Charles University in Prague

Faculty of Science

Neurobiology

The effect of structural changes in perineuronal nets and deep cooling on synaptic plasticity and memory of tauopathy mice

Efekt strukturálních změn v perineurálních sítích a hluboké hypotermie CNS na synaptickou plasticitu a paměť u myšího modelu tauopatie

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Diploma thesis

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Prague, 2020

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne

Podpis

Poděkování

Ráda bych poděkovala svému školiteli RNDr. Jiřímu Růžičkovi, Ph.D. za vedení, přátelský přístup a ochotu vždy pomoci a naučit veškeré techniky potřebné k úspěšnému zpracování mé diplomové práce. Dále bych ráda poděkovala své rodině a blízkým, kteří mě podporovali po celou dobu mého studia.

Abstrakt

Tauopatie je neurodegenerativní onemocnění charakteristické jak ztrátou neuronů, tak synapsí. Ztráta neuronů je ireverzibilní s velmi malou šancí funkční substituční terapie. Ztracené synapse však lze obnovit pomocí správné stimulace. Perineuronální sítě (PNNs) slouží jako ochranná bariéra neuronů, zároveň ale významně snižují jejich synaptickou plasticitu. Dočasné enzymatické rozštěpení struktury PNNs může vést k opětovnému zapojení synapsí a zlepšení procesu paměti a učení. Model chladem vyvolané plasticity vede k odpřažení signifikantního počtu synapsí v mozku. Následné obnovení synapsí bylo pozorováno jak u zdravých tak i nemocných zvířat a navíc došlo ke spuštění neuroprotektivních mechanismů vyvolaných exprimací proteinů studeného šoku (CSP). Tato diplomová práce je zaměřena na výše zmíněné formy modelů synaptické plasticity; umělé remodelace perineuronálních sítí a model synaptické plasticity vyvolané chladem. Oba tyto modely budou použity jako nástroj pro modulaci procesů paměti a učení u modelu P301S Tauopatie u myší. Práce se zaměří na změny v počtu synapsí v oblasti CA1 hipokampu, na změny hladin synaptických proteinů na úrovni celého hipokampu a na behaviorální změny předem trénovaného úkolu dlouhodobé paměti závislého na dorzálním hipokampu.

Klíčová slova: perineurální sítě, agrekan, chladem indukovaná synaptická plasticita, paměť, hipokampus

Abstract

Tauopathy is accompanied by both loss of neurons and synapses. The neuronal loss is irreversible with very low chance of functional replacement therapy. However, lost synapses could be restored with proper stimuli. Perineuronal nets (PNNs) are serving as a protecting barrier for neurons, on the other hand they are significantly decreasing the synaptic plasticity. Temporary disintegration of the PNNs by enzymatic therapy might lead to rewiring and accelerate processes of memory and learning. Model of Cold Induced plasticity leads to the withdrawal of significant number of synapses across the brain. The recovery of these could be followed in healthy and diseased animals. Moreover, it can stimulate Cold shock protein dependent neuroprotective mechanisms. This master thesis is focused on these two forms of synaptic plasticity models; forced remodeling of PNNs and model of cold induced synaptic plasticity. Both will serve as a tool to modulate processes of memory and learning in the P301S tauopathy, in mice. In detail, the work will follow changes in the number of synapses at the region of CA1 of hippocampus and synaptic protein levels at level of whole hippocampus and behavioral recovery of pre-trained long-term memory task dependent on dorsal hippocampus.

Key words: Perineuronal nets, aggrecan, cold-induced synaptic plasticity, memory, hippocampus

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List of abbreviations

AD	Alzheimer disease
ADAMs	a disintegrin and metalloproteases
ADAMTs	a disintegrin and metalloproteinase with thrombospondin motifs
AEV	average error value
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	5′ adenosine monophosphate-activated protein kinase
Bral2	brain link protein 2
C4ST-1	chondroitin 4-sulfotransferase-1
C6ST-1	chondroitin 6-sulfotransferase-1
chABC	chondroitinase ABC
CIP	cold-induced synaptic plasticity
CNS	central nervous system
CS	chondroitin sulfate
CSP	cold shock protein
CSPGs	chondroitin sulfate proteoglycans
CTRL1	cartilage link protein 1
ECM	extracellular matrix
ECS	extracellular space
EPSC	the excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
GABA	gamma-aminobutyric acid
GAD	glutamate decarboxylase
GAG	glycosaminoglycan
GlcA	glucuronic acid
GSK-3	glycogen synthase kinase-3
HA	hyaluronan
HAPLN	hyaluronan- and proteoglycan binding link protein gene family
HAS	hyaluronan synthase
IHC	immunohistochemistry
IPSP	inhibitory postsynaptic potential

KO	knock-out
LTD	long-term depression
LTP	long-term potentiation
MBDs	microtubule-binding domains
MMPs	matrix metalloproteases
MWM	Morris water maze
NFT	neurofibrillary tangle
NMDA	N-methyl-D-aspartate
ORT	object recognition test
Otx2	Orthodenticle homeobox 2
PGs	proteoglycans
PNNs	perineuronal nets
PP	phosphatase
PSD-95	post-synaptic density protein 95
PV+	parvalbumin positive
Sema3A	Semaphorin3A
SNAP25	synaptosomal nerve-associated protein 25
SNK	Student-Newman-Keuls test
TIMPs	tissue inhibitors of matrix metalloproteases
Tn	tenascin
VGAT	vesicular GABA transporter
VGLUT	vesicular glutamate transporter
VVA	Vicia villosa agglutinin
WFA	Wisteria floribunda agglutinin
WT	wild type

1. Introduction

Perineuronal nets (PNNs) are a specialized extracellular matrix (ECM) structure surrounding neurons from the dendrites of the soma to the endings of the axon (Celio & Blumcke, 1994) (Fig.1). The majority of PNNs are present with fast-spiking gamma-aminobutyric acid (GABA)-ergic parvalbumin positive interneurons (PV+) (W. Härtig et al., 1992) . A great number of studies has been made to capture the attributes of PNNs. Current knowledge shows PNNs properties range from physical support, ion buffering, stabilization of synaptic transmission to restricting of plasticity due to their association with inhibitory molecules (Gert Brückner et al., 1993; Celio & Blumcke, 1994; Kwok et al., 2015; Vo et al., 2013).

In a developing organism, the PNNs assembly coincides with the closure of the critical period causing the transition of the juvenile brain to the adult brain (Dityatev et al., 2007). Preventing the formation of PNNs prolongs the critical period (Carulli et al., 2010). Furthermore, their removal in adult organisms reopens the critical period window of high juvenile-like plasticity (Pizzorusso, 2002). This enables to create new synapses, to remodel the neuronal connectivity network and to enhance the learning process and memory retention (Romberg et al., 2013). The abolishing of PNNs is realized by invasive local injection of a bacterial enzyme, the chondroitinase ABC (chABC), or preventing the formation via various gene knock-out (KO) models (Lensjø, Lepperød, et al., 2017; Rowlands et al., 2018).

PNNs play a viable role in the function of a healthy central nervous system (CNS) and the reduction of PNNs together with the elevation of proteolytic enzymes of ECM molecules is usually associated with neurodegenerative or psychiatric diseases (Marín, 2012; Serrano-Pozo et al., 2011). PNNs have been mostly studied in association with Alzheimer disease (AD) since a reduction of PNNs densities is believed to result in the increased vulnerability of neurons and the progression of the disease. AD is characterized with a formation of neurofibrillary tangles composed of abnormally hyperphosphorylated protein Tau (Alonso et al., 2001). The Tau protein hyperphosphorylation was shown to be induced by low-temperatures (e.g. hypothermia) (Planel, 2004). On the other hand, low temperatures trigger the expression of cold shock proteins (CSP) that are known to serve as neuroprotectants and particularly RBM3 has been associated with neuroprotection in various models of cooling (Chip et al., 2011). Moreover, the induction of CSP

expression was found in parallel with the ability for structural plasticity and cooling helped to restore failed synaptic plasticity in an early stage of 5xFAD and prion mice model of AD (Peretti et al., 2015). Therefore, we use mice model of an early symptomatic stage of tauopathy P301S and apply above mentioned ChABC mediated PNN remodeling and cold induced plasticity, two different methods of restoration of synaptic plasticity, to examine the impact on memory and learning process in a hippocampal-based Morris water maze task and the effect on the overall progression of the disease.

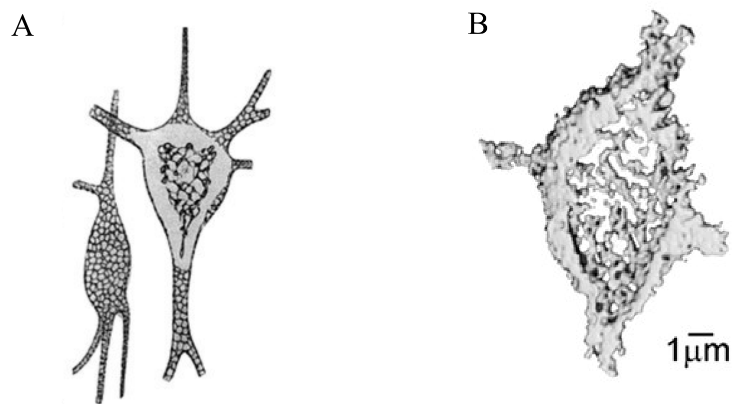

Figure 1 | Development of visualization of PNNs
A | Cerebral cells with short axons (adult cat), stained with reduced silver nitrate (Ramón y Cajal, 1909)
B | Confocal image of a rat somatosensory cortex interneuron labeled with fluorescent VVA (S. Miyata et al., 2005)

2. Extracellular matrix

ECM is a non-cellular structure filling the interstitial space of the CNS and is differing in its composition and density from tissue to tissue. It originates from cells embedded within it and it is composed of the molecules those cells are secreting. ECM consists of various molecules, predominantly proteoglycans (PGs) and fibrous proteins (e.g. collagens, elastins, fibronectins) (Galtrey et al., 2008). In the brain, ECM is taking up to 20% of the volume (Johnson & Jenkins, 1999). Both neurons and astrocytes contribute to the synthesis of the ECM components (Dzyubenko et al., 2016). Regarding the function, ECM has been traditionally considered as predominantly structural. Nevertheless, it is also involved in cell development, migration, maturation and differentiation, tissue homeostasis and tumor cell invasion proving its importance for the cells (Henderson et al., 1997; Hobohm et al., 1998).

2.1 Types of ECM

Depending on the ratio and structure of the ECM molecules, the ECM consistency can be simply divided into the loose and dense one, differing not just among brain areas but also within them (Hobohm et al., 1998) (Figure 2). The neural interstitials of brain are filled with diffused matrix and with growing proximity to specific CNS neurons ECM is becoming more condensate and specific in composition. For example, near synapses, the ECM unique pattern is termed as peri-synaptic ECM and the ECM molecules in this area contribute to synaptogenesis and plasticity (Ferrer-Ferrer & Dityatev, 2018). In the direct proximity of the body of specific neurons, the ECM components aggregate into a highly organized assembly, PNNs, and serve as a scaffolding (Celio & Blumcke, 1994). Around majority of nodes of Ranvier, a transition state of the ECM aggregation is observed and is resembling a PNN-like structure. This specific part of ECM is termed as perinodal ECM and due to its position, this one is associated with regulating of ion-exchange during axon conduction (Oohashi et al., 2002). The perinodal ECM and PNNs may appear similar, nevertheless, there are some differences resulting in structural and chemical diversity. For example, in the perinodal ECM, the predominant chondroitin sulfate proteoglycans (CSPGs) are brevican, versican and neurocan, whereas in PNNs it is rather an aggrecan (Bekku & Oohashi, 2010).

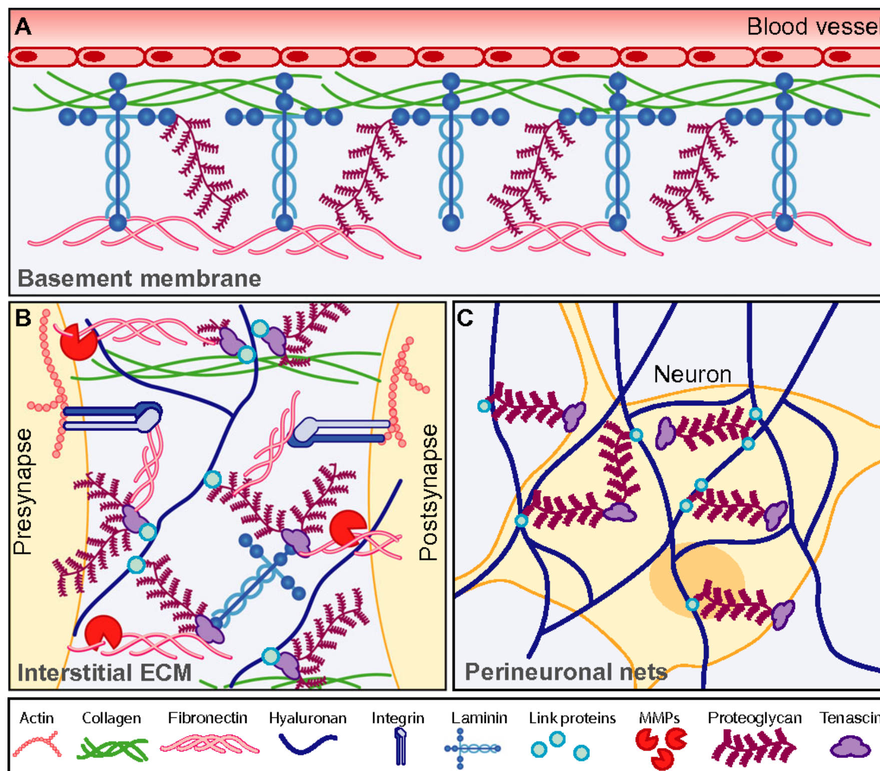

Figure 2 | Schematic diagram of types of ECM in the brain

A | ***ECM in the proximity of membrane located on the basolateral side of endothelial cells of blood vessels***

B | ***Interstitial matrix between neuronal cells of the brain***

C | ***PNNs forming lattice-like structures around neurons (Lasek, 2016)***

2.2 The ECM crosstalk

ECM is a highly dynamic structure that is undergoing constant restructuralization via engaging in the signaling crosstalk. Binding of specific factors on the cell membrane induces signaling pathways resulting in ECM remodeling, which in turn causes backwards interactions with cells. That is mediated via ECM receptors (e.g. integrins). Integrins are heterodimeric transmembrane receptors that mediate the linking of ECM to the actin cytoskeleton and serve as adhesion molecules between cells. This connection of integrins to the cytoskeleton can transmit ECM signals and trigger various intracellular signaling pathways and feedback loops (Richard O. Hynes, 2002). By this process, ECM can control cell activity (Turrigiano & Nelson, 2004).

2.3 Tetrapartite synapse

Neurons and astrocytes are known to form the tripartite synapse (Volterra & Meldolesi, 2005). However, recent evidences show there is a more advanced relationship and ECM appears to be another essential part of the synapse. The synapse communicates with ECM directly or through signaling molecules and this interaction gives a rise to tetrapartite synapse (Dityatev & Rusakov, 2011). Further examination of this concept could help to better understand the mechanism of how the brain processes and stores information.

3. PNNs

PNNs are a specialized extracellular matrix structure surrounding a specific subpopulation of neurons (R. O. Hynes & Naba, 2012). PNNs have been known for a long time and are dating back to the end of the 19th century. Camillo Golgi was the first one who noticed the extracellular coverage of neurons and provided the written description. Nevertheless, there was a long break and progress was made almost 100 years later when PNNs function was further detailed.

PNNs surround neurons from the dendrites of the soma to the endings of the axon (Celio & Blumcke, 1994). However, they are not present at the axonal boutons (Gert Brückner et al., 1993; Hockfield & McKay, 1983) and this specific absence of PNNs appears to enable neurons to make synaptic contacts (Celio & Blumcke, 1994; Zaremba et al., 1989). Regarding location, PNNs are not occurring around all types of neurons in the CNS. PNNs are preferably present with fast-spiking gamma-aminobutyric acid (GABA)-ergic PV+ interneurons (W. Härtig et al., 1992), which are cells responsible for establishing inhibitory circuits in the brain and scarcely present around other types of neurons, for example, excitatory pyramidal neurons in the hippocampus (Hausen et al., 1996; Wegner et al., 2003). The appearance of PNNs is co-occurring with the maturation of neurons they surround, implying their role in the termination of the critical periods and in the restriction of the brain plasticity (Dityatev et al., 2007; Pizzorusso, 2002).

3.1 The PNNs structure

PNNs resemble a tangle of molecules. Those are clustering and linking together and eventually creating a very stable assembly covering the surface of the neurons and serving as a scaffolding (Celio & Blumcke, 1994). PNNs are a very heterogeneous structure composed of predominantly glycosaminoglycans (GAG) (e.g. hyaluronan) (U. Rauch, 2004), link proteins, chondroitin sulfate proteoglycans (CSPGs) (e.g. lecticans) and glycoproteins such as tenascin-R (Tn-R) proteins (Carulli et al., 2010; Köppe et al., 1997; Kwok et al., 2010) (Figure 3).

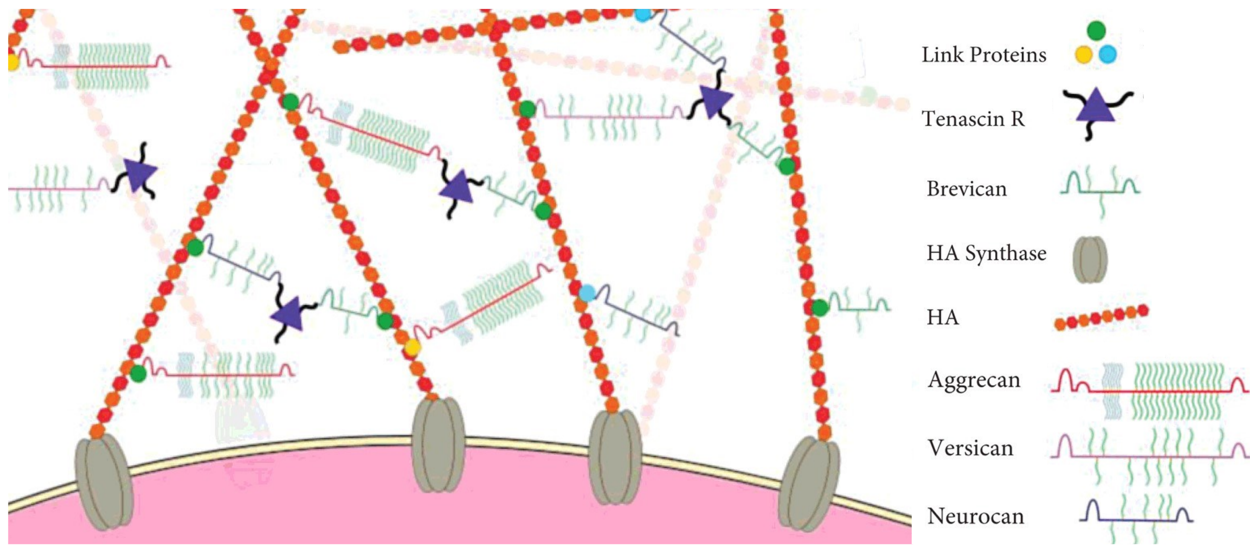

Figure 3 | Schematic PNNs structure
(Customized image, original acquired from Warren et al., 2018)

3.1.1 Hyaluronan backbone

Hyaluronan (HA) is a non-sulfated linear polymer of N-acetylglucosamine and glucuronic acid (GlcA) disaccharides present on a cell surface (Meyer et al., 1951). HA serves as a backbone of PNNs because it provides binding and interacting via link proteins with CSPGs it further branches promoting the formation of complex aggregates (Köppe et al., 1997). HA is an essential compartment of the PNNs assembly, and it fundamentally defines their final structure.

3.1.2 Hyaluronan synthase

Hyaluronan synthase (HAS) belongs to a family of glycosyltransferase enzymes creating the PNNs HA backbone. HAS exists in three isoforms HAS 1, 2 and 3 synthesizing HA polymers of different length and different speed. HAS is present on the inner surface of the cell membrane (Philipson & Schwartz, 1984) and is using free monosaccharides for the synthesis of the HA polymer. Since HA is necessary for the formation of PNNs, HAS is present on all the PNN-expressing neurons (Kwok et al., 2010).

3.1.3 CSPGs

CSPGs are composed of a core glycoprotein with GAG sugar chains. Different types of GAG are a result of sulfation modifications that are carried out on the sugars chains (S. Miyata & Kitagawa, 2015). The most prominent ones in the CNS are lecticans: non-CNS-specific aggrecan and versican and CNS-specific neurocan and brevican. Except brevican that is connected by glycosylphosphatidylinositol (GPI) anchor, all are secreted into the extracellular space (ECS) by glia, neurons or both (Hagihara et al., 1999; Hockfield & McKay, 1983; Köppe et al., 1997). Another CSPGs family molecule binding to PNNs are for example neuroglia 2 (NG2), CSPGs phosphacan, biglycan or decorin. Decorin binds transforming growth factor-β (TGF-β), hence contributing to the inhibition of the cell growth (Yu Yamaguchi et al., 1990).

3.1.3.1 Lecticans

Lecticans share some identical structural domains, for example N-terminal domain. It is a long central region containing covalently bound chondroitin sulfate (CS), that anchors them to the HA. Another mutual domain is a C-terminal globular domain that binds tenascin R (Tn-R) and some other various molecules (Aspberg et al., 1997).

CS chains are composed of repeating disaccharides of GlcA and N-Acetylgalactosamine (GalNAc). These places serve as a target for several chondroitin sulfotransferases that are responsible for sulfation-caused structural diversity of lecticans and contribute to the heterogeneity of PNNs (S. Miyata & Kitagawa, 2015; Shinji Miyata & Kitagawa, 2017). The central domain, however, differs radically in terms of size and sequence among each lecticans ranging from the longest central domain, with about 1700 amino acid residues for versican, to the shortest, with about 300 residues of brevican central domain (Y. Yamaguchi, 2000).

Nevertheless, not all the above mentioned lecticans are always present in the PNN. They are rather inconsistently distributed throughout each PNN structure in the brain. Only aggrecan was found to be present on all PNN-bearing neurons (Galtrey et al., 2008). Aggrecan is mostly expressed in a cartilage and although less frequently, in some other tissues including the brain. Nonetheless, in the CNS, aggrecan was found exclusively in PNNs, contrary to the other lecticans (Matthews et al., 2002). It was shown that without its presence, PNNs lose ability for assembly, thus, aggrecan is considered essential for the forming of normal PNNs (Rowlands et al., 2018). The other lecticans are present in various combinations and ratios or sometimes entirely lacking in the structure (Galtrey et al., 2008). Neurocan and brevican are only expressed in nervous tissue (Margolis et al., 1996; Hagihara et al., 1999). Regarding their exact location in a single PNN structure, aggrecan, brevican, and neurocan are present throughout the axonal coating. In detail, aggrecan surrounds synaptic contacts and brevican the synaptic cleft (Blosa et al., 2013), whilst versican is associated to nodes of Ranvier (Jäger et al., 2013).

3.1.3.2 Sulfation of CSPGs

The CSPGs are undergoing several modifications during the lifespan of an organism. One of the modifications is the sulfation that is realized by several sulfotransferases, for example, chondroitin 6-sulfotransferase-1 (C6ST-1) and chondroitin 4-sulfotransferase-1 (C4ST-1) (Mikami & Kitagawa, 2013). Those are producing 6-sulfation or 4-sulfation, respectively, on GalNAc residues of the repeating disaccharide units of CSPGs (Mikami & Kitagawa, 2013). Sulfation of different positions (e.g. C4S; C6S; C2,6S; and C4,6S, Figure 4) generates various patterns that are encoding functional information of CSPGs. Created binding sites are recognized by specific molecules (Gama et al., 2006).

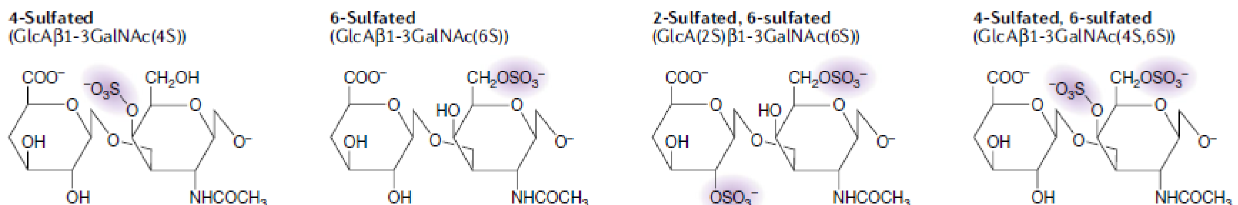

Figure 4 | Sulfation patterns of CSPGs
(Customized image, original acquired from Fawcett et al., 2019)

Since the ratio of C4S/C6S is changing throughout the different stages of life and depending on several biological processes, sulfation of CSPGs was addressed as a another regulator of the critical period plasticity (Gama et al., 2006). These CSPGs sulfation ratio shifts significantly influence the properties of PNNs. In an early postnatal stage, the predominantly sulfated position is C6S, and it allows high synaptic plasticity and neurite outgrowth (Lin et al., 2011). With the progression of development and maturation of CNS, C6S sites are progressively reduced due to C6ST-1 decreased activity while C4ST-1 activity is heightened and inducing sulfation of C4S position (Carulli et al., 2010; Mikami & Kitagawa, 2013). This C4S/C6S ratio shift is bearing restrictive properties for plasticity and promotes the end of the critical period. By adulthood, approximately 90 % of the CS-GAGs is sulfated on the C4S position (Carulli et al., 2010) and this site attracts inhibitory molecules such as Semaphorin3A (Sema3A) or Orthodenticle homeobox 2 (Otx2) (Beurdeley et al., 2012; Dick et al., 2013). The PNNs restrictive properties are therefore given also by predominant sulfation of C4S sites and their affinity for molecules of inhibitory nature.

3.1.4 Link proteins

The link proteins belonging to the hyaluronan- and proteoglycan binding link protein gene family (HAPLN) are characteristic with great binding capacity (Spicer et al., 2003). In PNNs, there are present two members of family: cartilage link protein Crtl1 (HAPLN1) and brain link protein Bral2 (HAPLN4) (Bekku et al., 2003; Carulli et al., 2007; Uwe Rauch et al., 2004). They are crucial for PNNs structural integrity and proper formation of PNNs since they mediate interaction of HA backbone with CSPGs (Bekku et al., 2012; Kwok et al., 2010).

3.1.5 Tenascins

Tenascins (Tn) are a member of oligomeric glycoproteins family, consisting of five forms : Tn-C, -R, -W, -X, and –Y (Aspberg et al., 1997, p.). Of those the ones present in the CNS are the Tn-R and Tn-C. Both Tn-R and Tn-C interact with different types of cellular receptors or components of the ECM. The complex of HA and CSPGs is connected through a trimeric Tn-R and it establishes a highly organized structure by connecting its fibronectin III repeats with the C-terminal globular G3 domain of the CSPGs core proteins (Aspberg et al., 1997; Jones & Jones, 2000).

The Tn-C is highly expressed during development of CNS and is decreasing postnatally with maturation (Garcion et al., 2004). Tn-C has both inhibitory and stimulatory effects on an axonal outgrowth in adult CNS, where is still expressed, although restricted to specific zones (Gates et al., 1995), and up-regulated during brain lesions or spinal cord injuries (Andrews et al., 2009; Dobbertin et al., 2010).

3.2 Visualization of PNNs

Several methods were developed to visualize PNNs and besides the presence, it is also possible to determine the state and composition (Figure 1). The plant lectins Vicia villosa agglutinin (VVA) and Wisteria floribunda agglutinin (WFA) are currently the most widely used markers, although they cannot visualize all PNNs. In the cortex, the VVA and WFA-positive structures ensheathe the PV+ GABAergic neurons (Kosaka et al., 1992). The lectins have an affinity for GalNAc and antibodies against CSPG core proteins, which are the compounds of PNNs. Yet, it is still unclear which domains they bind (W. Härtig et al., 1992).

Through capturing the fluorescence intensity, labelling serves as an indirect way to measure the maturity of PNNs. The more intense the staining appears, the more mature and complete PNNs are (Cabungcal et al., 2013; Carulli et al., 2013). The final image of VVA and WFA staining is similar but not identical and varies for example in the staining intensity of Ranvier nodes in some of the brain regions (Gert Brückner et al., 1993). The presence of PNNs is also possible to detect thanks to the use of monoclonal antibodies with affinity for lecticans, for example, Cat 301, that is used to detect aggrecan-positive PNNs (Matthews et al., 2002; Zaremba et al., 1989). This method is used for PNNs that are not able to bind WFA. Another way of detecting PNNs is to identify the presence of the HA backbone, and the biotinylated hyaluronan binding protein (HABP) is used for that purpose (Köppe et al., 1997).

PNNs are modified during the development, for example, due to CS, whose composition changes at different development stages (e.g. sulfation patterns and ratios). To capture these changes in the PNNs structure and map the CS distribution, various antibodies that visualize CS and the sulfation are used, for example, CS56 antibody (Shinji Miyata & Kitagawa, 2016).

3.3 Heterogeneity of PNNs within a brain

PNNs are an important element of the brain but interestingly, the areas expressing these structures are not to be found everywhere in the CNS. Moreover, the regions where PNNs are found are surrounding only a fraction of all the present neurons (Mueller et al., 2016). In Figure 5 is shown the percentage of neurons expressing PNNs in different brain areas of a macaque.

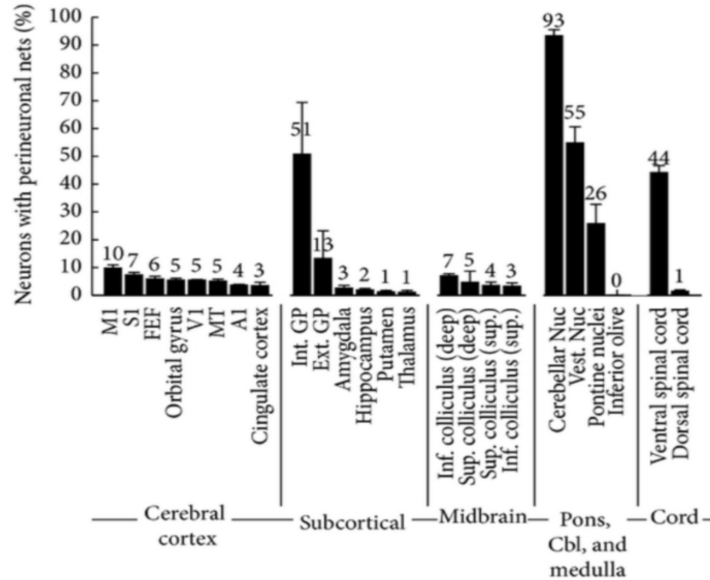

Figure 5 | Distribution of PNNs in the brain

Mean percentage of neurons surrounded by perineuronal nets in different parts of the brain (Mueller et al., 2016)

As we can see, the presence of PNNs does not differ only among the brain areas but even within them. It has been shown that PNNs expression of the specific brain areas and co-localization with specific cells differ also among species. In rats, compared to mice, the fast-spiking PV+ interneurons were shown to be mainly enveloped with PNNs in the visual cortex. In the CA2 of the hippocampus, PNNs co-localized predominantly with cells expressing CaMKII and PCP4-. (Lensjø, Christensen, et al., 2017)

This differences in expression of PNNs are additionally deepened due to CSPGs that are greatly participating in the heterogeneity of PNNs and vary in compositions in distinct CNS regions (Carulli et al., 2006; Galtrey et al., 2008). For example, versican and neurocan are found in the white matter in the adult CNS (Asher et al., 2000, 2002). Contrary aggrecan is present

throughout the whole CNS but the quantities of protein are varying during the development. Postnatally, there are low levels of aggrecan but the quantities are growing with the progress in development (Galtrey et al., 2008).

As mentioned earlier, CSPGs undergo various modifications, i.e. sulfation or glycosylation (Matthews et al., 2002; S. Miyata & Kitagawa, 2015; Shinji Miyata & Kitagawa, 2017). Those post-translation modifications equivalently contribute to the remarkable degree of biochemical heterogeneity of PNNs and for example, in the rat brain, at least seven different aggrecan glycoforms have been detected (Matthews et al., 2002).

3.4 Function of PNNs

As was already mentioned, PNNs create a scaffolding for specific neurons by enveloping their axons and somas. Therefore, this physical support serves as the force that maintains the neurons in their assigned position. Furthermore, they stabilize already existing synapses thanks to peri-synaptic localization around interneurons and inhibit the growth and synaptogenesis of matured neurons (Frischknecht et al., 2009; Hensch, 2003; Kwok et al., 2011). Fundamentally, PNNs could be considered to contribute to the maintenance of memory. Nevertheless, enveloping of the neurons and its physical support is not the only function PNNs serve for.

3.4.1 Ion buffering and antioxidant properties of PNNs

Another characteristic of the PNNs is that they are strongly negatively charged due to the presence of GAGs (Markus Morawski et al., 2015). This aspect may have a further effect on the diffusion and homeostasis of the local ions (Gert Brückner et al., 1993). Indeed, PNNs serve as an ion buffer thanks to the sulfate groups of the GAG chains and negatively charged HA and generate a highly polyanionic microenvironment around the neurons they enwrap. This buffering system can maintain the homeostatic ion balance by preventing calcium, sodium, and potassium ions from free diffusion and ultimately serve as a cation exchanger (Gert Brückner et al., 1993; Wolfgang Härtig et al., 1999). This is a crucial quality specifically for the PV+ inhibitory interneurons to sustain being fast spiking. Those neurons are engaged in microcircuits of the cortex and involved in the gamma oscillations (30-80 Hz), hence large ionic currents are occurring there and need stabilization (Freund, 2003; Ylinen et al., 1995).

Besides, PV+ cells are characterized by high metabolic demands and increased sensitivity to oxidative stress, and overproduction of superoxide causes impairments of those cells (Cabungcal et al., 2013; Hu et al., 2010). Even in this case, PNNs were found to serve as a protective shield thanks to their poly-anionic nature. For example, chelate iron present in PNNs is preventing the formation of iron-generated reactive hydrogen radicals. Additionally, hyaluronan and chondroitin serve as antioxidants by reducing hydroxyl radicals and prevent for example from the damage of DNA (Campo et al., 2004).

3.4.2 PNNs and restriction of plasticity

Although it is generally accepted that one of the properties of PNNs is restricting of the brain plasticity, the exact mechanism remains rather elusive. The function of PNNs as a physical barrier for neurons is one of the mechanisms of restriction as well as association with inhibition molecules, for example Sema3A. Sema3A is a signal molecule involved in many processes during development such as axon and dendrite growth, branching, axonal transport or apoptosis (Behar et al., 1996; Li, 2004). In some regions of the CNS this molecule stays active even in adulthood and works as a repulsive axon guidance molecule (Vo et al., 2013). It binds to the PNNs structure and then enhances the inhibition of neuronal growth (Dick et al., 2013). Sema3A is bound to the PNNs through GAGs and therefore it is possible to be removed by enzymatic treatment (Vo et al., 2013). When the Sema3A is inhibited it causes restoration of neuronal plasticity, thus, it demonstrates its control over brain plasticity as a PNNs effector (Boggio et al., 2019).

Another way how PNNs is restricting plasticity is through limiting lateral movement of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and suppressing the protein mobility. When PNNs are not present it causes a short-term synaptic plasticity and higher paired- pulse ration (Frischknecht et al., 2009) (Figure 6).

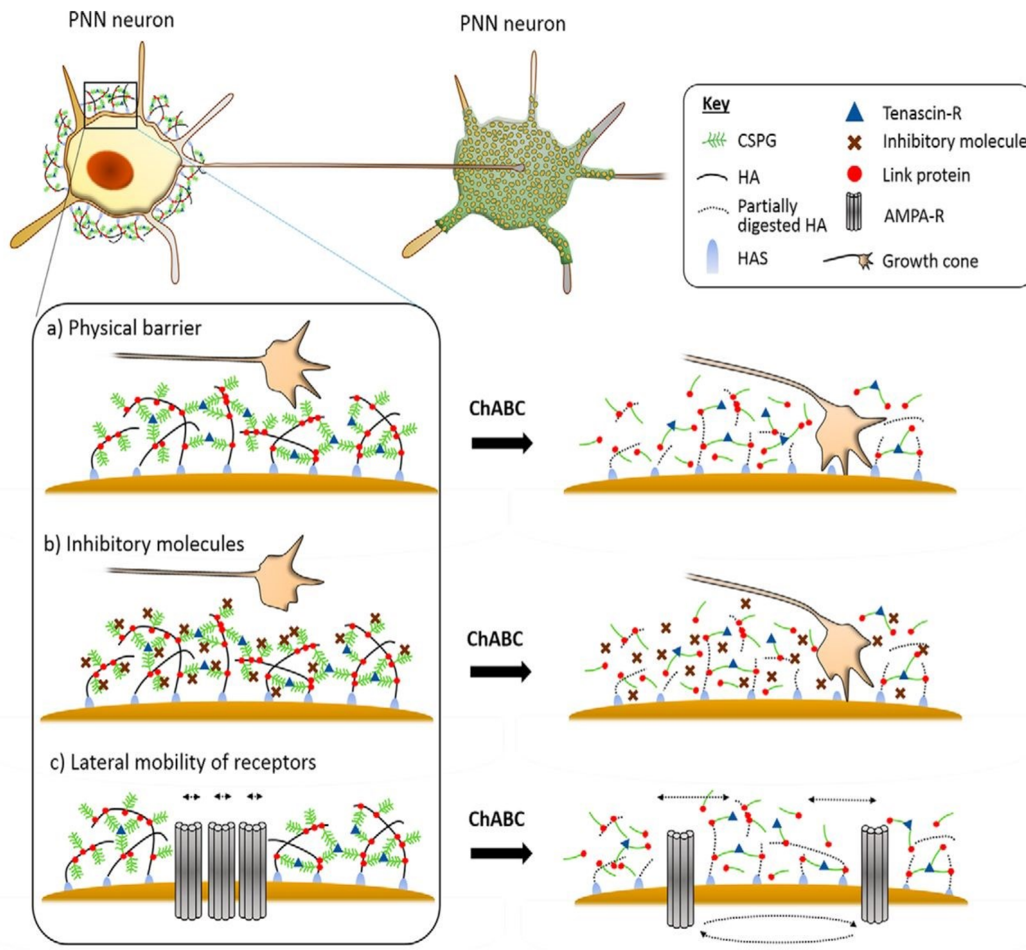

Figure 6 | Restriction of plasticity by PNNs

A | ***A physical barrier by PNNs to incoming synaptic inputs***
B | ***Binding of molecules via specific sites on CSPGs of PNNs (molecules, such as Sema3A, inhibit new synaptic inputs)***
C | ***Prevention of lateral diffusion of AMPA receptors, limiting the ability to exchange desensitized receptors in the synapse for new receptors from extrasynaptic sites. Treatment with Ch-ABC disrupts PNNs, reinstating juvenile-like states of plasticity (Sorg et al., 2016).***

3.4.3 PNNs and association with the critical period

A critical period is described as a period when the neurons are fully free to create new synapses, neuronal networks can develop and tune according to the external input and the whole system has a high plasticity (Hensch, 2003). During this time, the excitatory neurons are at their highest spontaneous activity and are responding to the input received from sensors like the first opening of an eye, smelling etc. The critical period is closing with the maturation of neurons, especially the maturation of the GABAergic fast-spiking PV+ inhibitory interneurons (Hensch, 2005). In different brain regions, the closure of the critical period is happening in different stages of organism development and it coincides with the assembly of PNNs (Carulli et al., 2010; Pizzorusso, 2002). This suggests that their formation and maturation may be dependent on neuronal activity and it has been demonstrated in different regions of the CNS, including the visual cortex, spinal cord motor neurons, or cerebellum (Dityatev et al., 2007). For such a spreading mechanism a general master switch would be ideal.

A growing body of evidence indicates that PNNs do not just occur when the PV+ inhibitory neurons reach maturity, but on the contrary, they directly promote and accelerate functional maturation of PV+ neurons through binding of homeobox protein Otx2. Otx2 plays the role of a transcription factor and is important for brain development, for example, while the formation of the sensory organs (Beurdeley et al., 2012; Lee et al., 2017). The presence of Otx2 homeobox on the PV+ interneurons is essential for termination of critical periods, assembly of PNNs and its maintenance in adulthood (Lee et al., 2017). Initially, the presence of Otx2 in this mechanism was observed in the visual cortex maturation, lately the extracellular biding of Otx2 in formation of PNNs has been observed in several other areas (Bernard & Prochiantz, 2016, p. 2) (Figure 7).

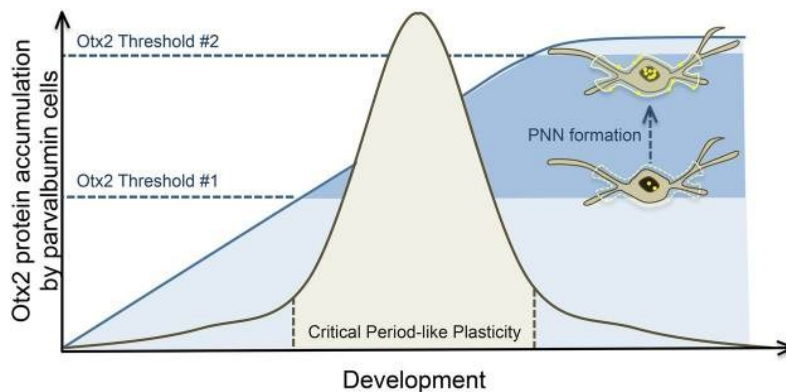

Figure 7 | Post-natal Otx2 accumulation and critical period plasticity

Sensory stimulation induces the formation of immature perineuronal nets (PNNs) around primarily PV+ neurons, which in turn increase internalization of Otx2. Critical period plasticity is initiated when Otx2 reach a certain threshold. Otx2 accumulation promotes the development and stabilization of PNNs. When Otx2 reaches second threshold, the critical period closes and PNNs mature. Another critical period-like plasticity is not initiated unless PNNs are destroyed, or accumulation of Otx2 is inhibited (Maheu & Ressler, 2017)

Once PNNs are formed around the neurons, they induce reductions of the synaptic plasticity, stabilization of neuronal excitability and firing properties (Kwok et al., 2015). Thanks to this mechanism, the brain is maintaining a stable structure establishing neural networks through experience-dependent learning and strengthening the neural connections. Nevertheless, dynamic synaptic plasticity is not fully restricted, hence learning and memory organization is ongoing throughout the whole lifespan (Max F.K. Happel & Frischknecht, 2016).

To reopen the critical period and re-establish the juvenile-like brain plasticity by abolishing PNNs is a useful way how to study synaptic plasticity and memory in the brain of young and ageing individuals as well as in models of neurodegeneration and impaired CNS.

4. Homeostatic plasticity

There is a balance that is essential to maintain in the CNS, termed as homeostatic plasticity. The stability of the system is crucial but at the same time it must remain flexible during development and learning. This maintenance is happening on the level of neurons and glia, and the key parameter for stabilization of the neural activity is a firing rate and the balance between excitation and inhibition within neuronal networks (Turrigiano & Nelson, 2004). That is assured as well by the ECM and PNNs that are constantly undergoing restructuring in response to the surrounding signals and external input. Although it could seem like an atypical event at the first glance, these changes in the structure through abolishing of the ECM components are a naturally occurring process in the brain. This process is essential for maintaining the endogenous homeostatic plasticity, for axons to grow, further branch and create new synapses among each other. This is concurrently projecting to a higher level that constitutes the plasticity of the neuronal circuits and brain in general. Without this mechanism, learning, re-learning, and rewiring of the circuits would be impossible and once the brain became mature, it would remain rigid. Therefore, many proteolytic molecules are involved in that matter and maintain the balance between the proteolytic degradation and inhibition of the restructuring process (Mott & Werb, 2004; Hensch, 2005; Knapska & Kaczmarek, 2016).

4.1 Proteolytic processing of ECM and PNNs

4.1.1 MMPs

The enzymes matrix metalloproteases (MMPs), so named for the presence of a conserved zinc-binding motif, are the main agents in the process of degradation and remodeling of the ECM (Sterchi, 2008). There is a lot of different MMPs and each can cleave a specific component of ECM. In general, all MMPs are able to digest every element of the ECM, including PNNs (Mott & Werb, 2004). Those proteolytic enzymes are synthesized as inactive pro-enzymes, secreted in ECS and activated by cleavage of the pro-peptide.

4.1.2 ADAMTs

Aggrecanases, the metalloproteases that have been shown to cleave aggrecans, are classified as ADAMTs (a disintegrin and metalloproteinase with thrombospondin motifs), and specified as ADAMTS-4/aggrecanase-1 and ADAMTS-5/aggrecanase-2 (Stanton et al., 2005; Tortorella, 1999). Those two enzymes are also proteolytically cleaving brevican since it shares some sequence similarities with the aggrecan core protein cleavage site (Nakada et al., 2005; Yamada et al., 1995). ADAMTS are expressed everywhere in the CNS and since their substrate of activity are lecticans, the important element of the PNN structure, they are undeniably contributing to the brain plasticity as well.

4.1.3 ADAMs

ADAMs (a disintegrin and metalloproteases) are another subgroup of the zinc protease superfamily. It is a minor group serving two main biological activities, proteolysis and adhesion. The substrates for ADAMs are mostly membrane-binding molecules such as receptors or integrins connected to PNNs, in contrast to MMPs, which are mostly involved in the degradation ECM proteins (Mullooly et al., 2016).

4.1.4 TIMPs

The inhibition of proteolysis is maintained via several MMPs activity inhibitors such as α2-macroglobulin and tissue inhibitors of matrix metalloproteases (TIMPs), which prevent from uncontrollable ECM degradation that could result in dysregulated cell growth or disease development (Overall & López-Otín, 2002).

In the hippocampus, the MMP-9 was observed to colocalize with pre- and postsynaptic markers (e.g. AMPARs and NMDARs) and jointly with its inhibitor TIMP-1, creating a peri-synaptic enzymatic system standing behind dynamic remodeling of the ECM. This process enhances the synaptic plasticity by remodeling the peri-synaptic environment, influencing the shape of dendrites and their function, thus, possibly playing a key role in regulating learning and memory (Knapska & Kaczmarek, 2016).

4.1.5 chABC

The ECM proteolytic enzymes serve as a way how to modify PNNs. In the same way works the application of an exogenous bacterial enzyme chABC, yet more robustly. The chABC is a broadly used enzyme that is cleaving the PNNs structure through recognizing and digesting CSPGs into disaccharides and to some extent HA as well (Kwok et al., 2008; Saito et al., 1968). That results into a collapse of the structure and loosening of the near environment. Although this enzyme is less specific, the degradation happens to be more localized and moreover, reversible (Pizzorusso, 2002).

The span of this artificially triggered PNN renewal might although differ from the juvenile brain. This duration was observed by the Norwegian team (Lensjø, Lepperød, et al., 2017) that established the rate at which PNNs regenerate after enzymatic degradation (Fig.8). They executed the brain dissection in several different times after the injection of chABC. The extent of regenerated PNNs was estimated by staining with WFA. On the third day after injection, there was almost no signal detected but after 14 days the intensity in WFA reached 30% compared to the control animal and almost 90% of intensity after 60 days of the treatment (Lensjø, Lepperød, et al., 2017).

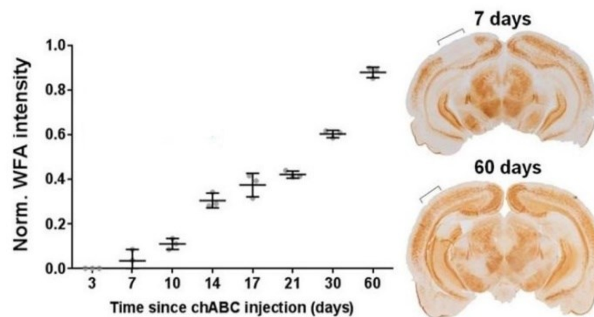

Figure 8 | Regeneration of PNNs
(Customized image, original acquired from Lensjø, Lepperød, et al., 2017)

The enzyme chABC is targeting not just the CSPGs in PNNs but also in the whole ECM. Therefore, there are other remarkable results of application of chABC that has been observed, for example, after spinal cord injury. The chABC treatment can promote functional recovery, axon regeneration and sprouting in the spinal cord (Fawcett & Asher, 1999; Bradbury et al., 2002). This is due to developing of the glial scar after spinal cord injury that is containing extracellular matrix molecules including CSPGs, the substrates for the chABC (Fawcett & Asher, 1999).

4.1.6 Hyaluronidase

Hyaluronidase is another enzyme cleaving the PNNs structure. Hyaluronidase specifically targets the HA chains and decomposes the HA backbone of PNNs, similarly resulting in a disruption of the whole structure like after application of chABC and in an increase of synaptic plasticity (Frischknecht et al., 2009; M. F. K. Happel et al., 2014). Nevertheless, usage of those enzymes affects a large area of the brain, which is not appropriate in some cases, for example when just a slight modification is required (van ’t Spijker & Kwok, 2017).

5. PNNs and plasticity

The phenomenon of the CNS plasticity has been a tremendously growing area of study within neuroscience in the past years. To a certain degree, the brain undergoes constant modifications throughout its whole lifespan. Every input or experience, either positive or negative, plays an important role in shaping the neuronal network of the brain, especially within its critical period. The brain is not a structure that once matured remains rigid but undergoes constant change. Nevertheless, certain restriction of plasticity is occurring and the relationship between PNNs, critical period and decrease in plasticity was well demonstrated on various models, for example on the classical model of the visual cortex or hearing cortex (Pizzorusso, 2002; Carulli et al., 2010).

Regarding the visual cortex, different outcomes were observed depending on open or closed critical period. One eye of an animal was closed (monocular deprivation) and the visual cortex did not receive normal visual input. When the visual cortex was still in the critical period, the ocular dominance shift was induced, and the open eye increased its responsiveness. When the same experiment was done after the end of the critical period, the change in neuronal connectivity was not observed (Pizzorusso, 2002).

As it implies, the removal of PNNs is the most illustrative way how to observe their function and importance. In many experiments, the neuronal network was reset to an immature state of high capability of plasticity and it demonstrated that it is possible to reopen the critical period and shift the adult brain to the state that corresponds to the traits of the juvenile brain (Lensjø, Lepperød, et al., 2017; Pizzorusso, 2002). One of the ways to reopen the critical period is the previously discussed enzymatic degradation of PNNs, for example, application of the bacterial enzyme chABC or hyaluronidases(Fawcett & Asher, 1999; Frischknecht et al., 2009).

Apart from the enzymatic treatment, there is another way how to reduce or prevent PNNs formation and maintain high neuronal plasticity. For that purpose, several animal knockouts and transgenic mice models were designed. Such as relatively new model of aggrecan gene deletion that caused restoration of juvenile plasticity in ocular dominance (Rowlands et al., 2018) or models lacking the link proteins Crtl1 and Bral2 , crucial components for the PNNs structural integrity (Bekku et al., 2012; Kwok et al., 2010). When those proteins are absent, PNNs fail to form properly and neuronal plasticity sustains. Besides the increase of the neuronal plasticity, the modulation of PNNs is associated also with altered EPSP (excitatory postsynaptic potential) and IPSP (inhibitory

postsynaptic potential) of the neurons they envelop. For instance, in the hippocampus, the highest density of PNNs is in the CA2 (Lensjø, Christensen, et al., 2017). Here, PNNs are present on the excitatory neurons and suppress plasticity of excitatory synapses. Upon removal of PNNs with ChABC, synaptic potentiation and the amplitude of the excitatory postsynaptic current (EPSC) increases (Carstens et al., 2016). That implies changes in synaptic strength and learning process as a consequence of PNNs modifications throughout the brain (Bukalo et al., 2001; Kim et al., 2007).

6. PNNs and memory

Learning and memory are on the cellular level established by long-term potentiation (LTP) and long-term depression (LTD) (Kandel et al., 2014) and there are many brain areas that are involved in the process of learning and memory formation, such as cortex, amygdala or hippocampus that is especially important in this process (LeDoux, 2000; Romberg et al., 2013; Shi et al., 2019). Those brain areas are also known to express PNNs and therefore, manipulating PNNs of those structures serve as a great tool for elucidating the function of PNNs in relation to the memory.

6.1 Hippocampus memory- related models

The hippocampus is generally associated with spatial memory. The hippocampal place cells have been demonstrated to play a role in representation of the spatial environment and lesions in this brain area can result in an impairment of the performance for example in a Morris water maze (MWM) (Broadbent et al., 2004; Moser et al., 2008).

A recent study focused on the role of PNNs in the hippocampus and examined the effect of their removal in relation to contextual fear memory. When conducting contextual fear conditioning before chABC treatment, they observed an increase of PV+ interneurons surrounded by PNNs in the hippocampus and protection of memories during consolidation and reconsolidation process. On the contrary after the chABC injection in the hippocampus, an impaired LTP and consolidation were observed after a recording of field excitatory postsynaptic potentials (fEPSPs) of CA1 pyramidal neurons evoked by stimulation of the Schaffer collateral pathway.

Their finding suggests that PNNs enhance theta oscillations that are regulated by PV+ interneurons expressing PNNs, contribute to consolidation and reconsolidation and protect recent and remote memories (Bukalo et al., 2001; Shi et al., 2019). That indicates a close relationship of PNNs with synaptic strength, neurotransmission, and learning.

6.2 Amygdala memory-related models

Amygdala serves as a storage of the fear memories and acquisition happens in the lateral or basal nucleus through AMPA and N-methyl-D-aspartate (NMDA) receptor-dependent LTP (LeDoux, 2000). The function of PNNs in the amygdala is associated with the plasticity of fear

memory and possible regulation of fear extinction. In the adult animals, fear conditioning paired with painful stimulus creates a permanent memory that can be with subsequent extinction training temporarily weakened yet is protected from complete erasure. On the contrary, before the closure of the critical period, fear memories in juvenile animals were completely erased by extinction training. Therefore, chABC was administrated to the adult animals to test if PNNs prevent unlearning of fear memories. Indeed, subsequent extinction training of chABC injected adult animals resulted in the acute and permanent loss of conditioned fear behavior (Gogolla et al., 2009). One of the proposed mechanisms of how exactly PNNs protect fear memories stored in the amygdala is that they make stimuli-potentiated synapses resilient to depotentiation, in other words they are protecting the synaptic strength to return to pre-LTP level, that is the mechanism necessary for memory extinction (Kim et al., 2007).

The observation of PNNs protecting fear memories was further expanded by a hypothesis of PNNs being the storage of very long-term memories. It proposes that the pattern position and sizes of holes in the PNN, created by enzymes (e.g. MMP-9), conserve the long-term memory and the strength of synapses. This mechanism could explain why some memories acquired in early childhood last the whole life despite the metabolic turnover of macromolecules associated with memory storage (Tsien, 2013).

6.3 Perirhinal cortex memory- related models

Another type of memory being studied in association with PNNs and plasticity is explicit memory, a memory when information can be consciously recalled. This type of memory is examined by an object recognition test (ORT).

When animals are exposed to familiar and novel objects, they tend to spend more time exploring the novel one. After PNNs are degraded via the application of chABC, animals maintain prolonged memory for familiar objects compared to control animals. For this kind of memory, synaptic plasticity in the perirhinal cortex is required and the degradation of PNNs that are known as a plasticity restrictors induced its enhancement (Romberg et al., 2013).

7. PNNs and neurodegenerative diseases

Homeostatic plasticity is essential for normal function of the brain; however, this balance is disrupted as a result of several CNS diseases and a decrease of PNNs due to neurodegeneration can be observed. Additionally, a reduction of PNNs densities and elevation of proteolytic enzymes of ECM molecules (MMPs) are also linked to diseases that are not necessarily connected with neurodegeneration, such as epilepsy, schizophrenia, and other psychiatric diseases (Marín, 2012; Pantazopoulos et al., 2015; Yamamori et al., 2013).

Since PNNs regulate the firing of PV+ interneurons, dysfunction of those cells can result in such disorders. Yet, it is not clear whether alternation of PNNs structure is caused by malfunction of PV+ interneurons or vice versa. Nevertheless, it appears that altered PNNs may not only be a consequence of neuronal dysfunctions but can also contribute to an onset of schizophrenia and bipolar disorder phenotypes (Testa et al., 2019).

PNNs in neurodegenerative disease have been mostly studied in association with AD that is characterized with a formation of neurofibrillary tangles composed of protein Tau, and extracellular β-amyloid plaques (Serrano-Pozo et al., 2011). In cortical areas of the AD brain, neurons expressing PNNs shown a significant reduction of CSPGs molecules due to neurofibrillary changes (G. Brückner et al., 1999) and in general, in comparison to normal brain, the AD brain samples are suffering from a decrease in densities of WFA-labeled PNNs (Baig et al., 2005). Overall, various studies suggest that ECM components and specifically PNNs are protecting neurons against Tau protein–caused lesions (G. Brückner et al., 1999; M. Morawski et al., 2010).

8. Tau protein

The tubulin-associated protein that was firstly identified in 1975 is known as the Tau protein (Weingarten et al., 1975). Tau is a microtubule-associated protein expressed in neurons where its primary role is to stabilize the cytoskeleton (Goedert et al., 1989; Müller et al., 1997).

Tau is in association with tubulin and its function has been proven to be essential for microtubule assembly by activating polymerization of tubulin followed with maintaining the structure of microtubules (Weingarten et al., 1975). Furthermore, the interaction of Tau with microtubules is a dynamic process that is crucial for structural remodeling of the neuronal cytoskeleton. It further influences neuronal plasticity which is based on the formation of synapses and growth of axons and spines, therefore Tau is directly involved in the formation of memory (Ahmed et al., 2014; Kimura et al., 2014).

8.1 Tau hyperphosphorylation

Due to alternative splicing, Tau exist in the brain in 6 different isoforms (ranging from 352 to 441 amino acids) that are possible to be distinguished by their specific binding domains (Johnson & Jenkins, 1999). Those specific repeat domains called MBDs (microtubule-binding domains) encoded by exons 9- 12 are enabling Tau to bind to microtubules (Fulga et al., 2007). Each of the MBDs contains a specific conserved motif in each repetitive domain where it can be phosphorylated on serine, threonine and tyrosine (Drewes et al., 1995). Tau phosphorylation is regulated by several kinases and phosphatases, for example, proline-directed kinases glycogen synthase kinase-3 (GSK-3) (Lovestone et al., 1994), and 5′ adenosine monophosphate-activated protein kinase (AMPK) (Thornton et al., 2011). Phosphatases that are in charge of Tau dephosphorylate include protein phosphatase-1, -2A, and -5 (PP1, PP2A, and PP5) (Liu et al., 2005).

It is believed that a disorder in the balance between kinases and phosphatases activities results in Tau hyperphosphorylation seen in the disease. The cause of Tau hyperphosphorylation was shown to be induced by direct phosphatase inhibition rather than by kinase activation (Planel, 2004). The exponential decrease in phosphatase activity was generated by hypothermia, thus low temperatures lead to the direct and rapid hyperphosphorylation of Tau protein that can be detected within a few minutes (Planel, 2004). Besides, an increase in body temperature in AD patients was

observed and is hypothesized to serve as a protective body response during AD pathogenesis to low-temperature-induced Tau hyperphosphorylation (Carrettiero et al., 2015; Harper et al., 2005).

The increased phosphorylation of Tau causes a decline in its affinity for microtubules. As a consequence, the neuronal cytoskeleton becomes destabilized (Dickey et al., 2007; Drewes et al., 1995). The cytoskeletal destabilization caused by dysfunctional Tau-protein results further in significant deterioration of functions. The vesicle and organelle transport, axonal growth, propagation of nervous signal along the network established by microtubules, all of these functions become impaired as a result of Tau hyperphosphorylation (Gendron & Petrucelli, 2009; Morfini et al., 2009). The Tau protein is involved in the myriad of interaction with various molecules and is found in different localizations that is suggesting additional roles beyond its basic function as microtubule regulating protein.

Under normal conditions, Tau protein is found predominantly in axons of matured neurons, in the nucleus, neuron membranes, and extracellularly. A little fraction of tau is localized as well in dendrites and synapses, where it regulates NMDA receptor phosphorylation and its interactions with post-synaptic density protein (PSD-95) (Ittner et al., 2010). Whilst under disease conditions, when Tau protein is hyperphosphorylated, a special event of liberation of Tau from microtubules and diffusion to spines was hypothesized to occur and bear further consequences for the synapses.

The hyperphosphorylated Tau in dendritic spines causes global disruption of anchoring of NMDA receptors, their uncoupling with PSD-95, reductions of AMPA receptor subtypes, and subsequently impairments in basal synaptic transmission and LTP (Hoover et al., 2010; Tackenberg & Brandt, 2009). Thus, Tau hyperphosphorylation, redistribution and translocation into the dendritic spines appear to cause synaptic function impairments and culminates into a synapse loss (Yoshiyama et al., 2007). Additionally, when Tau becomes abnormally hyperphosphorylated, it can further result in self-assembly and formation of NFT (neurofibrillary tangle) (Fig.9). NFT, also referred to as PHF (paired helical filaments), are found in Alzheimer's disease (AD) brains and patients suffering from some other tauopathies (Alonso et al., 2001).

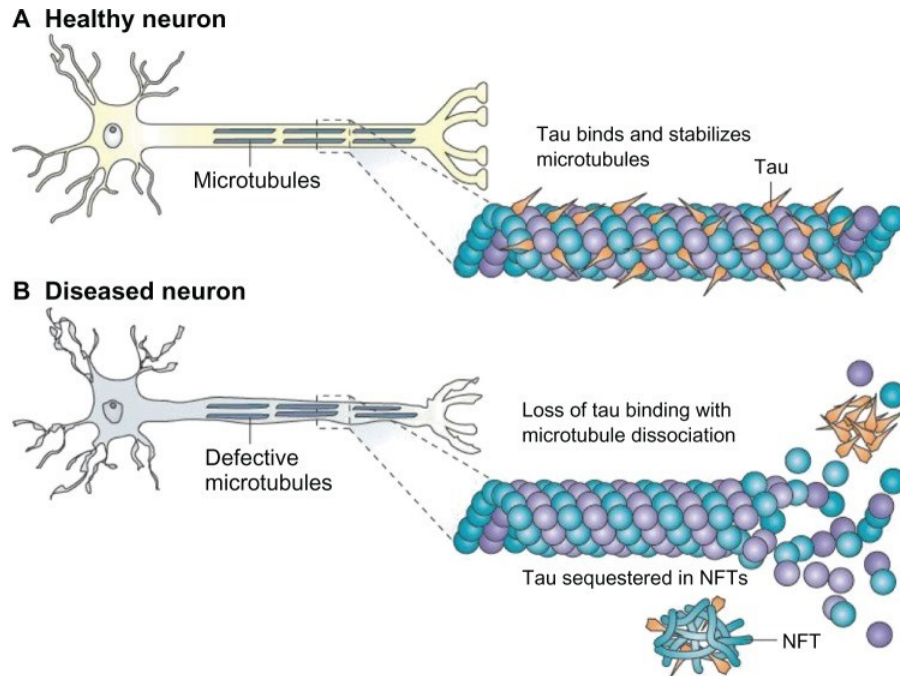

Figure 9 | Tau in healthy and diseased neuron

A | Tau facilitates the microtubule assembly and its stabilization
B | Microtubule destabilization and formation of NFT induced by tau hyperphosphorylation (Chen et al., 2013).

The exact mechanism of creation of tangles is not fully understood yet and some brain regions are affected by accumulation of hyperphosphorylated Tau earlier than the other. For example hippocampus is affected as one of the earliest sites (Braak et al., 1993). Moreover, it is not clear whether the tangles are predisposition to AD development, because the diagnosis depends upon their detection, or are a coexistent phenomenon. Besides, there has been no proof the NFT could not be some sort of expression of cellular adaptation to protect the rest of endangered neurons.

Together, the function of Tau protein is given by the level of phosphorylation (Iqbal et al., 2010; Köpke et al., 1993; Lindwall & Cole, 1984) and in general, the hyperphosphorylation of Tau protein diminishes or impairs its biological activity (Iqbal et al., 2010; Lindwall & Cole, 1984). To demonstrate the pathological effect of highly phosphorylated Tau, basal synaptic transmission was recorded on a mice model of tauopathy. As expected, the maximum of fEPSP was reduced, and synaptic conduction was impaired. That is believed to be caused by the synaptic loss, reduced plasticity and decrease in the number of firing fibers (Yoshiyama et al., 2007). This Tau-related

process of neurodegeneration is subsequently projecting also into behavior and a transgenic mice model of tauopathy with Tau hyperphosphorylation and reduction in LTP exhibit deterioration of spatial learning and memory (Mustroph et al., 2012).

8.2 Cold-induced Tau hyperphosphorylation

The Tau hyperphosphorylation temporarily occurs even in non-pathological cases like during development or hypothermia (Arendt et al., 2003; Lindwall & Cole, 1984; Planel, 2004). The only difference from pathological state is that the phosphorylation never reaches the degree that is occurring for example in AD brain.

For example, in hibernating animals during the torpor phase, Tau is hyperphosphorylated at several NFT-like epitopes. However, it does not result in neurodegeneration-associated fibril formation. Under these special conditions, Tau hyperphosphorylation seems to be tolerated and after arousal is fully reversible. Also, highly phosphorylated tau in CA3 neurons of the hippocampus correlated with an evident loss of synapses on terminals of mossy fibers but the changes in intrahippocampal connectivity were reversible. In a short period of time after re-warming of the organism, the synapses were restored. Moreover, the animals did not suffer from any impairments after this phase although reduction in synapses is associated with neurodegeneration (Magarinos et al., 2006; Popov & Bocharova, 1992; Selkoe, 2002). These observations point out to Tau protein and its level of phosphorylation to be involved as another factor influencing synaptic plasticity and not to be necessarily associated with pathological implications (Arendt et al., 2003; Magarinos et al., 2006; Popov & Bocharova, 1992). Once the research elucidates what is the potential threshold between Tau-related neuroplasticity and neuropathology observed during brain ageing, it could contribute to a discovery of successful treatment and prevention of AD and other Tauopathies.

Interestingly, a similar effect on brain plasticity and the capacity for synapse regeneration induced by low temperature was observed on non-hibernating laboratory rodents that underwent short artificial cooling in laboratory conditions (Peretti et al., 2015). The molecules observed during artificial cooling were cold-shock proteins (CSP). CSP belong to a group of DNA/RNA-binding proteins and their expression is induced as a response to a rapid decrease in temperature. During hibernation, global protein synthesis and cell metabolism are downregulated. On the

contrary, the expression of RNA- binding protein RBM3 is enhanced due to low temperature (Danno et al., 1997).

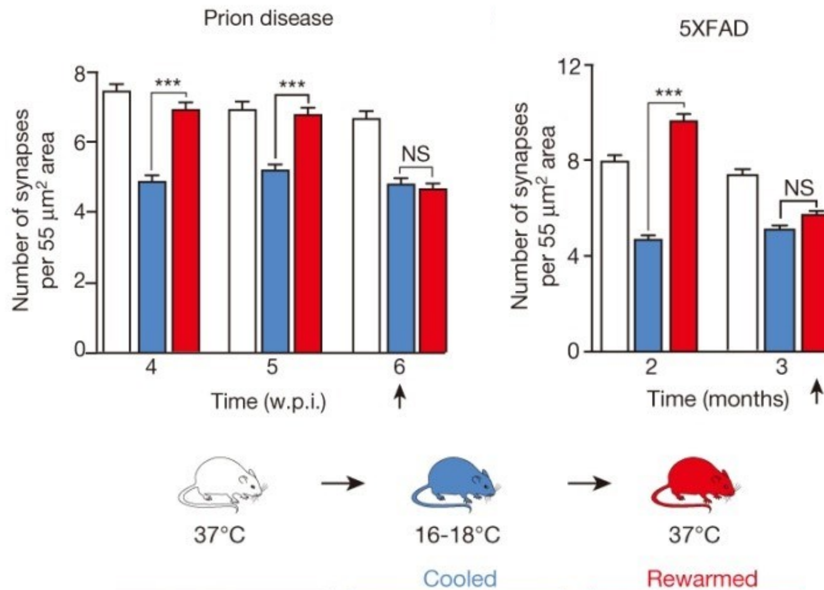

Figure 10 | The capacity for synaptic regeneration in Prion and 5XFAD disease

The capacity for synaptic regeneration after CIP in mice with Prion and 5XFAD disease is decreasing in time with the progression of the disease and with advanced neurodegeneration (Customized image, the original acquired from Peretti et al., 2015)

CSP are known to serve as neuroprotectants and particularly RBM3 has been associated with neuroprotection in various models of cooling (Chip et al., 2011). RBM3 induction of expression was found parallel with the ability for structural plasticity and an increase of endogenous levels of RBM3 via cooling helped to restore failed synaptic plasticity (Peretti et al., 2015). Moreover, in AD mice model, application of early cooling significantly prolonged their survival. On the other hand, application of cooling later in the disease progress, when the ability of RBM3 expression is lost due to progress in neurodegeneration, there was observed no positive effect on life-span or plasticity (Peretti et al., 2015) (Fig.10).

Indeed, there seems to be an involvement of CSP in restoring of the synaptic transmission and cell survival and it could serve as a possible therapeutic target for neurodegenerative disorders. Nevertheless, there is a missing link between CSP and tau-phosphorylation that needs to be determined, since Tau-hyperphosphorylation serves as a hallmark of the AD and, as was previously shown in other studies, is responsible for synapse retraction. For example, the

relationship between CSP and Tau-phosphorylating kinases could further extend previous work in this field. Nevertheless, an unpublished research suggests that in the early stages of neurodegenerative disease such as AD or Prion disease the cold induced hyperphosphorylated Tau is reversible whereas in the later stages is long term or even persistent.

At this moment, no treatment could modify the progress of neurodegeneration and memory loss in tauopathies or reverse the Tau pathology. The neuronal replacement is a therapy with very unpredictable outcome, however synaptic loss could be with proper treatment a reversible process. With respect to the reduced synaptic plasticity in AD and tauopathies, the possible contributors in modulation of disease progression and impact on processes of learning and memory is the restoration of juvenile plasticity and support of affected neuronal circuits. The relationship of Tau related diseases and PNNs in respect to synaptic plasticity has started to be closely studied. Lately, the mice model of tauopathy was injected with the chABC, suggesting that abolishing of PNNs might serve as an indirect way how to slow down a memory deterioration. The results of ORT shown a temporary improvement in memory even in the presence of advanced tau pathology (Yang et al., 2015).

The chABC does not cross the brain-blood barrier and must be injected directly in the brain, therefore it is not possible to use it in therapy of AD patients. However, these findings offer new perspectives on how to look at tauopathy and its possible interconnectedness with PNNs components that appear to have protective effects against Tau NFT and other hallmarks of AD. A clinically relevant application of PNNs modifying compound is being studied.

9. Aims of the thesis

The cold-induced synaptic plasticity as published in Peretti et al.,2015 is simulating a hibernation-like state with significant retraction of synapses observed in the CA1 area of the hippocampus and leads to hyperphosphorylation of Tau, which under conditions of the late stage of 5xFAD or Prion disease leads to long term hyperphosphorylated Tau and significantly lower recovery of synapse withdrawal. The effect of restoration of juvenile plasticity in various brain region have been observed after digestion of PNNs by Chondroitinase ABC application, generating a time window of increased rewiring. Under these conditions a significant impact on memory and learning has been observed.

In this thesis, I have focused on the impact of integrity of perineuronal nets under the condition of Cold-induced synaptic plasticity in the model of progressive tauopathy, in mice.

We aimed to define the impact of the combination of reinstated juvenile plasticity and Cold-induced synaptic plasticity on long-term memory of already trained Morris water maze task.

We focussed on the capability of animals with progressive tauopathy to re-learn the MWM task, and the impact of individual plasticity models and their combination on it.

To confirm the impact of the hyperphosphorylated Tau and digested PNNs on synapse recovery in the Cold-induced plasticity, we performed an analysis of excitatory and inhibitory synapses on PV+ inhibitory interneurons and general level of synaptic proteins in the hippocampal tissue.

One of the hallmarks of tauopathy is an accumulation of NFT of hyper-phosphorylated Tau protein. According to latest research, the composition of ECM and PNNs may play role in Tau integration and hyper-phosphorylation. We aimed to assess the Tau protein levels, general phosphorylation and specific AD related AT8 antibody positivity, and the impact of the treatment on it.

10. Material and methods

10.1 Experimental design scheme

The experimental animals were the mouse tauopathy model expressing the human P301S mutant tau protein (Yang et al., 2015). Mice of both genders were tested at the age of 4 months when the state of the disease was in an early symptomatic stage and animals did not manifest any impairments in locomotion. Nevertheless, the Tau hyperphosphorylation is already present and the onset of the disease starts around that age. Animals were randomly assorted into 2 cohorts. Experimental animals from one cohort were stereotactically injected with chABC and further sorted into two groups, one that underwent CIP procedure and one that did not. The other cohort of control animals was stereotactically injected with saline that has no effect on synaptic plasticity and served as a control of the effect of the surgery alone. The control animals were also divided into two groups, one that underwent CIP and one that did not and served as a control to observe the effect of cooling on tauopathy progression alone.

To assess the changes in the learning ability and memory of the mice model of tauopathy, the MWM test was used. First round of the behavioural test was performed the first week (training week) of the experiment, 1 week before the enzyme injection, to evaluate the ability of animals to learn and orientate in the maze. The second week, the stereotactical injection of chABC and Saline was done. There was a one-week break for the animals to recover and for the enzyme to fully digest PNNs before the next phase of the experiment. On the third week, half of the animals of each cohort underwent the CIP procedure of AMP-induced hypometabolism. Animals were left for the rest of the week to recover. On the fourth week, the second round of the behavioural test (experimental week) was performed to evaluate the effect of enzymatic therapy and effect of the CIP on the learning process and disease progression. At the beginning of the fifth week, the Y maze test was done to evaluate explorative behaviour and activity. At the end of the experiment, animals were sacrificed, and the brain tissue was used for immunohistochemistry (IHC) and quantitative protein analysis (Fig.11). The total number of animals in each group: chABC n =9, chABC+C n =11, Saline n =10, Saline+C n =10.

Animals had unlimited access to food and water and were maintained on a 12 h light/dark cycle. All behavioural tests were conducted during the light phase of the cycle. All the performed procedures were approved by the ethical committee of the Institute of Experimental medicine, Czech Academy of Sciences.

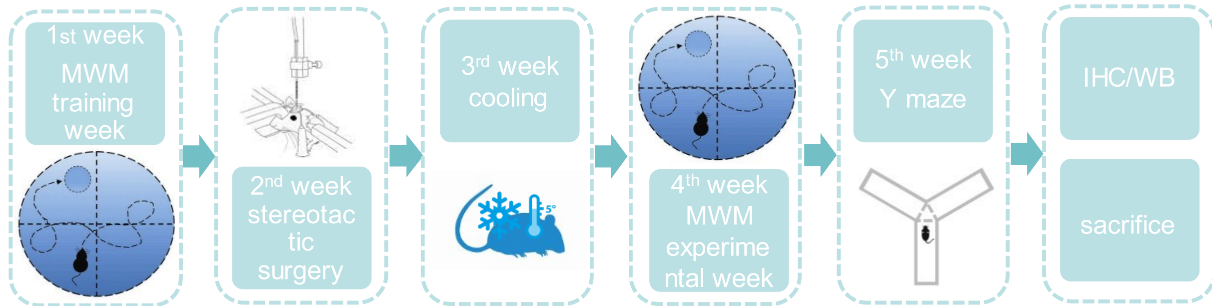

Figure 11 | Scheme of the experiment

10.2 Behavioral testing methods

10.2.1 Morris Water Maze

For the testing of an effect of cold-induced plasticity and the temporary removal of the PNN on memory, the MWM was chosen since it nicely demonstrates the spatial memory that is dependent on the hippocampus. Hippocampus is excessively developed in rodents (mice), pointing to the crucial importance of orientation in the environment for their survival. MWM is performed in an open circular pool that is filled approximately half-way with water. The animal is supposed to find a small platform hidden 1 to 2 cm below the water surface.

The Morris Water Maze was performed daily for two weeks, from day 1 to day 5 and from day 22 to day 26 of the experiment. At the start and end of each two MWM weeks the probe test (trial with removed target platform), of duration of 1 minute, was done. for the purpose of the probe test trial, the water maze arena is imaginary separated into target region and 4 different quadrants SQ, SE, NW, NE, surrounded with border zone. Probe test was done to set the baseline of parameters such as number of crossings of the target platform area (area C), time spent in the quadrants, or latency to reach the target. These parameters were then followed at the important

phase points of the study. After the probe test, the animals were left for 30 min to rest. When performing the MWM test itself, animals were released into the water at one of the 4direction - (N, S, W, E), at the water level and facing the tank. For the test recording a software VideoMot2 was used. The trajectory tracking program with time was started the moment the animal was released. The tracking stopped automatically once the animal reached the target area or after the passage of an interval of 1 minute.

10.2.2 Y maze test

To determine the condition of explorative behaviour of rodents in new environment, the Y Maze test was used. This test is based on a working memory and a natural behaviour of rodents to prefer to visit a new arm that was not investigated to the one visited previously. Among the parts of the brain engaged in this task are, for example, ventral hippocampus, septum, and prefrontal cortex. The test is performed in a Y-shaped maze with 3 arms at 120° angle. The animal is introduced to the central part of the maze and is free to explore the arms. The behaviour was recorded by the software VideoMot2 and the tracking of the exploration lasted for 5 minutes. One entry is counted all four limbs are within one arm. The system is based on counting of errors and was as following: 1 error point for an entry to a new arm, 2 error points for entry to previously visited arm and 3 error points for direct return to the same arm. These values of errors were summed up and divided by the number of entries to obtain an average error value (AEV) and the number of total entries was used as an index of activity.

10.3 Stereotactic injection

The chABC was used to digest PNNs in the hippocampus. The PNN digesting enzyme (Seikagaku Kogyo) was dissolved to 50 U/ml in 0.1% BSA. Mice were anaesthetized by isoflurane (3% induction, 1,5% maintenance) mixed with oxygen enriched air solution (0,2% flow rate), fixed to the stereotaxic frame via ear bars, and put on a heated mat to keep the body temperature of animals on constant level, as the body temperature declines during surgery due to anaesthesia. Ophtalmoseptonex was applied on the surface of the eyes as prevention for successive impairment and the parietal part of the head was shaved. The local painkiller (Rometar, xylasin, 30ul) was injected subcutaneously. The surface of the skin was cleaned by ethanol beforehand the 1cm longitudinal incision was done to expose the skull. To facilitate the injection of the enzyme, the

skull was drilled stereotactically using the motorized Drill and injection robot Stereo Drive (Neurostar GmbH) at eight sites in order to possibly access the largest area of the hippocampus. Afterwards, the agents were administered by stereotactic injection into every drilled site (0,5 ul each at 0,2 ul/min rate) using 10 µl Hamilton syringe with a 33 gauge needle. Before slow removal of the injection, the needle was left in situ for another 1 minute to prevent leakage of the enzyme and ensure its proper working in all sites. The skin incision was sewn up. To facilitate better healing and prevent infection, the wound was treated by the Novikov solution. The mice were left for recovery and a sufficient efficiency of the enzyme for 7 days after the surgery before conducting the next steps of the experiment. The control group of animals underwent the same procedure with the application of saline instead of chABC.

10.4 Cold-induced synaptic plasticity

To induce synapse retraction and changes in neuronal connectivity, a procedure of AMP (adenosine monophosphate) -induced metabolism was conducted. We applied the method of cold-induced synaptic plasticity (CIP) as was earlier conducted by Peretti et al., 2015, but on a different mice model. The experimental animals were selected with a weight >20g so a good tolerance to the drug was ensured and afterwards intra-peritoneally injected with a weight-based calculated treatment volume of 5´AMP solution. The final solution (5ml) was prepared from 5´AMP (0.5g) and $NaHCO_3$ (0.3g) and dissolved in sterile injection water (Braun). After the injection (20ml/g of solution), the mice were placed in a cooling cage in RT. The mice were monitored for 45 minutes and a decrease of the core body temperature was controlled by rectal thermometer until the temperature reached 25 °C. At this stage, the animals were almost at torpid state without active tremor thermogenesis. To induce full torpid state with significant withdrawal of synapses, mice were put into a 5°C pre-cooled glassed-door fridge with no further physical contact. Overall, all mice were cooled to 16–18 °C of the core body temperature for 45min interval. When the cooling finished, the animals were left in RT to slowly rewarm until they reached at least 25°C of core body temperature. The breathing frequency and potential spasms were carefully monitored. The animals were controlled after finished procedure for the next 24h until they have reached normal temperature (at least around 37°C).

10.5 Electrophoresis and Western blot analysis

The animals were sacrificed day 29 of the experiment and the dissection of hippocampus and cortex was quickly performed on a cooling tray, using cooled RIPA lysis buffer to maintain the sample at ideal condition. The number of animals was as following: Saline n=4 , chABC n=3, Saline+C n=4, chABC+C n=5. The proteins were isolated using modified RIPA lysis buffer: (137 mM NaC.l, 20 mM Tris (pH 7.8), 1mM MgCl2, 2.7 mM KCl, 1% Triton X-100, 1mM EDTA, 10% (w/v) glycerol, 1mM dithiothreitol, 0,1%NaDeoxycholate), which contains phosphatase inhibitor cocktail (Millipore) and protease inhibitor cocktail (Thermo Scientific). For determination of the total protein concentration of hippocampal brain homogenates, the bicinchoninic acid (BCA) assay (Pierce) was used.

To conduct western blot analysis, hippocampal protein samples were separated in 25µg aliquots using 4-15% gradient Mini-PROTEAN TGX Gels (Bio-Rad, cat. no. 456-1083), 50 mA per gel. The proteins were transferred on PVDF membranes (Life Technologies) at 350 mA for 60 minutes. The transfer was followed by blocking non-specific background of the membranes by 1-h incubation in 5% non-fat dry milk (9999S, Cell Signalling Technology) diluted in TBS-T, 50ml per membrane. After the blocking was finished, the membranes were washed with TBS-T three times for 5 minutes. Incubation with primary antibodies was conducted overnight at 4 °C, 20ml/membrane. The primary antibodies that were used included GAD65/67 antibody, SNAP 25 antibody, PSD95 antibody, VGLUT1 antibody, VGAT antibody, Gephyrin antibody and the beta-actin antibody (Table 1). Primary antibodies were diluted in the washing solution TBS-T. After the incubation with primary antibodies was finished, the membranes were recurrently washed three times for 5 minutes with TBS-T and another incubation for 1h at RT with rabbit or mouse secondary antibodies (1:15000 dilution in TBS-T) followed. Subsequently, the membranes were washed three times with TBS-T for the last time before the final visualisation. For the visualization of the protein bands, the ClarityTM Western ECL Substrate (170-5061, Rio-Rad) was used.

Afterwards, the detection of the chemiluminescence and visualization of the protein bands on the membrane was performed on Western blot imaging system Azur Biosystems c600. The relative signal intensity of the proteins was quantified with ImageJ software. Greyscale images were used and normalized to the background staining intensity and to the positive control protein

β-Actin staining intensity. The data were further normalized to a saline-injected group or to WT to isolate the effect of different substances and procedures.

Table 1 | List of antibodies used for Western blot analysis

Antibody	Type	Band size	Concentration	Incubating	Manufacturer	Catalog number
Anti-beta Actin Hrp conjugated	Mouse monoclonal	22	1:30000	Overnight	Abcam	ab49900
Anti-GAD65+ GAD67	Rabbit polyclonal	65/67	1:3000	Overnight	Abcam	ab11070
Anti-PSD95	Rabbit polyclonal	80- 85	1:1000	Overnight	Abcam	ab18258
Anti-SNAP25	Rabbit polyclonal	23- 26	1:1000	Overnight	Abcam	ab18258
Anti-VGAT	Rabbit polyclonal	53- 57	1:1000	Overnight	Synaptic Systems	131 002
Anti-VGLUT1	Mouse monoclonal	67	1:1000	Overnight	Synaptic Systems	135 302
Anti-Gephyrin	Mouse monoclonal	93	1:1000	Overnight	Synaptic Systems	147 011
Anti- AT8	Mouse monoclonal	50	1:1000	Overnight	Thermo Fisher	AB_223647
Anti-Tau5	Mouse monoclonal	50	1:1000	Overnight	Thermo Fisher	AB-2536235

10.6 Immunohistochemistry

To determine the synaptic withdrawal induced by low temperatures in the CA1 of hippocampus, the IHC staining was performed. The animals were divided into two cohorts and each cohort was composed of three different groups. First cohort was injected with chABC, one group was left as non-cooled control, two other groups were later subjected to CIP. First was euthanized acutely after the CIP procedure, second the next day after the animals rewarmed. The same procedure was applied to the second cohort injected with saline. Mice were anesthetized with intraperitoneal injection of ketamine (Narketan 10%, 50mg/kg) mixed with xylazine (Rometar 2%, 6mg/kg) and transcardially perfused with 0.1 M PBS and 4% paraformaldehyde (PFA) for fixation. Brains of the animals were placed in PFA and post fixed overnight. After washout with PBS the brains were saturated with 20% followed by 30% sacharose. Brains were frozen and cut with Leica CM1850 cryostat (Leica Microsystems GmbH, Vienna, Austria) into coronal slices 20µm thick.

Frozen sections were IHC stained for PV neurons using primary antibodies for VGAT, Gephyrin and Bassoon. The frozen brain sections were IHC stained following this protocol of 5 minutes washing in PBS (pH= 7,4) three times, for 10 minutes in 1% H2O2 that was repeated three times and finally three times in PBS for another 10 minutes. After the washing, the slices were placed in 0,05% Tween in PBS for 10 minutes and then a blocking buffer (3% goat serum) was added onto the sections and incubated for 2 hours. The blocking buffer was drained off and replaced with primary antibody, incubating the sections overnight at 4°C.

The staining with primary antibodies was followed by staining with secondary antibodies, with Streptavidin conjugated with Alexa Fluor 488 (1:400, S32354, Thermo Fisher) for 2h and with DAPI (1:1000, D9564, Sigma Aldrich) for 10 min. On a confocal microscope (Zeiss880 airyscan) 4 Z- stack (40x 1.5 zoom, 3channel imaging- 488nm (Green), 594nm (Red), 405nm (Blue), 1 line step, frame size 2112x2112,speed 0,82um/s) images per mice were taken and afterwards two-dimensional image analyses of signals was done.

The number of signals per each image was determined and the changes in the number and activity of neurons were assessed. For statistical analyses, the mean of on average 8 slices in each group was done. Three different IHC markers were used: VGAT, Gephyrin and Bassoon. Bassoon and VGAT served as a presynaptic excitatory and inhibitory marker, respectively. To determine the postsynaptic compartment on parvalbumin inhibitory interneurons the Gephyrin marker was used.

The same procedure was applied for slices stained to visualise PNNs, the used primary antibodies for this purposed were WSA (1:400, L1516, Sigma Aldrich).

10.7 Statistical analysis

One-Way ANOVA was used to compare the group means of the relative protein concentrations of the quantitative protein analysis and the group means of the synapse withdrawal. Behavioural test was analysed using Two-way ANOVA RM. The Student–Newman–Keuls (SNK), Tukey or Dunn´s test was used as a post hoc multiple comparison test, when recommended. Calculations were performed using GraphPad Prism 8.2.0 software. Results in the figures are displayed as arithmetic means ± standard error of mean. A probability level of $p < 0.05$ was considered statistically significant (marked by *, $p < 0.001$ marked by ***).

11. Results

11.1 Morris water maze

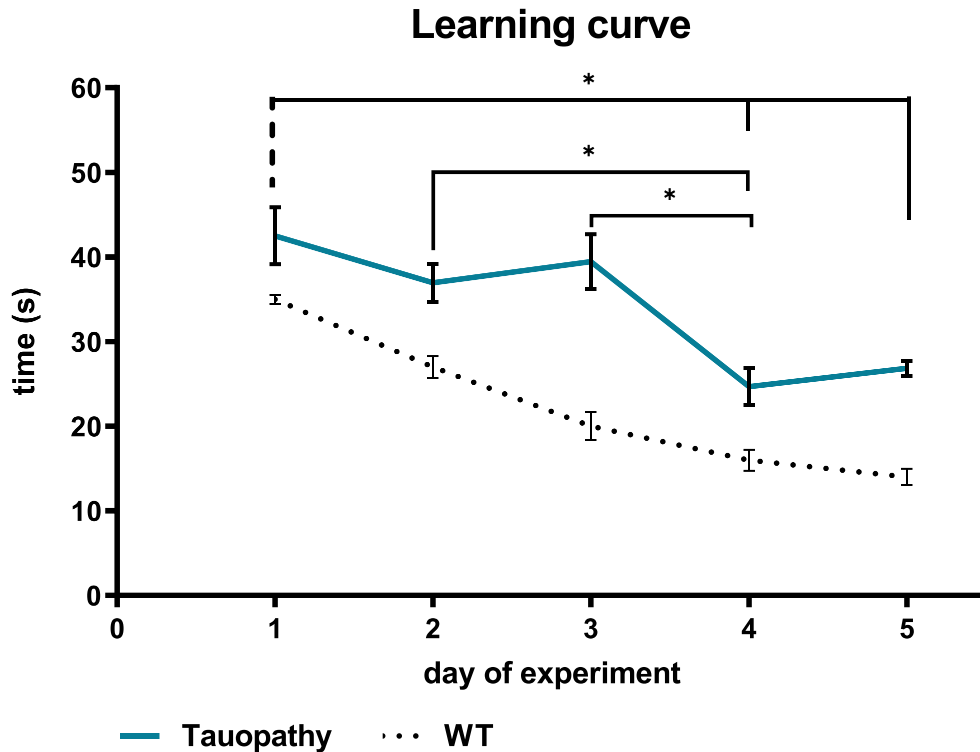

Figure 12 | The learning curve of naive mice with tauopathy

The first week of the experiment, we determined the learning ability in tauopathy without any therapy (Figure 12). Animals were able to successfully learn and remember the hidden platform. There was a statistically significant difference between training days. However, the learning process was already impaired compared to WT mice – dashed line inserted for illustration (data used from unpublished data) (Two Way RM ANOVA, *post hoc* SNK test, day 1 vs day 4; $q = 5.681$; $p<0.05$, day 1 vs day 5; $q = 5.234$; $p<0.05$, day 2 vs day 4; $q= 4.425$; $p<0.05$, and day 3 vs day 4, $q= 4.559$; $p<0.05$).

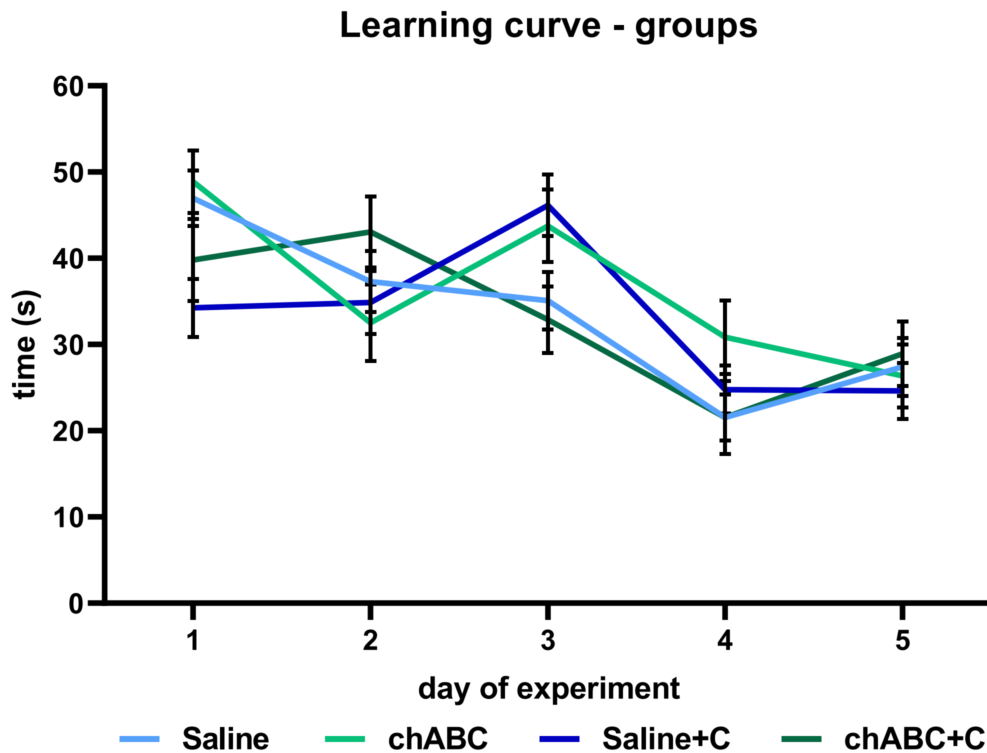

Figure 13 | Learning curve of naive mice with tauopathy – groups

Naive animals separated to groups for illustration of their initial learning ability.

Although we can observe some difference in the performance of individual groups, the differences were not statistically significant, and the groups are considered as same.

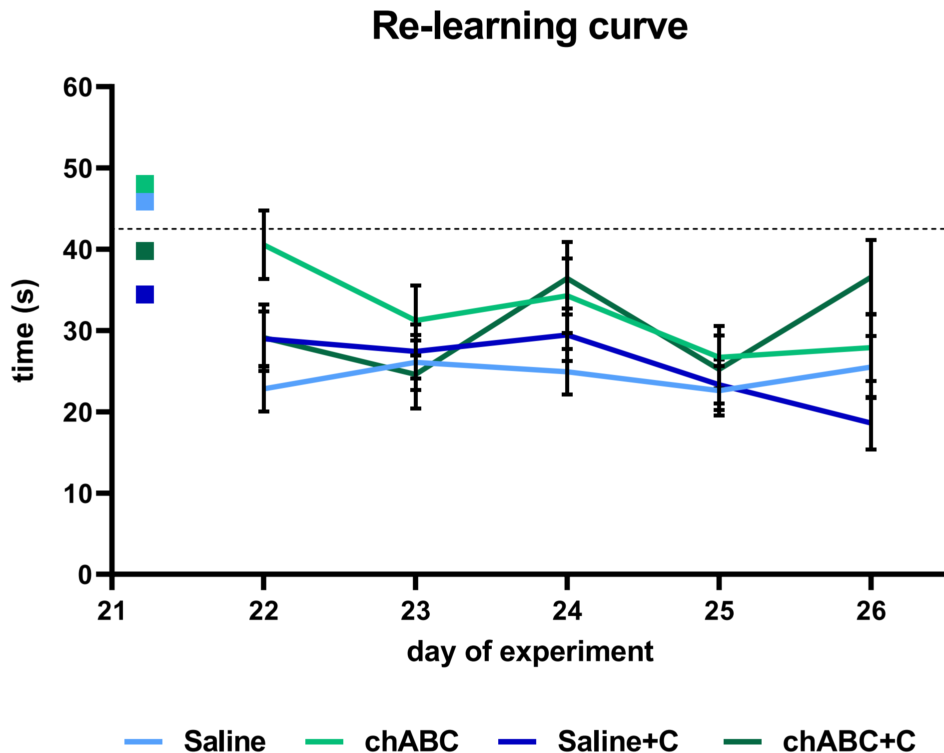

Figure 14 | The re-learning curve after therapy with chABC and CIP

In the experimental week after the surgery (Figure 14), saline failed to attain any further improvement in learning and the latency to reach the target remained constant over the week. On the other hand, animals treated with chABC performed relatively better at the end of the training week compared to the first day and despite the considerable deterioration of the memory retention after the therapy compared to other groups (Figure 15).

The remaining two groups captured the effect of the cooling itself (Saline+C) and in combination with enzymatic therapy (chABC+C) on the performance. In this case, the animals undergoing CIP performed better and their ability to learn improved compared to the training week. Although the difference in time to reach the target was not statistically significant. To the contrast, cooled animals injected with chABC were not able to learn and the latency to reach the target was prolonging despite the training. The dashed line illustrates the time to reach the target of a tauopathy naive mice, the dots at the beginning of the graph signify the starting time of reaching the target of each group in of the training week. None of the differences in the experimental training were statistically significant.

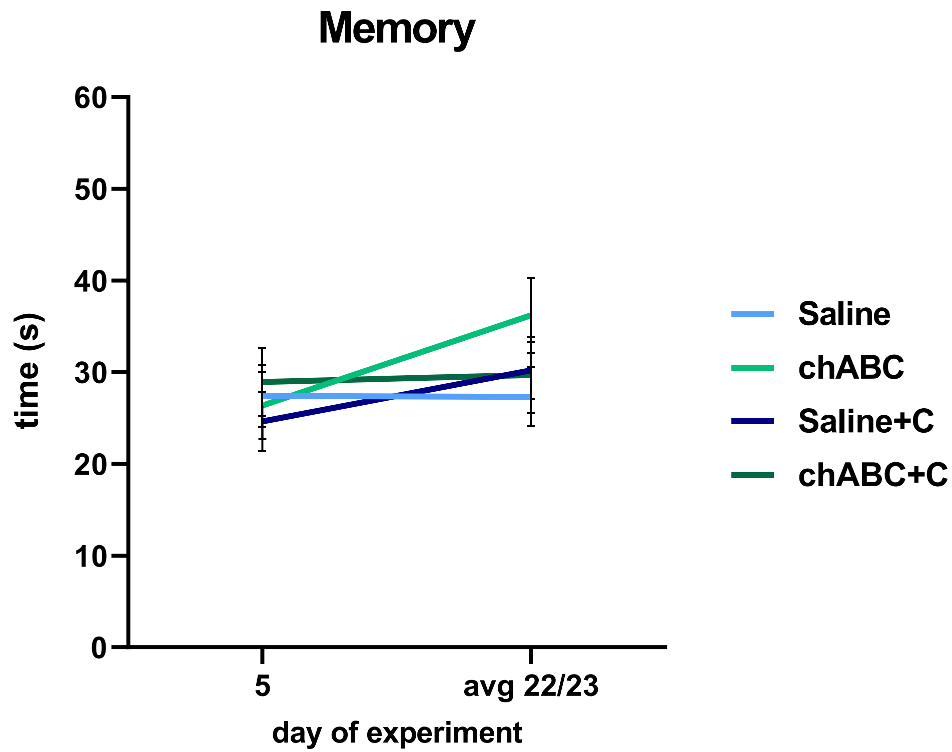

Figure 15 | The memory retention

In Figure 15, the retention of memory between the training and the experimental week is captured for each of the experimental groups. The chABC and Saline+C groups exhibit a partial memory loss after the time-lapse break of 2 weeks compared to other groups. None of the changes were statistically significant.

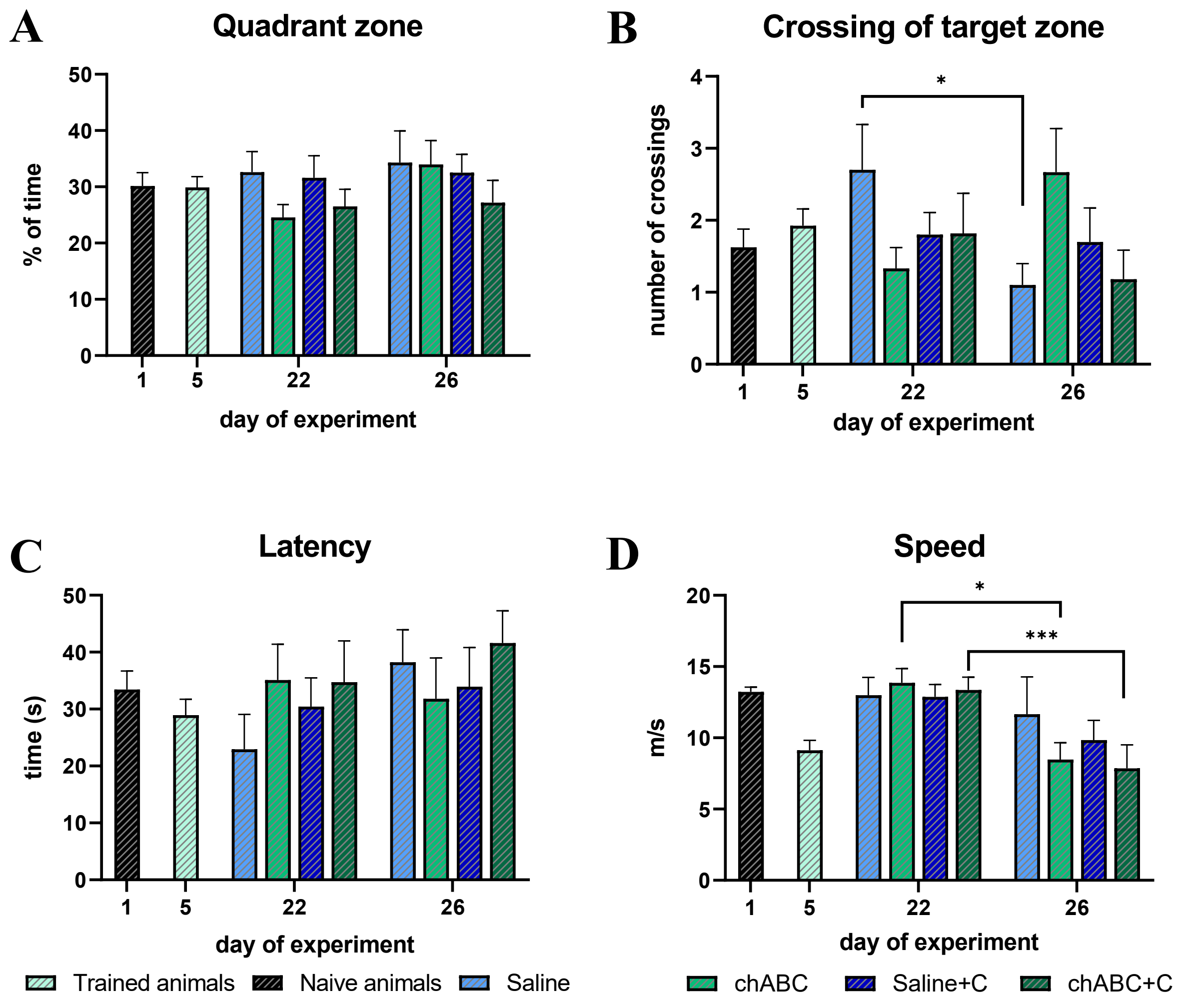

Figure 16 | Probe tests

In Figure 16, we can see the results of probe tests. The parameters of interest were the amount of time spent at the quadrant zone of the target platform, number of crossings of the target zone, latency to reach the target zone and speed of swimming. All groups tend to spend more time in the target quadrant zone (Figure 16A) with continuous training. In Figure 16B, which is the most accurate indicator of the spatial memory, the chABC mice show an increase in the number of crossings at the end of the experimental week compared to the training week before therapy. On the contrary, other groups exhibit a deterioration in performance despite training and Saline group shows a statistically significant decrease in the number of crossings. Latency to reach the target zone (Figure 16C) correlates with swimming speed (Figure 16D), which significantly decreased at 26 day of the experiment compared to the day 22, chABC and chABC+C groups show

a statistically significant decrease in speed (Crossings; Two Way RM ANOVA, *post hoc* SNK test Saline q= 2.909; p<0.05, Speed; Two Way RM ANOVA , *post hoc* SNK test chABC q=4.617; p<0.05, chABC+ C q=5.209; p<0.001).

11.2 Y maze test

The results of the second behavioural test were used to determine the condition of explorative behaviour in new environment among the groups that could, thus, also influence the performance in the MWM test. When the animals are very active and the AEV is closer to 1, it signifies no impairments. When the activity is high but the AEV is higher, animals are suffering from an impairment in memory and are easily distracted. Low activity and high AEV signifies an impairment of CNS, memory, changes in locomotion, and apathy towards the task.

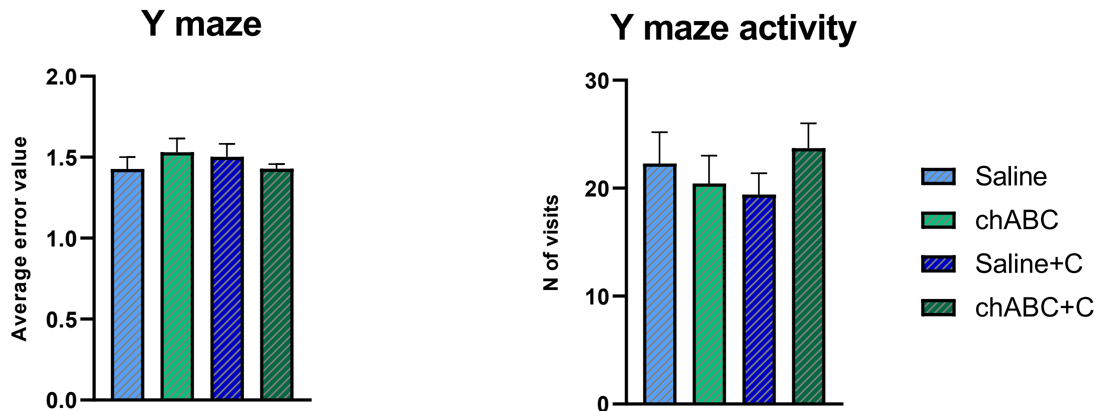

Figure 17 | Y maze test

In Figure 17, the data shows no statistically significant difference regarding either the AEV or the activity among groups that could also influence their performance in the MWM test. However, since the Y maze test was performed at the end of the experiment, the day 29, we can observe a trend of elevated AEV. It signifies a slight impairment in cognitive functions due to progress in tauopathy and its effect on activity and memory.

11.3 WFA visualization of PNNs

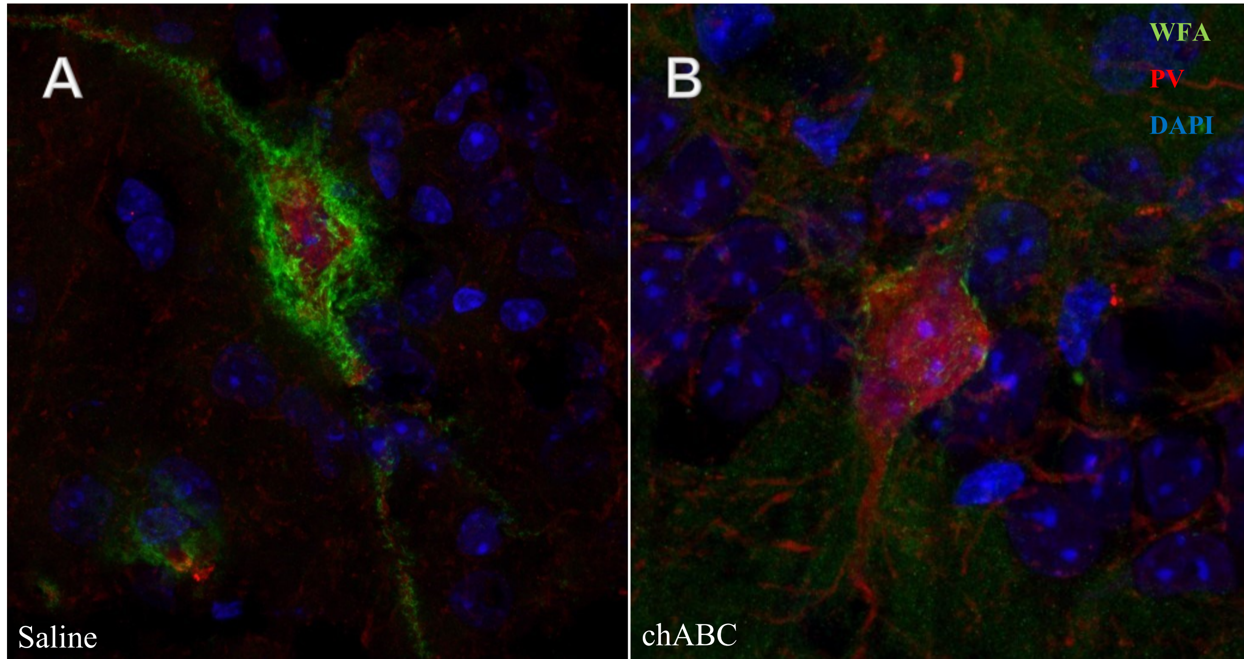

Figure 18 | IHC staining for visualization of PNNs on PV+ neurons in the CA1

A | ***PNNs ensheathing PV+ neuron of the saline injected mice***

B | ***PNNs residues on PV+ neuron after injection of chABC in the hippocampus***

The IHC staining images were acquired to visualize PNNs on the PV+ neurons in the CA1 area of the hippocampus to confirm the enzymatic digestion of the PNN structure after the application of chABC and compare with the saline-injected mouse. WFA stains PNNs along the soma and proximal dendrites and, in Figure 18A, we can see a dense coverage of PV neuron by PNNs. In Figure 18B, one week after the application of chABC, the PV+ neurons exhibit a disintegrated PNNs and significantly decreased WFA intensity, with some of the residues as a mark of ongoing PNN component production and secretion.

11.4 Synapses withdrawal

In this analysis, the same procedure of CIP was applied as used in the behavioural part of the study. In Figure 19, the CIP of different markers is visualised together with comparison of the groups of mice with and without PNNs.

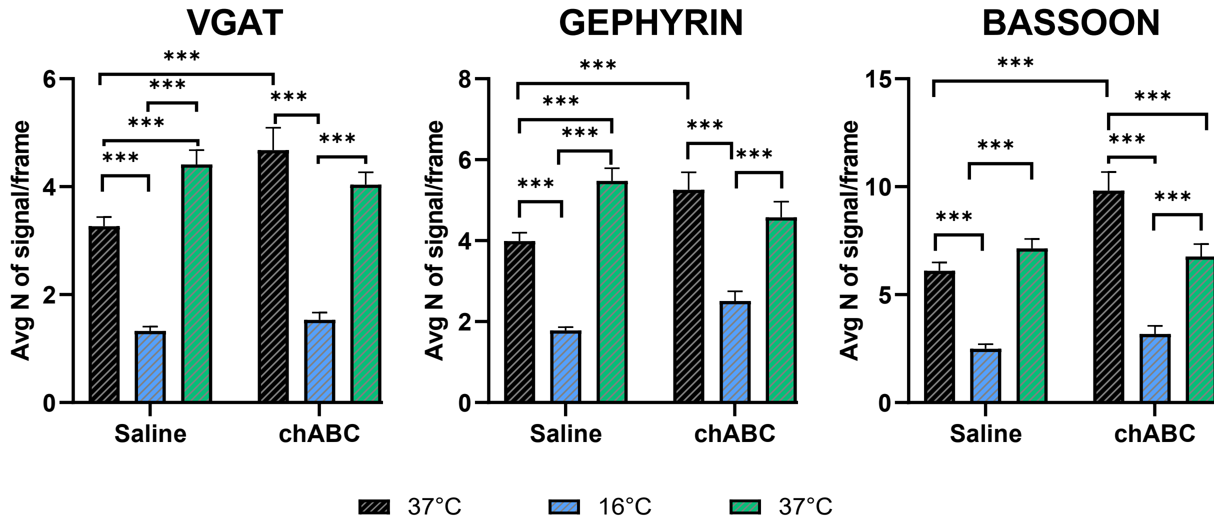

Figure 19 | Changes of the number of signals induced by low temperatures

In the **Figure 19**, we can observe a rapid loss of number of synaptic markers as a result of the exposition to low temperature (16°C) and subsequent regeneration of the signal when returned to the normal body temperature (37°C) in both chABC and Saline group. For VGAT, Gephyrin and Bassoon there was a statistically highly significant difference between initial 37°C and 16 °C and 16 °C and 37 °C rewarmed. Additionally, there was a statistically highly significant decrease in the number of synaptic markers between initial 37 °C and 37 °C rewarmed for chABC in Bassoon and on the contrary increase for Saline in VGAT and Gephyrin. Regarding differences between groups, there was a statistically highly significant difference in the number of signals between chABC injected group and Saline group at the initial 37°C for VGAT and Bassoon and statistically significant difference for Gephyrin (VGAT; Two Way ANOVA; *post hoc* SNK test, Saline – initial 37°C vs 16°C $q= 12.482$; $p< 0.001$, rewarmed 37°C vs 16°C $q= 6.824$; $p< 0.001$, Initial 37°C vs rewarmed 37°C $q=4.910$; $p< 0.001$, chABC - initial 37°C vs 16°C $q= 11.723$; $p< 0.001$, rewarmed 37°C vs 16°C $q= 9.342$; $p< 0.001$, chABC vs saline Initial 37°C $q=4.910$ $p< 0.001$. Gephyrin; Two Way ANOVA; *post hoc* SNK test, Saline - initial 37°C vs 16°C $q= 12.599$; $p< 0.001$, rewarmed 37°C vs 16°C $q= 7,524$; $p< 0.001$,

Initial 37°C vs rewarmed 37°C q=4.814; p= 0.001, chABC - initial 37°C vs 16°C q= 9.248; p< 0.001, rewarmed 37°C vs 16°C q= 6.958; p< 0.001, chABC vs saline Initial 37°C q=4.179 p< 0.01. Bassoon; Two Way ANOVA F= 9.047; p< 0.01, *post hoc* SNK test, chABC vs Saline q= 4.254; p< 0.01, Saline - initial 37°C vs 16°C q= 8.456; p< 0.001, rewarmed 37°C vs 16°C q= 6.878; p< 0.001, chABC - initial 37°C vs 16°C q= 12.214; p< 0.001, rewarmed 37°C vs 16°C q= 6.515; p< 0.001, initial 37°C vs rewarmed 37°C q= 5.957; p< 0.001. chABC vs saline Initial 37°C q=6.987; p< 0.001).

11.5 Western blot

11.5.1 Pre-synaptic markers

VGLUT (vesicular glutamate transporter) is mediating the transport of glutamate into the synaptic vesicles in the presynaptic terminals of the excitatory neurons. Similarly, VGAT (vesicular GABA transporter) is expressed in synaptic vesicles and loads the vesicles with GABA and glycine from the cytoplasm of the GABAergic neurons. The expression of the VGAT and VGLUT is typically associated with change of neuronal activity and synaptic plasticity since it directly regulates the release of the neurotransmitters.

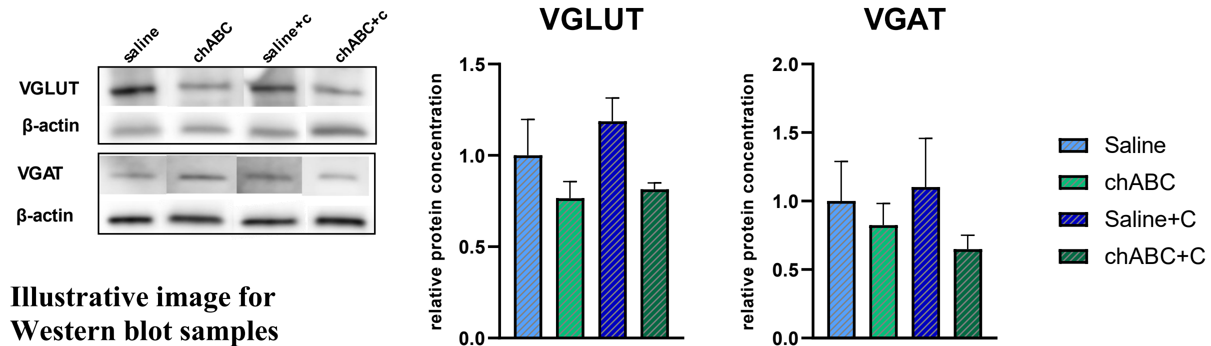

Figure 20 | Quantitative protein analysis of VGLUT and VGAT
Levels of presynaptic protein markers VGLUT and VGAT in hippocampus

The results display an increase in the VGLUT for the Saline+C group as a result of CIP. Nevertheless, the application of chABC shows a suppressive effect on excitatory neurons as well

in the chABC+C group. The levels of VGAT decreased in groups injected with chABC. Yet statistically, those changes are not significant.

11.5.2 Post-synaptic markers

The postsynaptic density protein 95 (PSD-95) level was analysed in order to quantify the postsynaptic density of the excitatory neurons that is regulated by changes in neuronal excitability and the decrease in PSD-95 serves as a marker to a loss of synapses. Gephyrin serves as a scaffold in the inhibitory synapses where it brings and stabilizes the inhibitory synaptic receptors and is associated with synaptic plasticity.

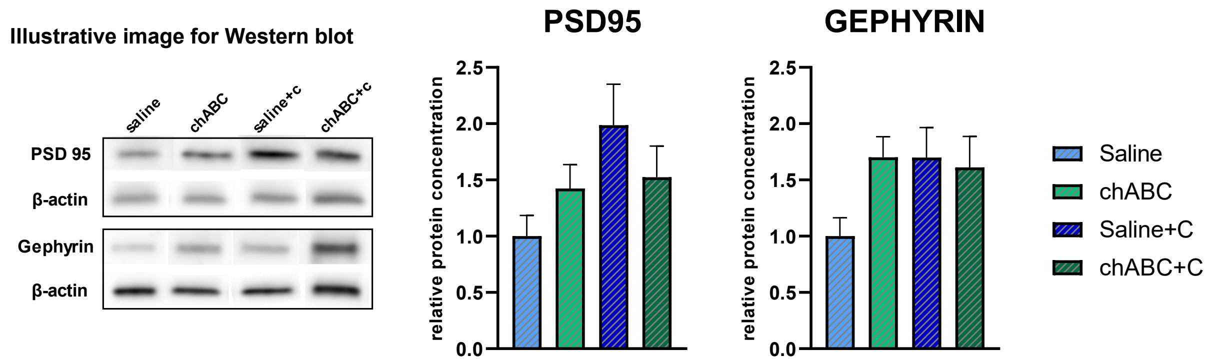

Figure 21 | Quantitative protein analysis of PSD95 and Gephyrin
Levels of presynaptic protein markers VGLUT and VGAT in hippocampus

The PSD-95 increased in all the groups compared to saline group. The effect on the increase of the protein level was even stronger for the Saline+C group. Regarding gephyrin, the protein level shows an increase in all experimental groups compared to saline suggesting both positive effect on inhibitory neuron function by CIP and chABC application. Nevertheless, the increase was not statistically significant.

11.5.3 Other markers

Synaptosomal nerve-associated protein 25 (SNAP25) is a component of the trans-SNARE complex and plays a crucial role in the fusion of the vesicular and plasmatic membrane of the neuron, facilitates the release of neurotransmitter from synaptic vesicles and therefore serves as an important synaptic marker. The protein GAD (glutamate decarboxylase) is present in two isoforms GAD65 and GAD 67. It is primarily expressed in GABAergic neurons where it synthesizes GABA from glutamic acid. Thus, GAD65/67 serves as an indicator of the synaptic activity of inhibitory neurons.

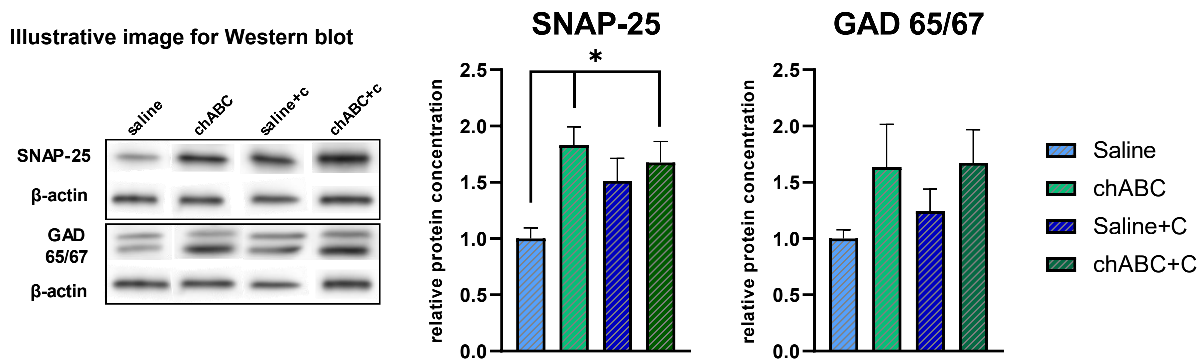

Figure 22 | Quantitative protein analysis of SNAP-25 and GAD 65/67
Levels of general protein markers SNAP-25 and GAD 65/67 in hippocampus

The results showed a statistically significant increase in the SNAP-25 protein level in both chABC injected groups indicating an increase in synaptic plasticity in consequence of the PNNs removal. Quantitative analyses of the protein GAD65/67 showed an increase of the protein level in the groups treated with chABC; CIP alone showed only a mild positive effect on the protein level (One Way ANOVA $F = 3.723$; $p < 0.05$, *post hoc* Tukey test Saline vs chABC $q = 4.225$; $p < 0.05$, Saline vs chABC+C $q = 3.904$; $p < 0.05$)

11.5.4 Tauopathy markers

11.5.4.1 Tau 5

The quantitative protein analysis was conducted also to determine the level of Tau protein in the mice model of tauopathy. The marker Tau 5 was used to quantify a total value of Tau protein and to assess the proportion between phosphorylated and non-phosphorylated form to see an effect of exposure to low temperature. The values above 1 signify higher proportion of phosphorylated form compared to non-phosphorylated and vice versa. Values that are equal to zero shows no presence of phosphorylated Tau form.

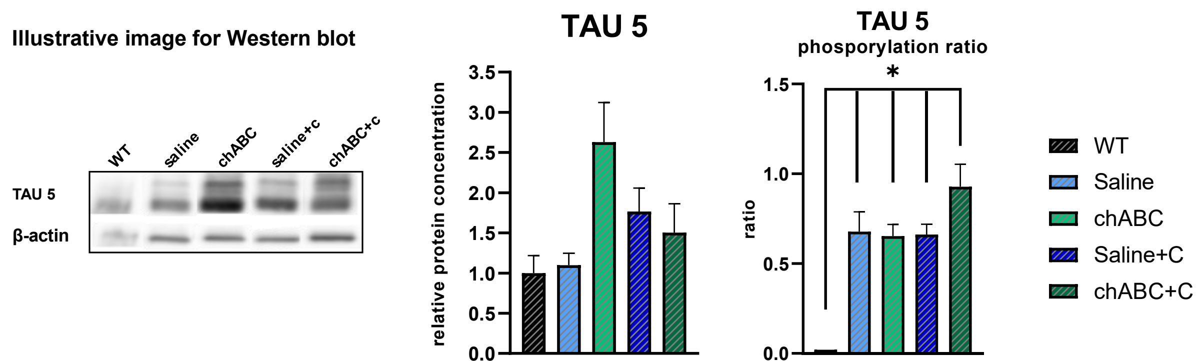

Figure 23 | Quantitative protein analysis of TAU 5
Levels of tauopathy protein markers TAU 5 and TAU 5 phosphorylation ratio

The results show an increase in a total value of Tau in all groups compared to WT, especially in the chABC group, nevertheless the differences were not statistically significant. Regarding the phosphorylated and non-phosphorylated proportion, in Figure 23, we can see a higher proportion of phosphorylated Tau form in the chABC+C compared to other groups, nevertheless, not exceeding the value 1, proving the tauopathy is not yet so progressed due to young age of the mice. There was no Tau 5 phosphorylation detected in the WT group (One Way ANOVA $F = 7.892$; $p < 0.001$, *post hoc* Tukey test WT vs Saline $q = 5.589$; $p < 0.05$, WT vs chABC $q = 5.106$; $p < 0.05$, WT vs Saline+C $q = 5.454$; $p < 0.05$, WT vs chABC+C $q = 7.942$; $p < 0.001$).

11.5.4.2 AT8

AT8 was used as a marker of Alzheimer disease since it recognises tau protein phosphorylated at both serine 202 and threonine 205.

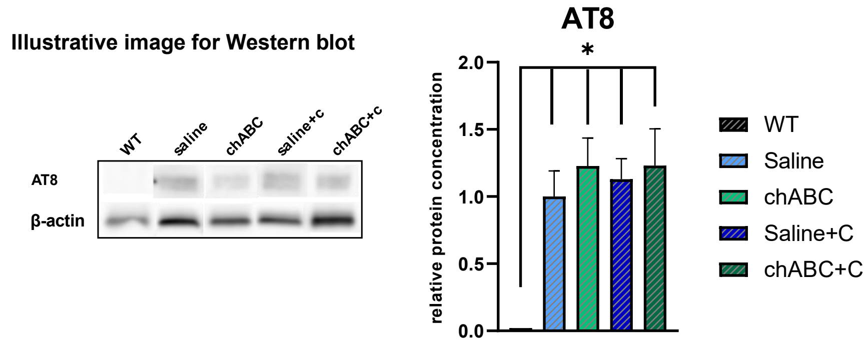

Figure 24 | Quantitative protein analysis of AT8
Levels of tauopathy protein marker AT8 in hippocampus

The results confirm presence of Tau phosphorylation on both serine 202 and threonine 205 sites and statistically highly significant difference in the protein level compared to WT control, where was no signal detected. The level of protein phosphorylation does not statistically significantly differ among groups suggesting that the animals are in a similar phase of the disease (One Way ANOVA $F = 3.328$; $p< 0.05$, *post hoc* Tukey test WT vs Saline $q = 3.776$; $p< 0.05$, WT vs chABC $q = 4.403$; $p< 0.05$, WT vs Saline+C $q = 4.267$; $p< 0.05$, WT vs chABC + C $q = 4.82$, $p< 0.05$).

12. Discussion

The role of PNNs has been a widely studied topic concerning the restoration of neuronal plasticity, memory enhancement, CNS regeneration and clinically applicable modifications in case of neurodegenerative diseases such as above discussed tauopathy (Fawcett & Asher, 1999; Pizzorusso et al., 2002, Morawski et al., 2010). In our study, we were examining the role of enzyme-induced structural changes of PNNs together with the model of CIP on the memory of the mice model of tauopathy P301S. We have chosen the early symptomatic stage of tauopathy when the progression of the disease could be potentially reversed or slowed down.

The injection of chABC previously demonstrated an improvement in explicit memory (ORT) and restoration of the synaptic plasticity in both WT mice and mice model of tauopathy (Romberg et al., 2013; Yang et al., 2015).

Although the digestion of PNNs restored the memory by enhancing the function of unaffected neurons, it did not have any effect on the progress of tauopathy (Yang et al., 2015).

In the same manner, we examined an effect of chABC therapy on hippocampal-based spatial memory of tauopathy using the MWM behavioural test and captured effects on learning and memory. Since the enzymatic removal of PNNs is not permanent and undergoes gradual regeneration (Lensjø, Lepperød, et al., 2017), there is a time window where the juvenile plasticity is stimulated, and neurons can easily create new connections due to various stimuli and new conditions the animals are exposed to (Kwok et al., 2008). This may result in an entire rewiring of the neuronal circuits. The chABC intervention can be thought of as a kind of "reset button" that restarts the entire system and could lead to an enhancement of the neuronal activity or, on the contrary, to a complete collapse in the case of later phases of neurodegenerative diseases.

Additionally, half of the animals (from both ChABC and Saline group) underwent a protocol of CIP that has been previously used with pre- and symptomatic stage of AD and it demonstrated dramatic plastic changes, reduction of dendrites, change of the number of synapses and neuroprotection via induced expression of the CSP RBM3 (Peretti et al., 2015). We aimed to assess the impact of CIP on the system that is affected by tauopathy since, in unpublished research, this combination of enzymatic cleavage of PNNs combined with CIP in WT mice contributed to an even more effective ability to re-learn the hippocampal-based task, when compared to CIP mice induced learning deficit. Besides, one of the hallmarks of tauopathy is an accumulation of NFT of

hyperphosphorylated protein Tau. Several studies have shown that Tau protein phosphorylation is increased by low temperatures (Arendt et al., 2003; Lindwall & Cole, 1984; Planel, 2004). Therefore, the combination of enzymatic treatment with deep cooling of an animal provided insight for what is the effect on the disease progression and the degree of involvement of PNNs in tauopathy.

The tauopathy disease is a continuous dynamic process and for our P301S mice model, the first few months are non-symptomatic. Nonetheless, around the age of 6 months, the disease is manifesting severe impairments in locomotion and memory and it restricts the swimming ability of the mice. Thus, we aimed to use animals of the same age, however, there was inevitably a difference in the date of birth ranging from one to two weeks. Although small difference, this could cause some differences in the progression of the disease and bring the variability among individual animals. Besides, regarding the progression of the disease, there are some sex-dependent differences. There were observed disturbances in performance in a study of a tauopathy mice model rTg4510 (Yue et al., 2011), and the pathology occurred in females earlier than in males. Although, in general, males perform in spatial tasks better than females (Berger-Sweeney et al., 1995), the Tau pathology amplifies the differences and suggests that cognitive deficits are sex-dependent appearing the progression of the disease to be more aggressive in females. The same phenomenon was observed even in our study (data not shown), where the females performed slightly worse compared to males and had difficulties with learning from the very beginning of the experiment. Nevertheless, despite statistically non-significant differences, we decided to connect males and females to achieve a sufficient number of individuals in the experimental groups. As a result, the performance and changes on the protein level can be influenced by the stage of tauopathy and the sex-differences. Hence the probability to unveil the significant effect of the treatments may be suppressed. From this aspect, successful therapy can be considered even when reaching a plateau in learning when the general deterioration is slowed down despite ongoing progression in the disease. Besides, an error emerged while creating experimental groups because the mice were divided randomly. We omitted the impact of the small number of animals and individual differences in performance since tauopathy also affects other brain areas. As a result, the groups were not uniform.

Concerning the behavioral tests, our results demonstrated a positive effect on memory and learning in chABC injected mice (Fig. 16B) as was previously showed also in other studies

(Romberg et al., 2013; Végh et al. 2014). From our results, animals were able to learn despite progress in the disease and with the highest level of overall Tau5 protein (Fig. 22). The Probe test revealed strong trend in frequency of crossing the platform area after the re-learning period. The absence of statistically significant difference from other groups could be well connected with statistically significant decrease of swim speed (Figure 16D), pointing out the early stage of symptoms and initial atrophy of muscles. Similarly, local chABC injection into hippocampus enhanced learning via induction of PV+ interneurons plasticity that was shown to be also involved in the regulation of learning and memory (Donato et al., 2013). The positive effect of chABC on memory is in agreement with another study of quadruple KO mice (Tn-C, Tn-R, brevican and neurocan) (Gottschling et al., 2019) that had significantly diminished PNNs and, as a consequence, this altered the proportion between excitatory and inhibitory synapses. The number of excitatory synapses was increased, and it caused heighten synaptic transmission in the neuronal network. Our results of the quantitative protein analysis also showed a statistically significant increase in the protein level of SNAP25 (Figure 22) as a result of chABC administration pointing out possible rewiring. Nevertheless, the quantitative protein analysis was not possible to be done only on PV+ neurons and the results demonstrate the changes in the protein level of the whole hippocampus including even neurons that were possibly not affected by the enzymatic treatment. In our study, we captured the effect of chABC promoting an increase of inhibitory markers but causing a decrease in presynaptic terminals. Besides, the WB analysis was done at the end of the experiment, approximately 3 weeks after chABC application, thus the PNNs already regenerated up to 40%, which could mitigate the significance of the effect in our results (Lensjø, Lepperød, et al., 2017). On the contrary, the Saline group reached its maximum learning capacity and failed to further improve with continuous training. Regarding the probe tests (Figure 16B), Saline demonstrated a statistically significant impairment of memory through a decrease in the number of the crossings of the target, showing a learning process deterioration with tauopathy progression without any therapy.

Similarly, we captured an improvement in the performance of the Saline+C group regarding its learning curve in the experimental week (Fig. 9). However, there was no change captured in the probe test and the group reached its plateau (Figure 16B). The quantitative protein analysis also indicates a trend of increase in the protein level of both presynaptic and postsynaptic markers due to CIP (Fig. 14 and Fig. 15). A decrease in body temperature was shown to have a

neuroprotective effect for the CNS (Darwazeh et al., 2013). Indeed, hypothermia induces expression of the CSP, for example, the RBM3, that was previously mentioned to be associated with neuroprotection in various models of cooling (Chip et al., 2011). Higher endogenous levels of RBM3 restored failed synaptic plasticity and prolonged the survival in neurodegenerative models of AD and Prion disease (Peretti et al., 2015). Similar outcome was observed in our results of synapse withdrawal when Saline group exhibited trend of increased number of synaptic signals after rewarming to normal body temperature (37°C) compared to initial state before undergoing cooling. Therefore, in the Saline+C group, there could be an effect of CSPs restoring the synaptic transmission, higher degree of rewiring and cell survival which could indicate CIP as a promising therapeutic target for neurodegeneration (Bastide et al., 2017). Nevertheless, there is a missing link between CSP and tau-phosphorylation and how they affect each other.

On the contrary, the combination of enzymatic degradation of PNNs and CIP lead to deterioration in learning and loss of memory in our model of tauopathy. The combination of those methods did not have any behavioural effect since the functional synapses failed to restore to the initial level, although the decrease was not statistically significant (Fig.19). On the other hand, in WT mice, this combination of chABC and CIP exhibited an enhancement in the learning ability of hippocampal-based task (unpublished research), pointing out the importance of current state of synaptic strength before the actual induction of CIP. The chABC+C group performed worse throughout the experimental week than at the beginning of the training week and this observation could be explained by the increased vulnerability of tauopathy neurons when exposed to synaptic stress and lacking the PNNs coverage. The PNNs are neuroprotective in many ways, e.g. as ion buffering or as antioxidants, and their absence or reduction in CSPGs coincides with neurofibrillary changes and with a higher susceptibility of neurons for synapse loss. Conversely, PNNs-ensheathed neurons exhibit higher resilience (M. Morawski et al., 2010).

A recent study has shown a potential link between ECM, specifically PNNs, and Tau protein (Suttkus et al., 2016). PNNs were found to be directly involved and play a role in the regulation of spreading and internalization of exogenous Tau protein, which is associated with progress in tauopathy. Abnormal PNNs or their absence cause that the rate of Tau spreading is elevated, and Tau internalization is heightened. On the contrary, neurons with PNNs displayed no Tau internalization (Suttkus et al., 2016). ECM is generally influencing the flow of the molecules by its diverse consistency and polyanionic character that is given mainly by hyaluronan and

aggrecan (Y. Yamaguchi, 2000). Application of chABC and cleavage of the CSPGs shown a drastic increase in an ion flow (Hrabetová et al., 2009) and therefore, the ECM components, specifically PNNs that are rich in CSPGs, could prevent the Tau from free disperse among cells in normal conditions. That was also shown in models of various ECM components KO mice (aggrecan, Tn-R, etc.), when the Tau protein dispersion was demonstrated to be significantly less restricted, and neurons were affected more extensively by Tau internalization compared to PNN-associated neurons (Suttkus et al., 2016).

Recently, an opinion that Tau spreading is predominantly happening in dendrites via the synaptic junction is becoming widely accepted. This theory points to the interconnectedness of Tau protein with PNNs, which serve as stabilization of synaptic transmission among other functions. This is in an agreement with the opinion that PNNs appear to be protecting neurons against AD-related abnormal Tau protein accumulation and may prevent neuronal death (M. Morawski et al., 2010). Thus, an alternation in the PNN structure that induces the short time window of unprotected PV+ cells combined with hyperphosphorylation of Tau induced by low temperature may contribute to accelerated Tau spreading, internalization and progression of tauopathies including AD. This was similarly observed in the late stage of animals suffering from 5xFAD or Prion disease (Perretti, unpublished research) and it could serve as an intriguing explanation for our case of impaired memory in chABC+C group.

In general, the amount of abnormal Tau in AD brain correlates with the stage of disease and symptoms (Hampel et al., 2005). In fact, the idea that combination of PNNs removal and CIP can cause acceleration in the progression of tauopathy was supported with our results from quantitative protein analysis of phosphorylated protein Tau 5. The ratio of phosphorylated versus non-phosphorylated form exhibits a trend of increase in chABC+C compared to other groups (Figure 24). Furthermore, the chABC+C group manifested a statistically highly significant decrease in speed of swimming ($p < 0.001$, Figure 16D) pointing out to the accelerated progress of tauopathy and early symptomatic atrophy of muscles. In addition, in latest publications, it has been shown that cooling can cause specific olfactory memory deficit, and the loss of olfactory function is also associated with an early stage of AD (Bullmann et al., 2019). Nonetheless, the increase of Tau phosphorylation ratio was not statistically significant and to support this idea the repetitive measure of Tau phosphorylation levels induced by CIP will be done.

We suggest that it could be an important index of tauopathy progression and it could serve as a tool to determine the effect of low temperature-induced hyperphosphorylation.

To sum up, when applied separately, the individual methods of synaptic plasticity, PNNs degradation and CIP have a positive effect on memory (Donato et al., 2013,Peretti et al., 2015; Yang et al., 2015, Bastide et al. 2017). The same was demonstrated in our study when the impact of the tauopathy on the learning processes was mitigated. However, when those procedures are combined, it caused a decrease of the effect (Figure 14). The presence of PNNs appeared to play a relevant role in the system and their removal caused a loss of protection for important neurons (M. Morawski et al., 2010). Furthermore, when exposed to synaptic stress, it resulted in a deterioration of learning and memory retention. Yet, changes on the synaptic level from the results of quantitative protein analyses that were observed in association with CIP or injection of chABC were detectable also in their combination following the trend of CIP. On the other hand, when the animals were exposed to the same stress conditions with enzymatically intact PNNs, the neuronal system seemed to remain unaffected.

The specific sites where PNNs are present may be an indication of importance to stabilize and protect these neurons. Conversely, areas with none or low expression of PNNs might indicate a possibility for a higher level of plasticity (Wang et al., 2012; Lensjø, Christensen, et al., 2017). Additionally, some lecticans present in PNNs like brevican and versican were found to be linked to memory retrieval in the MWM (Saroja et al., 2014. Hence, digesting PNNs complexly (as in our case) or when the PNN structure is missing various key components could decrease the full potential of the therapy (Jansen et al., 2017). There are various KOs of PNNs components that have been designed (Rowlands et al., 2018, Bekku et al., 2012; Kwok et al., 2010) and those suggest the way how to partially modify the structure of PNNs, the ratio and combination of individual components. That could induce increased plasticity of the system but at the same time assure the neuroprotection the PNNs provide.

The effect of CIP on the synaptic withdrawal and subsequent synaptic contact regeneration of PV+ neurons via the number of signals of synaptic markers was successfully performed (Figure 19). The results demonstrated a high capacity of synaptic plasticity in the Saline group as well as in the group with abolished PNNs when the environment affected by tauopathy is highly vulnerable. There was a statistically significant difference between the chABC and the Saline

group in the number of signals before undergoing CIP, again pointing out to the positive effect of chABC on the enhancement of the neuronal activity (Romberg et al., 2013). Nonetheless, the capacity of synaptic plasticity is limited and was shown to be impaired due to an advanced progression of the neurodegenerative disease (Peretti et al., 2015). Indeed, the number of signals of synaptic markers in the chABC group with highly diminished PNNs did not reach the same level of signal after rewarming as was initially, and for Bassoon the decrease of synapse regeneration was statistically highly significant. That indicates the increased vulnerability of PV+ neurons when lacking PNNs and decreased ability of synaptic plasticity when exposed to stress (Reichelt et al, 2019).

Although most of the differences among the groups were not significant and with a larger experimental animal groups the impact of the therapy would be more distinctive, we believe that the results outline the trend of the effect of each procedure on the memory and learning process of mice with tauopathy and demonstrate the viable importance of PNNs.

13. Conclusion

In this study, we have applied different methods of restoration of the synaptic plasticity such as enzymatic degradation of PNNs with chABC and CIP in a mice model P301S to possibly restore the memory and modify the progression of tauopathy. We successfully promoted enhanced synaptic plasticity in both cases as was similarly demonstrated in previous studies. Additionally, we have combined those methods to examine their positive effect on the restoration of failed synaptic plasticity and if the effect could be amplified. Nonetheless, in combination, we demonstrated that those promising therapeutic methods attenuate their individual effect, in P301S mice model of tauopathy.

In the current study, we targeted the whole brain area of the hippocampus, the manipulation of the synapses was less specific, and we were only looking at the overall effect. The subject of further research will be to define what synapses in neurodegenerative diseases are sensitive to retraction and are affected in the early stages. This will be followed by the mapping of neurons and synapses that are still functional and which are responsible for learning and their function could be therapeutically enhanced. Furthermore, the effect of CIP on PNNs and its structure regarding the composition and strength will be also defined.

Up to date, no treatment could stop or successfully modify the memory decline in AD and other Tauopathies. The effects of chABC might be a temporal solution but due to invasive application of the enzyme that must be directly injected in the brain, this method is impossible for clinical applications. From this aspect, the research should focus on the specific modification of PNNs through, for example, gene therapy. On the other hand, stimulation of expression of CSPs that might provide neuroprotection is a more easily applicable approach and mild hypothermia treatment should be clinically considered. This thesis contributes to the current state of knowledge by comparing various methods of enhancement of synaptic plasticity of tauopathy and might serve as a guideline for further research.

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