake M, Kado S, Matsuoka T, Nishigori H. ProMMP-9 (92 kDa gelatinase) in vitreous fluid of patients with proliferative diabetic retinopathy. Life Sci 1999;64:2307–2315.
66. Kowlulru RA, Zhong Q, Santos JM. Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9. Expert Opin Investing Drugs 2012;21:797–805.
67. Kowluru RA, Santos LM, Zhong Q. Sirt1 a negative regulator of matrix metalloproteinase-9 in diabetic retinopathy. Ophthalmol Vis Sci 2014;55:5653–5660.
68. Kowluru RA. Role of matrix metalloproteinase-9 in the development of diabetic retinopathy and its regulation by H-Ras. Invest Ophthalmol Vis Sci 2010;51:4320–4326.
69. Krushel LA, Sporns O, Cunningham BA, Crossin KL, Edelman GM. Neural cell adhesion molecule (N-CAM) inhibits astrocyte proliferation after injury to different regions of the adult rat brain. Proc Natl Acad Sci U S A 1995;92:4323–4327.
70. Krushel LA, Tai MH, Cunningham BA, Edelman GM, Crossin KL. Neural cell adhesion molecule (N-CAM) domains and intracellular signaling pathways involved in the inhibition of astrocyte proliferation. Proc Natl Acad Sci U S A 1998;95:2592–2596.
71. Limb GA, Daniels JT, Pleass R, Charteris DG, Luthert PJ, Khaw PT. Differential expression of matrix metalloproteinases 2 and 9 by glial Müller cells: response to soluble and extracellular matrix-bound tumour necrosis factor-alpha. Am J Pathol 2002;160:1847.
72. Linn LF, Doherty DH, Lile JD, Dektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 1993; 260:1130–1132.
73. Lobanovskaya N, Jürgenson M, Aonurm-Helm A, Zharkovsky A. Alterations in the polysialylated neural cell adhesioon molecule and retinal ganglion cell density in mice with diabetic retinopathy. Int J Ophthalmol 2018;11:1608–1615.
74. Lobanovskaya N, Zharkovsky A, Jaako K, Jürgenson M, Aonurm-Helm, Zharkovsky A. PSA modification of NCAM supports the survival of injured retinal ganglion cells in adulthood 2015;1625:9–17.
75. Lobanovskaya N, Zharkovsky A. A role of PSA-NCAM in the survival of retinal ganglion cells (RGCs) after kainic acid damage. Neurotoxicology 2019;72:101–106.
76. Luke MP, LeVatte TL, O'Reilly AM, Smith BJ, Tremblay F, Brown RE, Clarke DB. Effect of NCAM on aged-related deterioration in vision. Neurobiol Aging 2016b;41:93–106.
77. Luke MP, LeVatte TL, Rutishauser U, Tremblay F, Clarke DB. Polysialylated Neural cell adhesion molecule protects against light-induced retinal degeneration. Invest Ophthalmol Vis Sci 2016a;57:5066–5075.
78. Luskin MB. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 1993;11:173–189.
79. Mali RS, Cheng M, Chintala SK. Plasminogen activators promote excitotoxicity-induced retinal damage. FASEB J 2005;19:1280–1289.
80. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effect of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized ganglion cells. Proc Nail Acad Sci U S A 1994;91:1632–1638.

81. Monnier PP, Beck SG, Bolz J, Henke-Fahle S. The Polysialic acid moiety of the neural cell adhesioon molecule is involved in intraretinal guidance of retinal ganglion cell axons. Dev Biol 2001;229:1–14.
82. Muller D, Djebbara-Hannas Z, Jourdain P, Vutskits L, Durbec P, Rougon G, Kiss JZ. Brain-derived neurotrophic factor restores long-term potentiation in polysialic acid-neural cell adhesion molecule-deficient hippocampus. Proc Natl Acad Sci U S A 2000;97:4315–4320.
83. Muller D, Stoppini L, Wang C, Kiss JZ. A role for polysialylated neural cell adhesion molecule in lesion-induced sprouting in hippocampal organotypic cultures. Neuroscience 1994;61:441–445.
84. Muller D, Wang C, Skibo G, Toni N, Cremer H, Calaora V, Rougon G, Kiss JZ. PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 1996; 17:423–422.
85. Murphy KJ, O'Connell AW, Regan CM. Repetitive and transient increases in hippocampal neural cell adhesion molecule polysialylation state following mulltitrial spatial training. J Neurochem 1996;67:1268–74.
86. Murphy JA, Franklin TB, Rafuse VF, Clarke DB. The neural cell adhesion molecule is necessary for normal adult retinal ganglion cell number and survival. Mol Cell Neurosci 2007;36:280–292.
87. Murphy JA, Hartwick AT, Rutishauser U, Clarke DB. Endogenous polysialylated neural cell adhesion molecule enhances the survival of retinal ganglion cells. Invest Ophthalmol Vis Sci 2009;50:861–869.
88. Murphy JA, Daniels BA, Chauhan BC, Baldridge WH. NMDA-induced calcium dynamics are altered in retinas of adult mice deficient in the neural cell adhesion molecule. Invest Ophthalmol Vis Sci 2012;53:14.
89. Murray BA, Owens GC, Prediger EA, Crossin KL, Cunningham BA, Edelman GM. Cell surface modulation of the neural cell adhesion molecule resulting from alternative mRNA splicing in tissue-specific developmental sequence. J Cell Biol 1986;103:1431–1439.
90. Nadal-Nikolas FM, Jimenez-Lopez M, Sobrado-Calvo P, Nieto-Lopez L, Canovas-Martinez I, Salinas-Navarro M, Vidal-Sanz M, Aged M. Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic-nerve injured retinas. In vest Ophthalmol Vis Sci 2009;50:3860–3868.
91. Neiiendam JL, Kohler LB, Christensen C, Pedersen MV, Ditlevsen DK, Kornum MK, Kiseluov VV, Bock E. An NCAM-derived FGF-receptor agonist, the FGL-peptide, induces neurite outgrowth and neuronal survival in primary ret neutrons. J Neurochem 2004;91:920–935.
92. Nelson RW, Bates PA, Rutishauser U. Protein determinants for specific polysialylation of the neural cell adhesion molecule. J Biol Chem 1995;270:17171–17179.
93. O'Connell AW, Fox GB, Barry T, Murphy KJ, Fichera G, Foley AG, Kelly J, Regan CM. Spatial learning activates neural cell adhesion molecule polysialylation in a corticohippocampal pathway within the medial temporal lobe. J Neurochem 1997;68:2538–2546.
94. Olivares AM, Althoff K, Chen GF, Wu S, Morrisson MA, DeAngelis MM, Haider N. Animal models of diabetic retinopathy. Curr Diab Rep 2017;17:93.
95. Osborn NN, Ugarte M, Chao M, Childlow G, Bae JH, Wood JP, Nash MS. Neuroprotection in relation to retinal ischemia and relevance to glaucoma. Surv Ophthalmol 1999;43:102–128.

96. Owens GC, Edelman GM, Cunningham BA. Organization of the neural cell adhesion molecule (N-CAM) gene: alternative exon usage as the basis for different membrane-associated domains. Proc Natl Acad Sci U S A 1987;84:294–298.
97. Paratcha G, Ledda F, Ibanez CF. The neural cell adhesion molecule NCAM is an alternative signaling receptor for GDNF family ligands. Cell 2003;113:867–879.
98. Peinado-Ramon P, Salvador M, Villegas-Perez MP, Vidal-Sanz M. Effect of axotomy and intraocular administration of NT-4, NT-3 and brain-derived neurotrophic factor on the survival of adult retinal ganglion cells. A quantitative in vivo study. Invest Ophthalmol Vis Sci 1996;37:489–500.
99. Pernet V, DI Polo A. Synergistic action of brain-derived neurotrophic factor and lens injury promotes retinal ganglion cell survival, but leads to optic nerve dystrophy in vivo. Brain 2006;129:1014–1026.
100. Phillips GR, Krushel LA, Crossin KL. Developmental expression of two rat sialyltransferases that modify the neural cell adhesion molecule, N-CAM. Brain Res Dev Brain Res 1997;102:(143–155).
101. Puranen J, Haapaniemi AM, Lappalainen S, Kalesnykas G. Increased expression of PSA-NCAM in the optic nerve of experimental glaucoma. Invest Ophthalmol Vis Sci 2014;55:13.
102. Puro DG, Yuan JP, Sucher NJ. Activation of NMDA receptor-channels in human retinal Müller glial cells inhibits inward-rectifying potassium currents. Vis Neurosci 1996;13:319–326.
103. Rougon G, Deagostini-Bazin H, Hirn M, Goridis C. Tissue- and developmental stage-specific forms of a neural cell surface antigen linked to differences in glycosylation of a common polypeptide. EMBO J 1982;1:1239–1244.
104. Rougon G, Deagostini-Bazin H, Hirn M, Goridis C. Tissue- and developmental stage-specific forms of a neural cell surface antigen linked to differences in glycosylation of a common polypeptide. EMBO J 1982;10:1239–1244.
105. Rousselot P, Lois C, Alvarez-Buylla A. Embryonic (PSA) N-CAM reveals chains of migrating neuroblasts between the lateral ventricle and the olfactory bulb of adult mice. J Comp Neurol 1995;351:51–61.
106. Ruthishauser U, Landmesser L. Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. Trends Neurosci 1996;19:422–427.
107. Rutishauser U, Landmesser L. Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. Trends Neurosci 1996;19:422–427.
108. Santiago AR, Hughes JM, Kamphuis W, Schlingemann RO, Ambrosio AF. Diabetes changes ionotropic glutamate receptor subunit expression level in the human retina. Brain Res 2008;1198:153–159.
109. Sawaguchi A, Idate Y, Ide S, Kawano Ji, Nagaike R, Oinuma T, Suganuma T. Multistratified expression of polysialic acid and its relationship to VaChT-containing neurons in the inner plexiform layer of adult rat retina. J Histochem Cytochem 1999;47:919–928.
110. Schlosshauer B, Schwarz U, Rutishauser U. Topological distribution of different forms of neural cell adhesion molecule in the developing chick visual system. Nature 1984;310:141–143.
111. Seki T and Arai Y. Distribution and possible roles of the highly polysialylated neural cell adhesion molecule (NCAM-H) in the developing and adult central nervous system. Neurosci Res 1993;17:265–290.

112. Seki T, Arai Y. Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. Anat Embryol (Berl) 1991; 84:395–401.
113. Seki T, Rutishauser U. Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocamous. J Neurosci 1998;18:3757–3766.
114. Shchi K, Fujita-Hamabe W, Harada S, Mizoguchi H, Yanmada K, Nabeshima T, Tokuyama S. Involvment of matrix metalloproteinase-mediated proteolysis of neural cell adhesion molecule in the development of cerebral ischemic neuronal damage. J Pharmacol Exp Ther 2011;338:701–710.
115. Silver J, Rutishauser U. Guidance of optic axons in vivo by a preformed adhesive pathway on nuroepithelial endfeet. Dev Biol 1984;106:485–499.
116. Singh S, Tyagi SC. Metalloproteinases as mediators of inflammation and the eyes: molecular genetic underpinnings governing ocular pathophysiology. Int J Ophthalmol 2017;10:1308–1318.
117. Soroka V, Kolkova K, Kastrup JS, Diederichs K, Breed J, Kiselyov VV, Poulsen FM, Larsen IK, Welte W, Berezin V, Kasoer C. Structure and interactions of NCAM Ig 1–2–3 suggest a novel zipper mechanism for homophilic adhesion. Structure 2003;11:1291–1301.
118. Stier H, Schlosshauer B. Axonal guidance in the chicken retina. Development 1995;121:1443–1454.
119. Stone J, Makarov F, Hilländer H. The glial ensheathment of the soma and axon hillock of retinal ganglion cells. Vis Neurosci 1995;12:273–279.
120. Tao C, Zhang X. Development of astrocytes in the vertebrate eye. Dev Dyn 2014;243:1501–1510.
121. Thanos S, Bonhoeffer F, Rutishauser U. Fiber-fiber interaction and tectal cues influence the development of the chichen retinotectal projection. Proc Natl Acad Sci U S A 1984;81:1906–1910.
122. Thiery JP, Brackenbury R, Rutishauser U, Edelman GM. Adhesion among neural cells of the chick embryo. II. Purification and characterization of a cell adhesion molecule from neural retina. J Biol Chem 1977;10:6841–6845.
123. Tonade D, Liu H, Palczewski K, Kern TS. Photoreceptor cells produce inflammatory products that contribute to retinal vascular permeability in a mouse model of diabetes. Diabetilogia 2017;60:2111–2120.
124. Trupp M, Arenas E, Fainzilber M, Nilsson AS, Sieber BA, Grigorioi M, Kilkenny C, Salazar-Grueso E, Pachnis V, Arumäe U. Functional receptor for GDNF encoded by the c-ret proto-oncogene. Nature 1996;381:785–789.
125. Varea E, Nacher J, Blasco-Ibanez JM, Gomez-Climent MA, Castillo-Gomez E, Crespo C, Martinez-Guijarro FJ. PSA-NCAM expression in the rat medial prefrontal cortex. Neuroscience 2005;136:435–443.
126. Varea E, Belles M, Vidueira S, Blasco-Ibanez JM, Crespo C, Pastor AM, Nacher J. PSA-NCAM is expressed in immature, but not resently generated, neurons in the adult cat cerebral cortex layer II. Front Neurosci 2011;5:17.
127. Vecino E, Hernandez M, García M. Cell death in the developing vertebrate retina. Int J Dev Biol 2004;48:965–974.
128. Vutskits L, Djebbara-Hannas Z, Zhang H, Paccaud JP, Durbec P, Rougon G, Muller D, Kiss JZ. PSA-NCAM modulates BDNF-dependent survival and differentiation of cortical neurons. Eur J Neurosci 2001;13:1391–1402.

129. Vutskits L, Gascon E, Zgraggen E, Kiss JZ. The polysialylated neural cell adhesion molecule promotes neurogenesis in vitro. Neurochem Res 2006;31:215–225.
130. Worby CA, Vega QC, Zhao Y, Chao HH, Seasholtz AF, Dixon JE. Glial cell line-derived neurotrophic factor signals through the RET receptor and activates mitogen-activated protein kinase. J boil Chem 1996;271:23619–23622.
131. Yabe U, Sato C, Matsuda T, Kitajima K. Polysialic acid in human milk. CD36 is a new member of mammalian Polysialic acid-containing glycoprotein. J Biol Chem 2003;278:13875–13880.
132. Yin X, Watanabe M, Rutishuser U. Effect of polysialic acid on the behavior of retinal gangion cell axons during growth into the optic tract and tectum. Development 1995;121:3439–3446.
133. Zhang X, Cheng M, Chintala SK. Kainic acid-mediated upregulation of matrix metalloproteinase-9 promotes retinal degeneration. Invest Ophthalmol Vis Sci 2004a;45:2374–2383.
134. Zhang X, Cheng M, Chintala SK. Optic nerve ligation leads to astrocyte-associated metalloproteinase-9 induction in the mouse retina. Neurosci Lett 2004b;356:140–144.
135. Zhu X, Sun Y, Wang Z, Cui W, Peng Y, Li R. Expression of glial cell line-derived neurotrophic factor and its receptors in cultured retinal Müller cells under high glucose circumstance. Anat Rec (Hoboken) 2012;295:532–539.
136. Zuber C, Lackie PM, Catterall WA, Roth J. Polysialic acid is associated with sodium channels and the neural cell adhesion molecule N-CAM in adult rat brain. J Biol Chem 1992;267:9965–9971.

# SUMMARY IN ESTONIAN

## Polüsialüülitud närviraku adhesioonimolekuli (PSA-NCAMi) roll silma võrkkesta ganglionirakkude elulemusele täiskasvanueas

Närviraku adhesioonimolekul (NCAM) on glükoproteiin, mis kuulub immunoglobuliinide superperekonda. Kolm peamist NCAMi isovormi on jaotatud molekulmassi alusel (NCAM-180, NCAM-140, NCAM-120) ning need erinevad üksteisest rakumembraani läbiva domeeni poolest. Närviraku adhesioonimolekulide peamine roll on närvirakkude vaheliste kontaktide loomine ja nende stabiliseerimine. Polüsiaalhappe (PSA) seondumine NCAMi viienda immunoglobuliini domeeni külge toimub polüsialüültransferaas II (ST8SiaII) ja IV (ST8SiaIV) vahendusel, ning selle tulemusena väheneb rakkudevaheline adhesioon. Lisaks on näidatud, et PSA-NCAM ei mõjuta mitte üksnes rakkudevahelisi adhesiivseid omandusi, vaid sellel on oluline roll ka protsessides, mille kaudu reguleeritakse rakkude elulemust, proliferatsiooni, diferentseerumist, migratsiooni, aksonite väljakasvu ning sünaptilist plastilisust. PSA-NCAMi leidub valdavalt kesknärvisüsteemi neuronitel ning gliiarakkudel ja olles närvirakkudevahelise struktuurse dünaamilisuse vahendaja, omab ta olulist rolli aju plastilisuse regulatsioonis nii arenevas ajukoes kui ka täiskasvanueas. Silma võrkkestas esineb PSA-NCAMi täiskasvanueas kõige rohkem Mülleri rakkudel ja astrotsüütidel ganglionirakkude (RGC) juures. Silma võrkkest koosneb mitmetest erineva funktsiooniga rakukihtidest, millest ühe kihi moodustavad ganglionirakud. Selles kihis paiknevad RGCde kehad, mille aksonid moodustavad nägemisnärvi. On näidatud, et silma võrkkesta PSA-NCAM võib mõjutada RGC elulemust, kuid milline on PSA-NCAMi täpne roll täiskasvanueas silma võrkkesta funktsiooni tagamisel, pole teada.

**Töö eesmärgid:**

1. Tuvastada, kas NCAM, ST8SiaII, ST8SiaIV puudulikel hiirtel ja endosialidaas-N-i (Endo-N) indutseeritud PSA defitsiit metsiktüüpi hiirtel esinevad muutused PSA-NCAMi ekspressionis ja silma võrkkesta RGCde arvus.
2. Selgitada, kas ja kuidas mõjutab NCAM-/-, ST8SiaII-/-, ST8SiaIV-/- või Endo-N-iga tekitatud PSA kadu silma võrkkesta RGCde elulemust neurotoksiini kaiinhappe intravitreaalse manustamise järgselt.
3. Selgitada, millise mehhanismi vahendusel väheneb PSA-NCAMi tase ning tekib RGCde degeneratsioon kaiinhappe intravitreaalse manustamise järgselt.
4. Teha kindlaks PSA-NCAMi ja maatriksi metalloproteinaas-9 (MMP-9) roll RGCde elulemusele hiire streptosototsiiniga indutseeritud diabeedi mudelis.

**Töö tulemused:**

1. Antud töö tulemused näitasid, et vastsündinud ST8SiaII-/- ja ST8SiaIV-/- hiirtel on PSA-NCAMi ekspressioon oluliselt vähenenud, kuid täiskasvanud hiirtel ekspressioonis muutusi ei täheldatud. NCAM-/- hiirtel PSA-NCAMi

ei leitud. Tuvastati, et NCAM-/- hiirtel on RGCde arv oluliselt suurenenud, vastupidiselt ST8SiaII-/- hiirtele, kellel RGCde arv on vähenenud, kuid ST8SiaIV-/- hiirtel RGCde arvus muutusi ei esinenud. Samuti selgus, et PSA eemaldamine NCAMilt Endo-N-iga ei mõjutanud RGCde elulemust.

2. Ilmnes, et ST8SiaII-, ST8SiaIV- või NCAM-defitsiitsetel loomadel ei mõjutanud kaiinhappe poolt põhjustatud toksilisus RGCde elulemust, mille põhjuseks võib olla olemasolevate sialüültransferaaside aktivatsioon. Samas, Endo-N-iga tekitatud PSA kadu suurendas kaiinhappe toksilist toimet RGCdele, mis viitab, et polüsialüülitud NCAMi vorm on vajalik RGCde elulemuse tagamiseks.
3. Selgus, et MMP-9 osaleb kaiinhappest indutseeritud PSA-NCAMi taseme vähenemises, põhjustades PSA eemaldamist NCAMilt ning selle mehhanismi kaudu mõjutatakseb RGCde elulemust.
4. Tuvastati, et streptosototsiiniga indutseeritud diabeedi mudelis on RGCde arv vähenenud ja see on seotud PSA-NCAMi taseme vähenemisega ning MMP-9 aktivatsiooni suurenemisega RGCde piirkonnas.

# ACKNOWLEDGEMENTS

This study was performed in the Department of Pharmacology, University of Tartu, Estonia. The study was supported financially by the Estonian Science Council (Institutional research founding) grant IUT2-3, the Archimedes Foundation and the European Regional Development Fund.

Foremost, I am thankful to my supervisor Professor Alexsander Zharkovsky. First, he gave me an opportunity to perform my thesis in the Department of Pharmacology. Throughout the years his support, encouragement, council, discipline, and inspiring discussions were invaluable. I feel respect and very warm attitude to him as a person also.

I am very thankful to all my colleagues for their time, instructions, support, and companionship. Tamara Zarkovskaja, Külli Jaako, Anu-Aounurm Helm, Katrin Põld, Oili Suvi, Allen Kaasik, Akbar Zeb, Vinay Chouby, Malle Kuum, Dzamilja Safiulina, Kaili Anier – thank you all for being there.

My special thanks to Monika Jürgenson for her kindness, friendship and help with animal experiments; to Miriam Hickey for her friendship and help in linguistic correction of all our manuscripts; to Hiie Sarv for her goodness and help in all official issues.

My greatest gratitude to my parents for their constant belief in me, her encouragement, and help with children; to my husband for his patience, time, invaluable help in all technical questions with computer programs and for always being with me.

# PUBLICATIONS

# CURRICULUM VITAE


| | |
|---|---|
| **Name:** | Natalia Lobanovskaya |
| **Date of birth:** | November 30, 1980, Russia, Nizhny Tagil |
| **Citizenship:** | Russian |
| **Address:** | University of Tartu, Institute of Biomedicine and Translational Medicine, Department of Pharmacology, Ravila 19, Tartu 50411, Estonia |
| **E-mail:** | natalia.lobanovskaya@gmail.com |


**Education:**

| | |
|---|---|
| 2010–… | PhD, Medicine, University of Tartu, Department of Pharmacology |
| 1998–2004 | Smolensk State Medical Academy of the Healthcare Ministry of Russian Federation, Russia |

**Career:**

| | |
|---|---|
| 2017–… | University of Tartu, Institute of Biomedicine and Translational Medicine, Department of Pharmacology; specialist |
| 11.2011–12.2012, 2017–… | Ida Viru Central Hospital; ophthalmologist, laser surgeon |
| 2004–2010 | S. Fyodorov Eye Microsurgery Federal State Institution Kaluga Clinic, Russia; ophthalmologist, laser surgeon |

**Practical courses**

2011 Competence on care and use of laboratory animals; Central Animal Laboratory, Faculty of medicine, University of Turku, Finland

2013 IBRO course in neuroscience; Center for the cognitive neuroscience and semantics, University of Latvia. Riga, Latvia

**Professional organizations:**

Estonian Ophthalmological Society

**Publications:**


**Lobanovskaya N**, Zharkovsky A, Jaako K, Jürgenson M, Aonurm-Helm, Zharkovsky A. PSA modification of NCAM supports the survival of injured retinal ganglion cells in adulthood. Brain Res 2015;1625:9–17

**Lobanovskaya N**, Jürgenson M, Aonurm-Helm A, Zharkovsky A. Alterations in the polysialylated neural cell adhesioon molecule and retinal ganglion cell density in mice with diabetic retinopathy. Int J Ophthalmol 2018;11:1608–1615

**Lobanovskaya N**, Zharkovsky A. A role of PSA-NCAM in the survival of retinal ganglion cells (RGCs) after kainic acid damage. Neurotoxicology 2019; 72: 101–106

Konoplyanikov AG, Belyy YA, Tereshchenko AV, Volodin PL, **Lobanovskaya NV.** Development of combined technology of treatment of age related macular degeneration by using autologous mesenchymal stem cells. Actual problems of ophthalmology 2009, Abstracts

Bellyy YA, Tereshchenko AV, Volodin PL, Konoplyanikov AG, Plahotniy MA, **Lobanovskaya NV**. Treatment of age related macular degeneration by using mesenchymal stem cells. Russian Journal of immunology. 2008; vol2(11), Issue 23

# ELULOOKIRJELDUS


**Nimi:** Natalia Lobanovskaya
**Sünniaeg:** 30. november 1980, Nizhny Tagil, Venemaa
**Kodakondus:** Venelane
**Aadress:** Tartu Ülikool, Bio- ja Siirdemeditsiini Instituut, Farmakoloogia osakond
Ravila 19, Tartu 50411, Eesti
**E-post:** natalia.lobanovskaya@gmail.com


**Haridustee:**

| | |
|---|---|
| 2010–… | PhD, Meditsiin, Tartu Ülikool |
| 1998–2004 | Smolensk Akadeemia, Arstiteaduskond, Venemaa |

**Töökogemus:**

| | |
|---|---|
| 2017–… | Tartu Ülikool, Bio- ja Siirdemeditsiini Instituut, Farmakoloogia osakond; spetsialist |
| 11.2011–12.2012, 2017–… | SA Ida-Viru Keskhaigla; silmaarst, laserkirurg |
| 2004–2010 | S. Fyodorov Silmade Milrokirurgia Kliinik, Kaluga, Venemaa; silmaarst, laserkirurg |

**Praktilised koolitused:**

| | |
|---|---|
| 2011 | Katseloomakursus; Meditsiiniteaduste valdkond, Turku Ülikool, Soomemaa |
| 2013 | IBRO Neuroteadusekursus; Riia Ülikool, Läti |

**Erialaorganisatsioonid:**
Eesti Oftalmoloogide Selts

**Publicatioonid:**


**Lobanovskaya N**, Zharkovsky A, Jaako K, Jürgenson M, Aonurm-Helm, Zharkovsky A. PSA modification of NCAM supports the survival of injured retinal ganglion cells in adulthood. Brain Res. 2015;1625:9–17

**Lobanovskaya N**, Jürgenson M, Aonurm-Helm A, Zharkovsky A. Alterations in the polysialylated neural cell adhesioon molecule and retinal ganglion cell density in mice with diabetic retinopathy. Int J Ophthalmol 2018;11:1608–1615

**Lobanovskaya N**, Zharkovsky A. A role of PSA-NCAM in the survival of retinal ganglion cells (RGCs) after kainic acid damage. Neurotoxicology 2019; 72: 101–106

Konoplyanikov AG, Belyy YA, Tereshchenko AV, Volodin PL, **Lobanovskaya NV.** Development of combined technology of treatment of age related macular degeneration by using autologous mesenchymal stem cells. Actual problems of ophthalmology 2009, Abstracts

Bellyy YA, Tereshchenko AV, Volodin PL, Konoplyanikov AG, Plahotniy MA, **Lobanovskaya NV**. Treatment of age related macular degeneration by using mesenchymal stem cells. Russian Journal of immunology. 2008; vol2(11), Issue 23

# DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos**. The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer**. Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar**. Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik**. Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu**. Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar**. Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus**. Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar**. Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm**. On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross**. Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo**. Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik**. The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa**. Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk**. Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar**. Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi**. The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar**. Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang**. The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak**. Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki**. Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar**. Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel**. Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik**. Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk**. Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets**. Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik**. Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi**. Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo**. Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber**. *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla**. Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk**. Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf**. Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv**. Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi**. Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva**. Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin**. Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev**. Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik**. Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto**. Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar**. Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge**. Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg**. The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp**. Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots**. Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe**. Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv**. Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan**. Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik**. Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand**. Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi**. Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask**. Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson**. Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar**. Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl**. Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap**. Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg**. *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro**. Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi**. Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann**. Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke**. Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves**. Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda**. Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist**. Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik**. Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull**. Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos**. Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova**. Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda**. Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner**. *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi**. Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla**. Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas**. Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk**. Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen**. Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar**. Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep**. Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes**. Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu**. Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi**. Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa**. Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk**. Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.
84. **Helena Soomer**. Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin**. Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann**. Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu**. Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas**. Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba**. Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev**. CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja**. Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla**. Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid**. Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter**. Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa**. Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre**. Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev**. Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema**. Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet**. Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.
102. **Eduard Maron**. Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona**. *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk**. Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv**. Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun**. Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda**. Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo**. The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila**. Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla**. Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell**. Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme**. Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor**. Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm**. Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite**. Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll**. Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach**. Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell**. Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako**. Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtson**. Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld**. Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann**. Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar**. Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson**. Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus**. Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder**. Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru**. Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand**. Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo**. Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo**. The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild**. Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik**. Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller**. Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik**. Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals**. Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus**. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab**. Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme**. Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin**. The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik**. Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu**. Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu**. Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik**. Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets**. The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits**. Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam**. Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots**. Cationic regulation of Na-pump in the normal, Alzheimer's and $CCK_2$ receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp**. The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull**. Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre**. Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link**. Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre**. Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak**. Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich**. Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep**. Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk**. Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass**. L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai**. Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum**. Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova**. The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi**. Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi**. A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe**. Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm**. Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu**. Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont**. Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt**. Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru**. Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov**. Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg**. Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm**. Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg**. Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal**. Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass**. Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu**. Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.
205. **Oksana Jagur**. Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk**. Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal**. Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand**. Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma**. Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre**. Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp**. Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum**. Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson**. Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand**. Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma**. Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg**. Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit**. Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees**. Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik**. The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel**. Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna**. The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrid Liiv**. Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison**. Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress**. Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru**. Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu**. Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets**. The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome**. Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo**. Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin**. Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna**. Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase**. Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel**. Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere**. Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust**. Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman**. Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar**. Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson**. Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme**. High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part**. Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.
241. **Urve Paaver**. New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.
242. **Aleksandr Peet**. Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.
243. **Piret Mitt**. Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.
244. **Merli Saare**. Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.
245. **Kaja-Triin Laisaar**. People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.
246. **Eero Merilind**. Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.
247. **Jaanika Kärner**. Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.
248. **Kaido Paapstel**. Metabolomic profile of arterial stiffness and early biomarkers of renal damage in atherosclerosis. Tartu, 2016, 173 p.
249. **Liidia Kiisk**. Long-term nutritional study: anthropometrical and clinico-laboratory assessments in renal replacement therapy patients after intensive nutritional counselling. Tartu, 2016, 207 p.
250. **Georgi Nellis**. The use of excipients in medicines administered to neonates in Europe. Tartu, 2017, 159 p.
251. **Aleksei Rakitin**. Metabolic effects of acute and chronic treatment with valproic acid in people with epilepsy. Tartu, 2017, 125 p.
252. **Eveli Kallas**. The influence of immunological markers to susceptibility to HIV, HBV, and HCV infections among persons who inject drugs. Tartu, 2017, 138 p.
253. **Tiina Freimann**. Musculoskeletal pain among nurses: prevalence, risk factors, and intervention. Tartu, 2017, 125 p.
254. **Evelyn Aaviksoo**. Sickness absence in Estonia: determinants and influence of the sick-pay cut reform. Tartu, 2017, 121 p.
255. **Kalev Nõupuu**. Autosomal-recessive Stargardt disease: phenotypic heterogeneity and genotype-phenotype associations. Tartu, 2017, 131 p.
256. **Ho Duy Binh**. Osteogenesis imperfecta in Vietnam. Tartu, 2017, 125 p.
257. **Uku Haljasorg**. Transcriptional mechanisms in thymic central tolerance. Tartu, 2017, 147 p.
258. **Živile Riispere**. IgA Nephropathy study according to the Oxford Classification: IgA Nephropathy clinical-morphological correlations, disease progression and the effect of renoprotective therapy. Tartu, 2017, 129 p.

259. **Hiie Soeorg**. Coagulase-negative staphylococci in gut of preterm neonates and in breast milk of their mothers. Tartu, 2017, 216 p.
260. **Anne-Mari Anton Willmore**. Silver nanoparticles for cancer research. Tartu, 2017, 132 p.
261. **Ott Laius**. Utilization of osteoporosis medicines, medication adherence and the trend in osteoporosis related hip fractures in Estonia. Tartu, 2017, 134 p.
262. **Alar Aab**. Insights into molecular mechanisms of asthma and atopic dermatitis. Tartu, 2017, 164 p.
263. **Sander Pajusalu**. Genome-wide diagnostics of Mendelian disorders: from chromosomal microarrays to next-generation sequencing. Tartu, 2017, 146 p.
264. **Mikk Jürisson**. Health and economic impact of hip fracture in Estonia. Tartu, 2017, 164 p.
265. **Kaspar Tootsi**. Cardiovascular and metabolomic profiling of osteoarthritis. Tartu, 2017, 150 p.
266. **Mario Saare**. The influence of AIRE on gene expression – studies of transcriptional regulatory mechanisms in cell culture systems. Tartu, 2017, 172 p.
267. **Piia Jõgi**. Epidemiological and clinical characteristics of pertussis in Estonia. Tartu, 2018, 168 p.
268. **Elle Põldoja**. Structure and blood supply of the superior part of the shoulder joint capsule. Tartu, 2018, 116 p.
269. **Minh Son Nguyen**. Oral health status and prevalence of temporomandibular disorders in 65–74-year-olds in Vietnam. Tartu, 2018, 182 p.
270. **Kristian Semjonov**. Development of pharmaceutical quench-cooled molten and melt-electrospun solid dispersions for poorly water-soluble indomethacin. Tartu, 2018, 125 p**.**
271. **Janne Tiigimäe-Saar**. Botulinum neurotoxin type A treatment for sialorrhea in central nervous system diseases. Tartu, 2018, 109 p.
272. **Veiko Vengerfeldt.** Apical periodontitis: prevalence and etiopathogenetic aspects. Tartu, 2018, 150 p.
273. **Rudolf Bichele.** TNF superfamily and AIRE at the crossroads of thymic differentiation and host protection against *Candida albicans* infection. Tartu, 2018, 153 p.
274. **Olga Tšuiko**. Unravelling Chromosomal Instability in Mammalian Preimplantation Embryos Using Single-Cell Genomics. Tartu, 2018, 169 p.
275. **Kärt Kriisa.** Profile of acylcarnitines, inflammation and oxidative stress in first-episode psychosis before and after antipsychotic treatment. Tartu, 2018, 145 p.
276. **Xuan Dung Ho.** Characterization of the genomic profile of osteosarcoma. Tartu, 2018, 144 p.
277. **Karit Reinson.** New Diagnostic Methods for Early Detection of Inborn Errors of Metabolism in Estonia. Tartu, 2018, 201 p.

278. **Mari-Anne Vals.** Congenital N-glycosylation Disorders in Estonia. Tartu, 2019, 148 p.
279. **Liis Kadastik-Eerme**. Parkinson's disease in Estonia: epidemiology, quality of life, clinical characteristics and pharmacotherapy. Tartu, 2019, 202 p.
280. **Hedi Hunt**. Precision targeting of intraperitoneal tumors with peptide-guided nanocarriers. Tartu, 2019, 179 p.
281. **Rando Porosk**. The role of oxidative stress in Wolfram syndrome 1 and hypothermia. Tartu, 2019, 123 p.
282. **Ene-Ly Jõgeda**. The influence of coinfections and host genetic factor on the susceptibility to HIV infection among people who inject drugs. Tartu, 2019, 126 p.
283. **Kristel Ehala-Aleksejev**. The associations between body composition, obesity and obesity-related health and lifestyle conditions with male reproductive function. Tartu, 2019, 138 p.
284. **Aigar Ottas**. The metabolomic profiling of psoriasis, atopic dermatitis and atherosclerosis. Tartu, 2019, 136 p.
285. **Elmira Gurbanova.** Specific characteristics of tuberculosis in low default, but high multidrug–resistance prison setting. Tartu, 2019, 129 p.
286. **Van Thai Nguyeni.** The first study of the treatment outcomes of patients with cleft lip and palate in Central Vietnam. Tartu, 2019, 144 p.
287. **Maria Yakoreva**. Imprinting Disorders in Estonia. Tartu, 2019, 187 p.
288. **Kadri Rekker**. The putative role of microRNAs in endometriosis pathogenesis and potential in diagnostics. Tartu, 2019, 140 p.
289. **Ülle Võhma**. Association between personality traits, clinical characteristics and pharmacological treatment response in panic disorder. Tartu, 2019, 121 p.
290. **Aet Saar**. Acute myocardial infarction in Estonia 2001–2014: towards risk-based prevention and management. Tartu, 2019, 124 p.
291. **Toomas Toomsoo**. Transcranial brain sonography in the Estonian cohort of Parkinson's disease. Tartu, 2019, 114 p.
292. **Lidiia Zhytnik**. Inter- and intrafamilial diversity based on genotype and phenotype correlations of Osteogenesis Imperfecta. Tartu, 2019, 224 p.
293. **Pilleriin Soodla.** Newly HIV-infected people in Estonia: estimation of incidence and transmitted drug resistance. Tartu, 2019, 194 p.
294. **Kristiina Ojamaa.** Epidemiology of gynecological cancer in Estonia. Tartu, 2020, 133 p.
295. **Marianne Saard.** Modern Cognitive and Social Intervention Techniques in Paediatric Neurorehabilitation for Children with Acquired Brain Injury. Tartu, 2020, 168 p.
296. **Julia Maslovskaja.** The importance of DNA binding and DNA breaks for AIRE-mediated transcriptional activation. Tartu, 2020, 162 p.