THE EXTRACELLULAR MATRIX AND PERINEURONAL NETS IN MEMORY

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ABSTRACT

All components of the CNS are surrounded by a diffuse extracellular matrix (ECM) containing chondroitin sulphate proteoglycans (CSPGs), heparan sulphate proteoglycans (HSPGs), hyaluronan, various glycoproteins including tenascins and thrombospondin, and many other molecules that are secreted into the ECM and bind to ECM components. In addition, some neurons, particularly inhibitory GABAergic parvalbumin-positive (PV) interneurons, are surrounded by a more condensed cartilage-like ECM called perineuronal nets (PNNs). PNNs surround the soma and proximal dendrites as net-like structures that surround the synapses. Attention has focused on the role of PNNs in the control of plasticity, but it is now clear that PNNs also play an important part in the modulation of memory. In this review we summarize the role of the ECM, particularly the PNNs, in the control of various types of memory and their participation in memory pathology. PNNs are now being considered as a target for the treatment of impaired memory. There are many potential treatment targets in PNNs, mainly through modulation of the sulphation, binding, and production of the various CSPGs that they contain or through digestion of their sulphated glycosaminoglycans.

INTRODUCTION

All components of the CNS are surrounded by a diffuse extracellular matrix (ECM) containing chondroitin sulphate proteoglycans (CSPGs), heparan sulphate proteoglycans (HSPGs), hyaluronan, various glycoproteins including tenascins and thrombospondin, and many other molecules that are secreted into the ECM and bind to ECM components. In addition, some neurons, particularly inhibitory GABAergic parvalbumin-positive (PV) interneurons, are surrounded by a more condensed cartilage-like ECM called perineuronal nets (PNNs). PNNs surround the soma and proximal dendrites as net-like structures that surround the synapses. Attention has focused on the role of PNNs in the control of plasticity, but it is now clear that PNNs also play an important part in the modulation of memory. In this review we summarize the role of the ECM, particularly the PNNs, in the control of various types of memory and their participation in memory pathology. PNNs are now being considered as a target for the treatment of impaired memory. There are many potential treatment targets in PNNs, mainly through modulation of the sulphation, binding, and production of the various CSPGs that they contain or through digestion of their sulphated glycosaminoglycans. [1]

EXTRACELLULAR MATRIX BIOLOGY

Extracellular matrix

Extracellular matrix (ECM) refers to a collection of extracellular molecules that provides physical and biochemical support to cells. Studies on the ECM mainly focus on the intricate network of ECM formed by macromolecular assembly. The ECM in the central nervous system (CNS) is mainly composed of proteoglycans, glycosaminoglycans (GAGs) and glycoproteins such as tenascins and thrombospondin that interact with them [2]. Diffusion of molecules such as neurotransmitters, ions, guidance molecules, and metabolites are tightly regulated by this network.

Proteoglycans are a family of large ECM molecules whose basic structure comprises linear GAG chains covalently attached to a core protein. There are five types of GAGs, chondroitin sulphates (CS), heparan sulphates (HS), keratan sulphates, dermatan sulphates, and hyaluronan [3, 4]. Chondroitin sulphate proteoglycans (CSPGs) and heparan sulphate proteoglycans are the key proteoglycans in CNS function[3]. Research in the last three decades has elucidated the inhibitory functions of CSPGs in neurite extension, path-finding, plasticity and neural regeneration [5-9]. CSPG function is strongly influenced by the pattern of sulphation of the GAG chains, with 4-sulphated GAGs being inhibitory and 6-sulphated GAGs being permissive to axon growth and plasticity [10]. **Synthesis of GAGs and their sulfation occurs in the Golgi, sulfation being determined by the activity of sulfotransferases that sulphate CS and HS chains in various positions on the constituent disaccharides** [11]. In addition to being a key inhibitory molecule in the diffuse ECM, CSPGs around some classes of neurons also interact with other brain ECM molecules, self-assembling into aggregate structures called perineuronal nets (PNNs) [12-14]. **The lectican family of CSPGs (also called aggrecan-family CSPGs) are found within PNNs, including aggrecan, brevican, neurocan, and versican** [1].

While diffuse CNS ECM surrounds all structures in the CNS, perineuronal nets (PNNs) with a cartilage-like structure surround some classes of neurons. PNNs are reticular CSPG-containing ECM structures surrounding the soma and proximal dendrites of a subpopulation of CNS neurons and important for controlling neuroplasticity [1, 13]. **Hyaluronan is synthesized by the transmembrane enzyme hyaluronan synthase (HAS), which anchors the nascent hyaluronan chains to the neuronal surface [15] together with ankyrin-R [16] and RPTPzeta/phosphacan** [17]. The long hyaluronan chains provide a scaffold for the assembly of CSPGs, hyaluronan, hyaluronin and proteoglycan link proteins (Hapln), and tenascins [15, 18]. While binding of the N-terminal of CSPG to hyaluronan chains is stabilised by Hapln, the C-terminals of three CSPG molecules will link with the trimeric tenascin-R [19-21]. These interactions enable the formation of a stable PNN. **However, the diffuse ECM also affects neuronal activity. Hyaluronan is a regulator of extracellular volume, and hyaluronan deficiency causes altered neuronal activity and seizures** [22].

PNN components and their interactions with other ECM molecules such as OTX2, neuronal pentraxin 2 (Nptx2, also called Narp) and semaphorin3A (Sema3A), contribute to the functions of PNNs in neuroplasticity [23-27]. While OTX2 and Sema3A bind to 4,6 disulphated GAGs in the PNNs [23, 28], Nptx2 binds to both 4,6 sulphated GAGs and HA [29]. The soluble transcription factor OTX2 binds to PNNs and is internalised, leading to maturation of PV neurons and maintenance of PNNs in adulthood [23, 26]. Nptx2 is an activity-regulated protein that interacts with the extracellular domain of AMPA receptors to facilitate receptor clustering and insertion of GluR4 on the postsynaptic membrane of neurons, strengthening synaptic communication [24]. Removal of CS by chondroitinase ABC (ChABC) abolishes these effects of Nptx2. Sema3A is a chemorepulsive molecule, and prevention of its binding to PNNs reinstates ocular dominance and cerebellar plasticity in adult mice [30, 31]. Moreover, a recent study has also shown that Sema3A binding to CS GAGs induces rigidification of the CS matrix, which may alter the mechanical properties of PNNs and ultimately affect neuroplasticity [32]. Proteoglycans also exert effects through binding to a cell surface phosphatase, **protein tyrosine phosphatase sigma** (PTPσ), which can exert inhibitory effects from CSPGs and permissive effects from HSPGs [33].

ECM and synapses

The substrate of memory is synaptic strength and connectivity. All synapses are embedded in ECM, either the general interstitial ECM found throughout the CNS or the specialized ECM of PNNs, where they can interact with CSPGs, HSPGs, tenascin-C, tenascin-R, thrombospondin, and laminins **(Figure 1).** These molecules in turn bind and present other active molecules to neurons**. Synaptic ECM molecules also interact directly with receptors and ion channels, modulating their migration and properties** [34, 35]. **The most-studied ECM molecules that affect synapses** and memory are CSPGs. The ECM can be modified rapidly by the release of proteases [36], by microglial action and by internalization, all of which can be activated by memory events [37-39].

Experimentally, much of our knowledge of the effects of CSPGs and PNNs stems from their modification by digestion of the GAG chains using ChABC. At postsynaptic sites, ChABC enhances dendritic spine number and motility, while presynaptic terminals tend to show enhanced sprouting and synapse numbers [40-42]. In the perirhinal cortex and hippocampus, ChABC digestion increases inhibitory inputs to PV interneurons [43-45] while in the entorhinal cortex, ChABC reduces inhibitory inputs, and in V1 visual cortex, ChABC decreases both excitatory and inhibitory inputs to PV interneurons [46, 47]. The deep cerebellar nucleus, where PNNs surround most neurons, has been a

fertile region for PNN research: Purkinje cell terminals sprout after ChABC digestion [48], and in the frontal cortex, the number of inhibitory connections to pyramidal cells is decreased [49]. Digestion of HA with hyaluronidase modulates synaptic function by increasing AMPA receptor mobility (reviewed in [50] **and surface expression of NMDA receptors** [35, 51]. PNN function can be modulated by removing individual components. Deletion of link proteins leads to fewer Purkinje synapses, decreases inhibitory transmission in the deep cerebellar nucleus [52], and facilitates long-term depression in the perirhinal cortex [53]. Manipulation of individual CSPG proteins can also affect synapses and synaptic function [34, 54]. A relevant function of CSPGs in PNNs is to present semaphorins to synapses; absence of semaphorin 4C (sema4C) prevents the increase in spine number during fear learning [55, 56]. Moreover, knockout mice deficient in PNN component tenascin-R have abnormal synapse formation and synaptic plasticity after injury [54, 57]. **An important mechanism of plasticity is modification of the CNS ECM by activity-related release of metalloproteinases, which can cause rapid changes in PNNs in region of synapses, enabling local changes in synaptic properties [38, 58]**.

Electrophysiological effects

Digestion or transgenic attenuation of PNNs has various effects on electrophysiological properties that are dependent on brain region and type of PNN manipulation [59]. Most studies in the hippocampal CA1 region show that PNN degradation or attenuation decreases long-term potentiation (LTP). [60-65]. Similarly, LTP is also affected by CSPG sulphation, with loss of 6-sulphation causing loss of LTP in the perirhinal cortex and CA1 [45]. However, the effects of reducing PNNs may be dependent on the cell type surrounded by PNNs. For example, in the CA2, an area associated with social memory and which usually does not exhibit LTP, PNN depletion enables LTP [66]. Long-term depression (LTD) is also altered after PNN degradation, with both increases [67] and decreases [53, 60] reported. However, in general, there is an overall increase in network activity when PNNs are depleted or attenuated [53, 59, 68], possibly due to an overall reduction in inhibitory activity. In line with this, digestion of CSPGs in the primary visual cortex in rats or deletion of aggrecan in mice decreases inhibitory activity, causing the network to revert to an immature juvenile state and an increased level of activity-dependent plasticity [47, 69]. Enhanced learning of eyeblink conditioning is also observed after ChABC digestion in the deep cerebellar nucleus, although here it is induced by increased GABAergic transmission [31, 70].

The variable effects of PNN attenuation could be related to cell-specific expression patterns of PNNs. While PNNs predominantly enwrap PV inhibitory neurons in most brain areas, they surround

158 excitatory neurons in the CA2[66, 71]. Moreover, individual CSPGs have distinctive effects that may also
159 contribute to the large variation in effects of PNN depletion. For example, brevican affects mainly
160 excitatory synapses, regulating both AMPA receptors and potassium channels and the speed and
161 duration of synaptic potentials, leading to impaired hippocampal LTP observed in brevican knockout
162 animals [34, 65, 72]. In contrast, aggrecan affects inhibitory synapses on PV interneurons (Ruzicka
163 unpublished observations), and mice deficient in neurocan showed impaired hippocampal LTP [64].
164 Tenascin-R deficient mice also have a disrupted PNN structure, impaired LTP in the hippocampus [63],
165 and show reduced active zones in inhibitory synapses [73]. Lastly, animals deficient in tenascin-C show
166 impaired L-type calcium channel dependent LTP [74].

167

168 **Types of memory and memory models**

169 ***Associative learning***

170 To examine PNN function in associative memories, we focus on fear conditioning and eyeblink
171 conditioning, two well-studied phenomena (see [75] for review). Fear and eyeblink memory are similar
172 in that a conditioned stimulus (usually a tone, visual cue or context) is linked to an unconditioned
173 stimulus; electric shock in the case of fear memory, a puff of air to the cornea for eyeblink memory.
174 After a training period during which both stimuli are given simultaneously, the conditioned stimulus
175 alone will cause animals to freeze (fear memory) or blink their eyes. The neural pathways differ, but
176 both involve the auditory or visual pathways. Other forms of conditioning, such as that associated
177 with drugs of abuse, are also discussed below.

178

179 *Eyeblink conditioning*

180 Delayed eyeblink conditioning is a type of associative conditioning that requires neurons in the deep
181 cerebellar nuclei (DCN) [76], many of which are surrounded by PNNs [77]. The acquisition of eyeblink
182 conditioning reduces the intensity of PNNs in the DCN, whereas longer training (to plateau levels)
183 restabilizes PNN intensity [31]. Injection of ChABC [70] or viral vector-containing ChABC to provide long-
184 term depletion of PNNs increases acquisition of eyeblink conditioning [31] but slightly decreases
185 retention of this response when tested about three weeks later. This is consistent with reduced
186 firing of these neurons, an increased number of inhibitory terminals and reduced excitatory
187 terminals[31], and greater inhibition of DCN neurons [70]. The increased acquisition is in contrast to
188 another study [78] that showed a reduced conditioned response and no change during extinction. The
189 differences between studies may be due to differences in species, strength of the unconditioned
190 stimulus, or the method of ChABC delivery. Sema3A is associated with PNNs around Purkinje cell

terminals and may influence remodelling of synapses and in turn the impact of ChABC on eyeblink conditioning [56]**(Figure 2A).**

Fear conditioning

Fear conditioning is often used as a model for posttraumatic stress disorder (PTSD), a psychiatric disorder characterized by hyperarousal, intrusive memories of traumatic events, and avoidance of reminders of those events [79]. While many studies focus on the basolateral amygdala (BLA), cortical regions also process threats associated with anxiety [80]. Studies in rodent models have focused on fear conditioning because PTSD in humans is believed to arise from abnormal activation of fear circuitry [81]. Fear memory was the first type of memory to be linked to PNNs and the ECM. Gogolla et al. [82] showed that PNN removal in the BLA in adult mice allowed for a subsequent extinction training to diminish expression of fear, similar to what occurs in juvenile mice prior to PNN development **(Figure 2A).**

Since then, other studies have shown that PNN degradation in the hippocampus, medial prefrontal cortex (mPFC), anterior cingulate cortex, BLA, or auditory cortex impairs the expression of fear conditioning [62, 83-85]. The effectiveness of ChABC implicates CSPGs, but digestion of hyaluronan also reduces fear memory retrieval [61]. Fear conditioning increases PNNs or mRNA encoding PNN components in the auditory cortex [84], hippocampus, and anterior cingulate cortex [62], and activates PNN-surrounded neurons [86]. **PTPσ associates with PNNs and restricts plasticity by signalling through the receptor for brain-derived neurotrophic factor, TrKB [87, 88]. Sema4C, which also** associates with PNNs (see above), is increased in the hippocampus and ACC following fear conditioning, and sema4C knockout mice show deficits in conditioned fear memory recall [55].

The formation and recall of fear memories and other associative memories involve many connected brain areas and need to be considered in the context of precisely timed brain oscillations synchronizing neural activity within and across brain regions. PV neurons are essential for these oscillations, and the impact of PNNs on learning and recall is likely to be tightly linked to their influence on the PV neuron network [89-92]. For instance, coherence (phase alignment) between theta oscillations in the secondary visual cortex (V2) and the BLA is necessary for successful recall of remote fear memories [93, 94] . Attenuation of PNNs in V2 weeks after training reduces theta coherency between BLA and V2 and prevents recall of a remote fear memory [94]. Moreover, Shi et al. [62] found that the increased theta power in the hippocampus and anterior cingulate cortex during fear

conditioning is prevented by ChABC treatment, while overexpression of the PNN protein **hapln1** increases theta power.

Spontaneous object recognition memory

The spontaneous novel object recognition (SOR) memory task measures discrimination between a novel and a familiar object presented at the same time. Novelty detection is an innate rodent behaviour that can be impaired during ageing or neurodegeneration [53, 95, 96]. The test is usually performed in a Y-maze, in which two test objects are placed in the Y arms. The times during which animals interact with the objects through whisking and smelling are measured; animals spend more time with objects that they perceive as novel. A variation is object-place memory testing in which objects are moved within a test arena, and animals recognize objects that have been moved to a new position. The brain regions **that participate in the behaviour** have been identified based on early gene *c-Fos* and *Arc* expression and lesion studies. For the Y-maze SOR test, a key brain area is the perirhinal cortex and the neighbouring visual association area TE: animals with lesions in these areas have impaired SOR [97]. Variations of the task where animals actively explore and dissociate the objects also involve the CA1 and CA3 areas of hippocampus whose rhythms are synchronised during generation of SOR memory [98, 99]. Object-place memory is primarily associated with the hippocampus, and may be preserved after perirhinal lesions [99]. Increased activity occurs in several regions during task performance, including the CA1 and CA3, perirhinal cortex, insular cortex, and medial PFC [98, 100]. Both hippocampus and perirhinal cortex are rich in PV interneurons enwrapped with PNNs, and PNNs are also present on some hippocampal pyramidal neurons [46 101, 102]. Genetic or enzymatic attenuation of PNNs can increase synaptic transmission and facilitate long-term depression (LTD) in the perirhinal cortex [53] or CA1 region [67], and this correlates with enhanced recognition memory. Similarly, disaggregation of PNNs by genetic deletion of aggrecan shifts the population of PV inhibitory interneurons toward a juvenile-like plasticity state, accompanied by increased performance in the SOR memory task [69]. Another component of PNNs - brevican - regulates the localization of potassium channels and AMPA receptors on PV interneurons, and intact brevican is required for short-term, but not long-term SOR memories [34] **(Figure 2B).**

Spatial memory

Spatial memory is a form of episodic memory that depends on a distributed network of brain areas including the hippocampus, parahippocampal areas, and connected areas. The rich diversity of spatially modulated neurons in these areas - including place cells of the hippocampus [103] and grid

cells [104, 105] of the medial entorhinal cortex as well as neurons estimating the distance to an object [106] or speed of the animal's movement [107] - represent a rare window into neural correlates of complex behaviours and memories. Flexible spatial learning requires both dorsal and ventral hippocampus together with their connection to the mPFC [108], with the medial entorhinal cortex necessary for place navigation using a global reference frame [109].

The complexity of behaviours and the many brain regions involved makes it difficult to dissociate contributing elements. Nevertheless, regulation of PNNs and proper excitatory/inhibitory balance of these brain areas seem to be essential for spatial memory processing. Overexpression of ECM/PNNs in the CA1 area of hippocampus, either due to dysregulation of NPY-Y1 receptor signalling [110], targeted deletion of hyaluronan binding protein **that mediates** hyaluronan depolymerization (HYBID) [111], or defeat-induced persistent stress [112], leads to decreased spine density and deficits in spatial learning. In contrast, digestion of hippocampal PNNs with ChABC promotes re-learning of a once-trained Morris water maze task (Ruzicka et al., 2021, unpublished results). Similarly, significantly enhanced working memory and reversal learning in the Morris water maze task is found in TNR -/- global knockout mice [113]**(Figure 2C).** However, degradation of PNNs in medial entorhinal cortex, where PV positive neurons are enwrapped in particularly dense PNNs, destabilizes the grid cell networks leading to impaired representations of new environments [46]. The new representations also interfere with the map of familiar places. **Following PNN removal in entorhinal cortex there are distorted spatial representations in downstream hippocampal neurons (Figure 2C)** [46]. This suggests that PNNs contribute to ensure a rigid grid cell network, which is essential for new representations to form, and that the heightened network plasticity caused by PNN removal interferes with stored spatial representations and perhaps memories.

The mPFC has an integrative role in object, place and time information [114, 115] as well as reward-regulated mechanisms of spatial learning [116, 117]. A robust approach to test the role of mPFC for spatial working memory is the mPFC-dependent trial-unique nonmatching-to-location assay (TUNL) task, a hippocampus-dependent automated test of location memory [118]. Infusion of ChABC into the mPFC improves performance on the touchscreen TUNL task [119]**(Figure 2D).**

Social memory

Social memory is explored using several experimental approaches. The basic principle is based on the propensity of rodents to investigate an unfamiliar subject more thoroughly than a familiar one.

Commonly used is the habituation/dishabituation test, in which the animal repetitively explores the same subject animal with a time delay between explorations [120]. Another variant is the social discrimination paradigm [121], which has an initial exploration phase, but in the second phase, both familiar and novel subjects are presented at the same time. The task, usually performed in a three-chamber maze, shows high sensitivity for measuring social recognition in rodents [120, 122].

Social recognition memory is probably consolidated through the activation of cAMP response element-binding protein (CREB)-mediated gene expression in the hippocampus, mPFC, anterior cingulate cortex, and amygdala [123, 124]. Whereas the mPFC, anterior cingulate cortex, and amygdala are needed for coordination of brain activity during social interaction, the hippocampus **serves as one of the mediators of social recognition memory** 'and as a connection hub between the various brain areas [123, 125]. The dorsal CA2 is the key centre for encoding, consolidation and recall phases of social memory [125-128]. CA2 also participates in social novelty discrimination [128] and modulates social aggression [129]. All the social memory associated regions are highly populated with PNN-surrounded PV neurons [46, 66, 130, 131]. Unusually in CA2 and the basolateral amygdala, PNNs are found around many excitatory pyramidal cells [47, 66], and calbindin-positive inhibitory interneurons [130]. PNNs play a distinct role in social memory, since mice with deficient social memory (BTBR mice) have atypical PNNs, and their degradation can partially restore social memory [132]. PNNs are usually associated with restriction of synaptic plasticity on inhibitory PV neurons, but in CA2, the PNNs also suppress LTP in excitatory synapses on pyramidal neurons [66]. However, PNNs in CA2 can also be permissive for inhibitory LTD (iLTD) in CA2, through maturation of PNNs and ErbB4 signalling at PV synapses [133]. This appears at the end of adolescence and correlates with social memory maturation. PNN degradation, in contrast, impairs social memory as well as iLTD induction [133, 134]. PNNs in CA2 are also upregulated during early postnatal exposure to an enriched environment, which opens the possibility of an early critical period synaptic plasticity in hippocampus [66]**(Figure 2E).**

Auditory plasticity/memory

The auditory pathway has tonotopic maps in the cortex and inferior colliculus that become refined during the critical periods for plasticity. As in other topographically arranged projections, PNNs **contribute to** the closure of these critical periods, with auditory experience and the diffusible transcription factor OTX2 **which is a key factor** in the initiation of PNN formation [135-137]. The timing of this transition at 3.5 years in deaf children is important for successful cochlear implants [138]. Learning of song in birds occurs either once or seasonally when PNNs are downregulated, and song

is crystallized when PNNs appear [139]. In adult mammalian life, auditory learning is limited, but cortex-dependent auditory relearning **regains the agility of the juvenile state** after ECM digestion [140]**(Figure 2F).** In the auditory cortex, the levels of brevican, which surrounds synapses in PNNs, **changes over the course of** auditory learning, **with an initial decrease followed by a transient increase during consolidation** [141]. Location of sounds is achieved in part by comparison of the timing of signals from each ear through the cochlear nucleus via the medial nucleus of the trapezoid body and lateral superior olive. In the trapezoid body there are massive synapses onto the principal cells called the Calyx of Held. These are specialized for very rapid and reliable transmission, and learning sound location **requires** these synapses. The CSPG brevican is enriched in the perisynaptic space of the Calyx, and knockout of brevican slows **pre-to-postsynaptic action potential transmission** and prolongs pre- and postsynaptic potentials [72].

The above-mentioned experiments describe the effects of attenuating PNNs, **either naturally (as occurs during learning), by enzymatic degradation of PNNs, or by genetic disruption of PNN components,** and suggest that PNNs may act as a brake on adult brain plasticity and perhaps learning and memory performance. **It is important to note that abolishing PNNs by enzymatic approaches** may not reflect processes occurring under physiological conditions in the brain. Rather, another suggestion is that learning induces slight changes to the ECM composition, either via incorporation of specific CSPGs [136, 142] , metalloproteinase activity [143], or **recycling of PNN components** [144]. We are far from understanding the full complexity of this system. The outstanding richness and complexity of the ECM landscape, its components, and evolutionarily conserved endogenous regulators point to a fine-tuned regulation contributing to the brain's ability to adapt and respond to a changing environment.

EXTRACELLULAR MATRIX AND MEMORY PATHOLOGY

Stress

Several studies have examined how acute and chronic stress exposures not involving fear conditioning influence PNNs. Spijker et al. [145] provide an excellent review on the impact of stress on PNNs. Although there are exceptions, in general, early life/adolescent stress reduces PNNs when examined early after stress, while these changes disappear or increases are found weeks after discontinuing stress. For example, decreases in PNNs around PV neurons are found in the hippocampus after chronic mild stress or maternal separation during adolescence, but an increase is observed several weeks post-stress [146-148]. In addition to time-dependent effects of stress, sex- and

hemispheric-dependent differences have also been identified: early life chronic stress in rodents during postnatal days 1-10 increases BLA PNNs in males but not in females and show a hemispheric specificity [149]. In adults, often no changes or increases are found after discontinuing stress. For example, chronic stress increases PNN numbers in the mPFC and habenula [150]. Social defeat stress combined with social isolation for 2 months (producing a depression-like phenotype) increases the number of PNNs around PV neurons and PNN components in the dorsal hippocampus. Moreover, removal of PNNs with ChABC restores impaired memory and electrophysiological changes induced by this stress [112]. Consistent with the longer-term effects of stress on PNNs, another study on social defeat stress in young rodents showed biphasic effects, with decreases in PNN-enwrapped PV neurons and PNN components in the CA1 early after stress exposure but increases 2 months after stress exposure [151] **(Figure 3A).** Overall, both early life and adult stress produce brain region-dependent changes in PNNs. The decreases in the intensity or number of PNNs found after early stress may reduce PV neuron activity or function, leading to enhanced output from brain regions such as the BLA that mediate fear responses [152].

Drugs of Abuse

Several studies have shown that drugs of abuse can either decrease or increase PNNs (see [153] for review). Several classes of drugs, including ethanol, nicotine, cocaine, and heroin, alter the intensity or number of PNNs in various brain regions, including the mPFC [154, 155], anterior cingulate cortex [131], orbitofrontal cortex [156], barrel cortex [157], insula [158], hypothalamus [159, 160], ventral tegmental area [156], and cerebellum [161-164]. Several examples of opposing direction of changes in PNNs are the following. 1) Acute vs. repeated cocaine injections produce opposite responses in PNN intensity in the mPFC [154]. 2) Extended exposure to cocaine self-administration increases PNN intensity in the cerebellum over abstinence time [162]. **3) Long-term abstinence times** (2-3 weeks) or extinction from heroin self-administration reduces PNN components in the mPFC and/or nucleus accumbens, but even a short reinstatement session in these animals reverses PNN increases [165]. This latter finding suggests that the changes can be rapid (within several minutes). Other work examining the effects of cocaine and heroin self-administration also supports opposing effects of abstinence time on the number of PNNs in the mPFC (dorsal prelimbic and infralimbic, respectively) [166]. In the cerebellum, repeated cocaine exposures **followed by an additional cocaine exposure one week later** increase PNN intensity within DCN neurons [164], whereas similar treatment reduces PNN intensity 1 month later [161]. Cocaine conditioned place preference (CPP) training decreases PNN intensity in DCN neurons but increases PNN intensity in Golgi neurons, the latter of which is correlated with place preference. A binge model of alcohol in adolescents increases PNN intensity and PNN components in the orbitofrontal

cortex in adult mice [167]. Extended ethanol drinking in adult mice increases PNN intensity in the mouse insular cortex after 6 weeks, but not after 1 week of exposure [158]. Short-term abstinence from nicotine self-administration also decreases PNN intensity in the orbitofrontal cortex and ventral tegmental area a few days after discontinuing exposure [156]. Thus, in general, short-term abstinence reduces PNNs, whereas long-term abstinence increases PNNs. However, as with stress, the changes are dependent on brain area, drug dose and class, exposure duration, and abstinence time from drug exposure **(Figure 3B).**

Several studies have demonstrated that removal of PNNs with ChABC alters behavioural responses to drugs. For example, removal of PNNs with ChABC in the mPFC or lateral hypothalamus prior to training for conditioned place preference (CPP) attenuates acquisition of the CPP memory [117, 159], and removal after CPP training also attenuates memory reconsolidation [117]. Removal of PNNs in the amygdala after training for morphine, cocaine CPP or heroin self-administration but before extinction reduces drug-primed reinstatement, but has no impact on reconsolidation, retrieval, or long-term morphine CPP memory [168]. Moreover PNN depletion in the lateral hypothalamus blocks cue-induced reinstatement in cocaine self-administering rats [160]. Depletion of the PNN component brevican in knockout mice enhances cocaine CPP 3 weeks after training, which is normalized by overexpressing this protein in the hippocampus prior to CPP training [169]. Extended ethanol exposure increases PNN intensity in the insular cortex, as mentioned, and removing PNNs in this brain region allows mice to become sensitive to the aversive effects of quinine added to ethanol, suggesting that increases in PNN intensity may **contribute to** the plasticity needed for compulsive ethanol seeking behaviour [170]. Interestingly, several of these studies found an effect only for drugs of abuse but not for non-drug rewards such as sucrose or food (e.g., [160, 166, 170]). Thus, the impact of PNN removal appears to be specific for plasticity induced by the learning/memory aspects of drugs of abuse.

Overall, stress or drugs of abuse bring about short-term changes in PNN numbers and/or intensity, while long-term increases in PNN may be related to loss of flexibility induced by subsequent natural stimuli, as previously considered for chronic exposure to stress [145] or drugs of abuse [171] **(Figure 3B).** The time of day PNNs are measured also may be critical due to daily rhythmicity in PNNs[172, 173]. PNN removal may enhance plasticity induced by weak stimuli or prevent metaplasticity induced by strong stimuli (stress or drugs of abuse). Whether these changes are beneficial or detrimental may depend on task demands, the neurons surrounded by PNNs (see [86]), the circuit that underlies task completion, and whether there is a need for sustained flexibility vs. stability after learning a particular task.

Ageing

Cognitive impairment and memory loss are common changes in ageing. To maintain normal cognitive and memory functions in the face of scattered neuronal dysfunction, the nervous system needs a certain level of neuroplasticity to allow for adjustments in circuitry through changes in synaptic strength and the formation of new synapses [172].

Chondroitin sulphates and their sulphation pattern **can determine whether or not there is** memory loss in ageing. While chondroitin 4-sulphates (C4S) are inhibitory [5], chondroitin 6-sulphates (C6S) are more permissive to plasticity and regeneration [174, 175], and the balance between C6S and C4S regulates neuroplasticity. The sulphation pattern changes in the aged brain, having remained fairly constant since the end of the juvenile critical periods. Analysis of the PNN CSPGs in the aged rat and mouse brain showed that C6S almost disappears after 20 months while the level of C4S remains stable [45, 176] **(Figure 3C).** The effect of removing C6S on memory can be tested in transgenic mice with C6-sulfotransferase knockout, giving very low C6S levels. These animals showed a very early deficit in object recognition memory and spontaneous alternation memory as young as 3-months old, similar to the performance of 20-month old aged mice [45]. The importance of C6 sulphation for memory was confirmed by virus-induced or transgenic expression of C6-sulfotransferase, leading to the restoration of the C6S level in aged mice and restoring or preventing age-related object recognition memory loss. **As mentioned below in the neurodegenerative disease section**, neutralisation of the inhibitory C4S with anti-C4S antibody restores object memory in a mouse tauopathy model [96]. These results indicate that the ratio of C6S:C4S is key to regulation of memory by PNNs.

Hyaluronan is another PNN component which demonstrates age-related changes in the brain. Long chain hyaluronan on the neuronal surface provides binding sites for the lectican family of CSPGs, which have a hyaluronan binding site, enabling hyaluronan to act as the backbone of the PNN [15]. Many studies show that the functions of hyaluronan depend on chain length. For example, low molecular weight hyaluronan is pro-inflammatory while high molecular weight hyaluronan is anti-inflammatory [177]. Changes in hyaluronan quantity have been reported in different pathological conditions such as ischemic and traumatic brain injury as well as in ageing [3, 178, 179]. A recent biochemical analysis of hyaluronan recovered from the PNNs in aged brains has shown its degradation into smaller fragments. This degradation has led to a release of other PNN components such as aggrecan into the soluble ECM [180]. Whether these age-related changes in hyaluronan affect memory is yet to be shown **(Figure 3C).**

PNNs in genetic cognitive disorders

Rett syndrome is a neurodevelopmental disorder characterised by normal early development but then profound regression in cognitive, motor, and social function. It is caused by a loss-of-function mutation in the gene methyl-CpG–binding protein 2 (MECP2). The condition is associated with larger denser PNNs around PV interneurons in the cortex and many neurons in hippocampal CA2 (which mediates social behaviours), possibly due to decreased secretion of the metalloproteinase MMP-9. The increased PNN density causes loss of LTP in hippocampal neurons, which can be restored by ChABC digestion. In the cortex there is also an increase in the number and complexity of PNNs around PV interneurons in a Rett syndrome model, altering cortical excitability [102, 181]. Fragile X syndrome is a heritable condition causing intellectual disability and autism, modelled in mice by Fmr1 knockout. In these mice, there is a decrease in PNNs and impaired PV interneuron development in the cortex, hippocampus, amygdala and elsewhere. As well as general disability, the animals have a loss of tone-associated fear memory. The PNN decrease is associated with increased production of MMP-9, and genetic reduction or inhibition of MMP-9 production restores normal auditory responses and normalizes behaviour [182]. Schizophrenia, which is associated with various memory disorders, is also associated with a decrease in PNN numbers and density in the amygdala, thalamic reticular nucleus, entorhinal cortex and prefrontal cortex of patients [183]. Schizophrenia is linked to abnormalities in PV+ interneurons and an imbalance between glutamatergic and GABAergic transmission. A current hypothesis is that loss of the neuroprotective activity of PNNs renders the fast-firing PV+ oxidant-generating neurons vulnerable to oxidative stress [184].

ECM memory in neurodegenerative disease

The main neurodegenerative disease associated with memory loss is Alzheimer's disease, and most of the data linking the ECM to neurodegeneration apply to this condition. The ECM, in particular heparan sulphate proteoglycans (HSPGs) and CSPGs, are implicated in the progression of Alzheimer's in several ways. In A-beta amyloid pathology, HSPGs bind to A-beta and are associated with plaques, affecting beta-amyloid precursor protein processing [185] and clearance [186]. Tau aggregation is promoted by proteoglycans [187], which are present in tangles, and are involved in the prion-like spread of tau pathology [188]. PNNs exclude tau pathology from the neurons that they surround, inhibiting tau uptake [189]. However, PNNs are themselves affected in Alzheimer's disease [190] and in Huntington's disease partly through engulfment by activated microglia [191, 192] **(Figure 3D).**

There is currently no treatment to prevent the progression of Alzheimer's disease. However, the condition leads to the malfunction or death of scattered neurons, so functional compensation requires plasticity, some aspects of which, including spine and synapse loss, are impaired in Alzheimer's, and some interventions aimed at synaptic transmission restore normal function [193]. From the perspective of the ECM, overall levels of plasticity can be restored to the levels normally associated with critical periods by manipulation of PNNs. Thus, digestion of PNNs in the perirhinal cortex of tauopathy mice restores object memory, ChABC digestion in A-beta pathology mice restores hippocampal function, and antibody blockade of the inhibitory 4-sulphated glycans of PNN CSPGs restores object memory [96, 193, 194]. Modification of PNNs in **Alzheimer's disease** could also come about through the action of activated microglia or secretion of metalloproteinases, both of which can occur in this condition [192, 195]. Reelin is an ECM-associated protein with effects on plasticity, and overexpression of this molecule restores memory in a tauopathy model [196]. Although much is yet to be understood about the role of PNNs in Alzheimer's disease progression and cognitive decline, these investigations point important and mostly uncovered territory to understand this disease and identify much-needed new drug targets.

CONCLUSION

The descriptions above show that the brain ECM, and particularly PNNs, play an important part in the regulation of memory and in memory pathology across a wide range of types of memory. This leads to the question of whether treatments that target PNNs could be useful for memory defects. At present, most of the evidence that memory can be modulated in useful ways comes from injections of ChABC into the CNS. This treatment is useful for proof-of-principle experiments, but is impracticable for long-term treatment of memory problems. However, there are many potential treatment targets in PNNs. An antibody that blocks inhibitory C4S has been effective at restoring memory in an Alzheimer's model, and AAV-mediated expression of C6-sulfotransferase to reinstate C6S levels has restored memory in ageing. Other potential targets are small molecule inhibitors of C4S synthesis or activators of C6S synthesis, hyaluronan production by hyaluronan synthases, viral-mediated knockdown of aggrecan, and modulation or blocking of the diffusible transcription factor OTX2 [1, 197]. Future research holds promise for further insight into the function of the ECM in cognition and for the development of novel treatments.

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REFERENCES

1. Fawcett JW, Oohashi T, Pizzorusso T. The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nat Rev Neurosci* 2019; **20**(8)**:** 451-465.

2. Venstrom KA, Reichardt LF. Extracellular Matrix 2: Role of extracellular matrix molecules and their receptors in the nervous system. *The FASEB Journal* 1993; **7**(11)**:** 996-1003.

3. Jenkins HG, Bachelard HS. Developmental and Age-Related Changes in Rat Brain Glycosaminoglycans. *Journal of Neurochemistry* 1988; **51**(5)**:** 1634-1640.

4. Kadomatsu K, Sakamoto K. Sulfated glycans in network rewiring and plasticity after neuronal injuries. *Neurosci Res* 2014; **78:** 50-54.

5. Wang H, Katagiri Y, McCann TE, Unsworth E, Goldsmith P, Yu ZX *et al.* Chondroitin-4-sulfation negatively regulates axonal guidance and growth. *Journal of cell science* 2008; **121**(Pt 18)**:** 3083-3091.

6. Bradbury EJ, Moon LDF, Popat RJ, King VR, Bennett GS, Patel PN *et al.* Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 2002; **416**(6881)**:** 636-640.

7. Asher RA, Morgenstern DA, Moon LDF, Fawcett JW. Chondroitin sulphate proteoglycans: inhibitory components of the glial scar. *Progress in Brain Research*, vol. Volume 132. Elsevier2001, pp 611-619.

8. Moon LDF, Asher RA, Rhodes KE, Fawcett JW. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. *Nat Neurosci* 2001; **4**(5)**:** 465-466.

9. McKeon RJ, Höke A, Silver J. Injury-Induced Proteoglycans Inhibit the Potential for Laminin-Mediated Axon Growth on Astrocytic Scars. *Experimental Neurology* 1995; **136**(1)**:** 32-43.

10. Yu P, Pearson CS, Geller HM. Flexible Roles for Proteoglycan Sulfation and Receptor Signaling. *Trends Neurosci* 2018; **41**(1)**:** 47-61.

11. Mikami T, Kitagawa H. Biosynthesis and function of chondroitin sulfate. *Biochim Biophys Acta* 2013; **1830**(10)**:** 4719-4733.

12. Lander C, Zhang H, Hockfield S. Neurons Produce a Neuronal Cell Surface-Associated Chondroitin Sulfate Proteoglycan. *J Neurosci* 1998; **18**(1)**:** 174-183.

13. Miyata S, Kitagawa H. Formation and remodeling of the brain extracellular matrix in neural plasticity: Roles of chondroitin sulfate and hyaluronan. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2017; **1861**(10)**:** 2420-2434.

14. Yamaguchi Y. Lecticans: organizers of the brain extracellular matrix. *Cellular and Molecular Life Sciences CMLS* 2000; **57**(2)**:** 276-289.

15. Kwok JCF, Carulli D, Fawcett JW. In vitro modeling of perineuronal nets: hyaluronan synthase and link protein are necessary for their formation and integrity. *J Neurochem* 2010; **114**(5)**:** 1447-1459.

16. Stevens SR, Longley CM, Ogawa Y, Teliska LH, Arumanayagam AS, Nair S *et al.* Ankyrin-R regulates fast-spiking interneuron excitability through perineuronal nets and Kv3.1b K(+) channels. *Elife* 2021; **10:** e66491.

17. Eill GJ, Sinha A, Morawski M, Viapiano MS, Matthews RT. The protein tyrosine phosphatase RPTPzeta/phosphacan is critical for perineuronal net structure. *J Biol Chem* 2020; **295**(4)**:** 955-968.

18. Giamanco KA, Matthews RT. Deconstructing the perineuronal net: cellular contributions and molecular composition of the neuronal extracellular matrix. *Neuroscience* 2012; **218:** 367-384.

19. Aspberg A, Miura R, Bourdoulous S, Shimonaka M, Heinegård D, Schachner M *et al.* The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein– protein interactions independent of carbohydrate moiety. *Proc Natl Acad of Sci USA* 1997; **94**(19)**:** 10116-10121.

20. Morawski M, Dityatev A, Hartlage-Rübsamen M, Blosa M, Holzer M, Flach K *et al.* Tenascin-R promotes assembly of the extracellular matrix of perineuronal nets via clustering of aggrecan. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 2014; **369**(1654)**:** 20140046.

21. Oohashi T, Edamatsu M, Bekku Y, Carulli D. The hyaluronan and proteoglycan link proteins: Organizers of the brain extracellular matrix and key molecules for neuronal function and plasticity. *Experimental Neurology* 2015; **274, Part B:** 134-144.

22. Arranz AM, Perkins KL, Irie F, Lewis DP, Hrabe J, Xiao F *et al.* Hyaluronan deficiency due to Has3 knock-out causes altered neuronal activity and seizures via reduction in brain extracellular space. *J Neurosci* 2014; **34**(18)**:** 6164-6176.

23. Beurdeley M, Spatazza J, Lee HH, Sugiyama S, Bernard C, Di Nardo AA *et al.* Otx2 binding to perineuronal nets persistently regulates plasticity in the mature visual cortex. *J Neurosci* 2012; **32**(27)**:** 9429-9437.

24. Chang MC, Park JM, Pelkey KA, Grabenstatter HL, Xu D, Linden DJ *et al.* Narp regulates homeostatic scaling of excitatory synapses on parvalbumin-expressing interneurons. *Nat Neurosci* 2010; **13**(9)**:** 1090-1097.

25. De Wit J, De Winter F, Klooster J, Verhaagen J. Semaphorin 3A displays a punctate distribution on the surface of neuronal cells and interacts with proteoglycans in the extracellular matrix. *Mol Cell Neurosci* 2005; **29**(1)**:** 40-55.

26. Spatazza J, Lee HH, Di Nardo AA, Tibaldi L, Joliot A, Hensch TK *et al.* Choroid-plexus-derived Otx2 homeoprotein constrains adult cortical plasticity. *Cell Rep* 2013; **3**(6)**:** 1815-1823.

27. Vo T, Carulli D, Ehlert EME, Kwok JCF, Dick G, Mecollari V *et al.* The chemorepulsive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. *Mol Cell Neurosci* 2013; **56:** 186-200.

28. Dick G, Tan CL, Alves JN, Ehlert EM, Miller GM, Hsieh-Wilson LC *et al.* Semaphorin 3A binds to the perineuronal nets via chondroitin sulfate type E motifs in rodent brains. *J Biol Chem* 2013; **288**(38)**:** 27384-27395.

29. Van't Spijker HM, Rowlands D, Rossier J, Haenzi B, Fawcett JW, Kwok JCF. Neuronal Pentraxin 2 Binds PNNs and Enhances PNN Formation. *Neural Plasticity* 2019; **2019:** 6804575.

30. Boggio EM, Ehlert EM, Lupori L, Moloney EB, De Winter F, Vander Kooi CW *et al.* Inhibition of Semaphorin3A Promotes Ocular Dominance Plasticity in the Adult Rat Visual Cortex. *Molecular neurobiology* 2019; **56**(9)**:** 5987-5997.

31. Carulli D, Broersen R, de Winter F, Muir EM, Meskovic M, de Waal M *et al.* Cerebellar plasticity and associative memories are controlled by perineuronal nets. *Proc Natl Acad Sci U S A* 2020; **117**(12)**:** 6855-6865.

32. Djerbal L, Vivès RR, Lopin-Bon C, Richter RP, Kwok JCF, Lortat-Jacob H. Semaphorin 3A binding to chondroitin sulfate E enhances the biological activity of the protein, and cross-links and rigidifies glycosaminoglycan matrices. *bioRxiv* 2019**:** 851121.

33. Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM *et al.* Modulation of the proteoglycan receptor PTPsigma promotes recovery after spinal cord injury. *Nature* 2015; **518**(7539)**:** 404-408.

34. Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sanchez-Aguilera A, Mantoan L *et al.* Activity-Dependent Gating of Parvalbumin Interneuron Function by the Perineuronal Net Protein Brevican. *Neuron* 2017; **95**(3)**:** 639-655 e610.

35. Frischknecht R, Heine M, Perrais D, Seidenbecher CI, Choquet D, Gundelfinger ED. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nat Neurosci* 2009; **12**(7)**:** 897-904.

36. Ethell IM, Ethell DW. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. *J Neurosci Res* 2007; **85**(13)**:** 2813-2823.

37. Beroun A, Mitra S, Michaluk P, Pijet B, Stefaniuk M, Kaczmarek L. MMPs in learning and memory and neuropsychiatric disorders. *Cell Mol Life Sci* 2019; **76**(16)**:** 3207-3228.

38. Mitlohner J, Kaushik R, Niekisch H, Blondiaux A, Gee CE, Happel MFK *et al.* Dopamine Receptor Activation Modulates the Integrity of the Perisynaptic Extracellular Matrix at Excitatory Synapses. *Cells* 2020; **9**(2)**:** 260.

39. Nguyen PT, Dorman LC, Pan S, Vainchtein ID, Han RT, Nakao-Inoue H *et al.* Microglial Remodeling of the Extracellular Matrix Promotes Synapse Plasticity. *Cell* 2020; **182**(2)**:** 388-403 e315.

40. Orlando c, Ster J, Gerber U, Fawcett JW, Raineteau O. Peridendritic chondroitin sulfate proteoglycans restrict structural plasticity in an integrin-dependent manner. *J Neurosci* 2012; **32:** 18009-18017.

41. de Vivo L, Landi S, Panniello M, Baroncelli L, Chierzi S, Mariotti L *et al.* Extracellular matrix inhibits structural and functional plasticity of dendritic spines in the adult visual cortex. *Nat Commun* 2013; **4:** 1484.

42. Oray S, Majewska A, Sur M. Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. *Neuron* 2004; **44**(6)**:** 1021-1030.

43. Donato F, Rompani SB, Caroni P. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 2013; **504**(7479)**:** 272-276.

44. Ruzicka J, Dalecka M, Safrankova K, Peretti D, Mallucci G, Jendelova P *et al.* Perineuronal nets affect memory and learning after synapse withdrawal. *bioRxiv* 2021.

45. Yang S, Gigout S, Molinaro A, Naito-Matsui Y, Hilton S, Foscarin S *et al.* Chondroitin 6-sulphate is required for neuroplasticity and memory in ageing. *Mol Psychiatry* 2021.

46. Christensen AC, Lensjo KK, Lepperod ME, Dragly SA, Sutterud H, Blackstad JS *et al.* Perineuronal nets stabilize the grid cell network. *Nat Commun* 2021; **12**(1)**:** 253.

47. Lensjo KK, Lepperod ME, Dick G, Hafting T, Fyhn M. Removal of Perineuronal Nets Unlocks Juvenile Plasticity Through Network Mechanisms of Decreased Inhibition and Increased Gamma Activity. *J Neurosci* 2017; **37**(5)**:** 1269-1283.

48. Corvetti L, Rossi F. Degradation of chondroitin sulfate proteoglycans induces sprouting of intact purkinje axons in the cerebellum of the adult rat. *J Neurosci* 2005; **25**(31)**:** 7150-7158.

49. Sullivan CS, Gotthard I, Wyatt EV, Bongu S, Mohan V, Weinberg RJ *et al.* Perineuronal Net Protein Neurocan Inhibits NCAM/EphA3 Repellent Signaling in GABAergic Interneurons. *Sci Rep* 2018; **8**(1)**:** 6143.

50. Choquet D, Opazo P. The role of AMPAR lateral diffusion in memory. *Semin Cell Dev Biol* 2022; **125:** 76-83.

51. Schweitzer B, Singh J, Fejtova A, Groc L, Heine M, Frischknecht R. Hyaluronic acid based extracellular matrix regulates surface expression of GluN2B containing NMDA receptors. *Sci Rep* 2017; **7**(1)**:** 10991.

52. Edamatsu M, Miyano R, Fujikawa A, Fujii F, Hori T, Sakaba T *et al.* Hapln4/Bral2 is a selective regulator for formation and transmission of GABAergic synapses between Purkinje and deep cerebellar nuclei neurons. *J Neurochem* 2018; **14**(6)**:** 748-763.

53. Romberg C, Yang S, Melani R, Andrews MR, Horner AE, Spillantini MG *et al.* Depletion of Perineuronal Nets Enhances Recognition Memory and Long-Term Depression in the Perirhinal Cortex. *J Neurosci* 2013; **33**(16)**:** 7057-7065.

54. Heikkinen A, Pihlajaniemi T, Faissner A, Yuzaki M. Neural ECM and synaptogenesis. *Prog Brain Res* 2014; **214:** 29-51.

55. Simonetti M, Paldy E, Njoo C, Bali KK, Worzfeld T, Pitzer C *et al.* The impact of Semaphorin 4C/Plexin-B2 signaling on fear memory via remodeling of neuronal and synaptic morphology. *Mol Psychiatry* 2021; **26**(4)**:** 1376-1398.

56. Carulli D, de Winter F, Verhaagen J. Semaphorins in Adult Nervous System Plasticity and Disease. *Front Synaptic Neurosci* 2021; **13:** 672891.

57. Apostolova I, Irintchev A, Schachner M. Tenascin-R restricts posttraumatic remodeling of motoneuron innervation and functional recovery after spinal cord injury in adult mice. *J Neurosci* 2006; **26**(30)**:** 7849-7859.

58. Wiera G, Mozrzymas JW. Extracellular Metalloproteinases in the Plasticity of Excitatory and Inhibitory Synapses. *Cells* 2021; **10**(8)**:** 2055.

59. Wingert JC, Sorg BA. Impact of Perineuronal Nets on Electrophysiology of Parvalbumin Interneurons, Principal Neurons, and Brain Oscillations: A Review. *Front Synaptic Neurosci* 2021; **13:** 673210.

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

61. Kochlamazashvili G, Henneberger C, Bukalo O, Dvoretskova E, Senkov O, Lievens PM *et al.* The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca(2+) channels. *Neuron* 2010; **67**(1)**:** 116-128.

62. Shi W, Wei X, Wang X, Du S, Liu W, Song J *et al.* Perineuronal nets protect long-term memory by limiting activity-dependent inhibition from parvalbumin interneurons. *Proc Natl Acad Sci U S A* 2019.

63. Saghatelyan AK, Dityatev A, Schmidt S, Schuster T, Bartsch U, Schachner M. Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R. *Mol Cell Neurosci* 2001; **17**(1)**:** 226-240.

64. Zhou XH, Brakebusch C, Matthies H, Oohashi T, Hirsch E, Moser M *et al.* Neurocan is dispensable for brain development. *Mol Cell Biol* 2001; **21**(17)**:** 5970-5978.

65. Brakebusch C, Seidenbecher CI, Asztely F, Rauch U, Matthies H, Meyer H *et al.* Brevican-deficient mice display impaired hippocampal CA1 long-term potentiation but show no obvious deficits in learning and memory. *Mol Cell Biol* 2002; **22**(21)**:** 7417-7427.

66. Carstens KE, Phillips ML, Pozzo-Miller L, Weinberg RJ, Dudek SM. Perineuronal Nets Suppress Plasticity of Excitatory Synapses on CA2 Pyramidal Neurons. *J Neurosci* 2016; **36**(23)**:** 6312-6320.

67. Khoo GH, Lin YT, Tsai TC, Hsu KS. Perineuronal Nets Restrict the Induction of Long-Term Depression in the Mouse Hippocampal CA1 Region. *Molecular neurobiology* 2019; **56**(9)**:** 6436-6450.

68. Gottschling C, Wegrzyn D, Denecke B, Faissner A. Elimination of the four extracellular matrix molecules tenascin-C, tenascin-R, brevican and neurocan alters the ratio of excitatory and inhibitory synapses. *Sci Rep* 2019; **9**(1)**:** 13939.

69. Rowlands D, Lensjo KK, Dinh T, Yang S, Andrews MR, Hafting T *et al.* Aggrecan Directs Extracellular Matrix-Mediated Neuronal Plasticity. *J Neurosci* 2018; **38**(47)**:** 10102-10113.

70. Hirono M, Watanabe S, Karube F, Fujiyama F, Kawahara S, Nagao S *et al.* Perineuronal Nets in the Deep Cerebellar Nuclei Regulate GABAergic Transmission and Delay Eyeblink Conditioning. *J Neurosci* 2018; **38**(27)**:** 6130-6144.

71. Lensjo KK, Christensen AC, Tennoe S, Fyhn M, Hafting T. Differential Expression and Cell-Type Specificity of Perineuronal Nets in Hippocampus, Medial Entorhinal Cortex, and Visual Cortex Examined in the Rat and Mouse. *eNeuro* 2017; **4**(3)**:** ENEURO.0379-0316.2017.

72. Blosa M, Sonntag M, Jager C, Weigel S, Seeger J, Frischknecht R *et al.* The extracellular matrix molecule brevican is an integral component of the machinery mediating fast synaptic transmission at the calyx of Held. *J Physiol* 2015; **593**(19)**:** 4341-4360.

73. Nikonenko A, Schmidt S, Skibo G, Bruckner G, Schachner M. Tenascin-R-deficient mice show structural alterations of symmetric perisomatic synapses in the CA1 region of the hippocampus. *J Comp Neurol* 2003; **456**(4)**:** 338-349.

74. Evers MR, Salmen B, Bukalo O, Rollenhagen A, Bosl MR, Morellini F *et al.* Impairment of L-type Ca2+ channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C. *J Neurosci* 2002; **22**(16)**:** 7177-7194.

75. Carulli D, Verhaagen J. An Extracellular Perspective on CNS Maturation: Perineuronal Nets and the Control of Plasticity. *Int J Mol Sci* 2021; **22**(5).

76. Freeman JH, Steinmetz AB. Neural circuitry and plasticity mechanisms underlying delay eyeblink conditioning. *Learn Mem* 2011; **18**(10)**:** 666-677.

77. Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K *et al.* Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. *J Comp Neurol* 2006; **494**(4)**:** 559-577.

78. O'Dell DE, Schreurs BG, Smith-Bell C, Wang D. Disruption of rat deep cerebellar perineuronal net alters eyeblink conditioning and neuronal electrophysiology. *Neurobiol Learn Mem* 2021; **177:** 107358.

79. *American Psychiatric Association: Diagnostic and statistical manual of mental disorders.* 5th edn, 2013.

80. LeDoux JE, Pine DS. Using Neuroscience to Help Understand Fear and Anxiety: A Two-System Framework. *Am J Psychiatry* 2016; **173**(11)**:** 1083-1093.

81. Maeng LY, Milad MR. Post-Traumatic Stress Disorder: The Relationship Between the Fear Response and Chronic Stress. *Chronic Stress (Thousand Oaks)* 2017; **1:** 2470547017713297.

82. Gogolla N, Caroni P, Luthi A, Herry C. Perineuronal nets protect fear memories from erasure. *Science* 2009; **325**(5945)**:** 1258-1261.

83. Hylin MJ, Orsi SA, Moore AN, Dash PK. Disruption of the perineuronal net in the hippocampus or medial prefrontal cortex impairs fear conditioning. *Learning & Memory* 2013; **20**(5)**:** 267-273.

84. Banerjee SB, Gutzeit VA, Baman J, Aoued HS, Doshi NK, Liu RC *et al.* Perineuronal Nets in the Adult Sensory Cortex Are Necessary for Fear Learning. *Neuron* 2017; **95**(1)**:** 169-179 e163.

85. Gunduz-Cinar O, Brockway E, Lederle L, Wilcox T, Halladay LR, Ding Y *et al.* Identification of a novel gene regulating amygdala-mediated fear extinction. *Mol Psychiatry* 2019; **24**(4)**:** 601-612.

86. Morikawa S, Ikegaya Y, Narita M, Tamura H. Activation of perineuronal net-expressing excitatory neurons during associative memory encoding and retrieval. *Sci Rep* 2017; **7:** 46024.

87. Lesnikova A, Casarotto PC, Fred SM, Voipio M, Winkel F, Steinzeig A *et al.* Chondroitinase and Antidepressants Promote Plasticity by Releasing TRKB from Dephosphorylating Control of PTPsigma in Parvalbumin Neurons. *J Neurosci* 2021; **41**(5)**:** 972-980.

88. Lesnikova A, Casarotto P, Moliner R, Fred SM, Biojone C, Castren E. Perineuronal Net Receptor PTPsigma Regulates Retention of Memories. *Front Synaptic Neurosci* 2021; **13:** 672475.

89. Engel AK, Fries P, Singer W. Dynamic predictions: oscillations and synchrony in top-down processing. *Nat Rev Neurosci* 2001; **2**(10)**:** 704-716.

90. Uhlhaas PJ, Roux F, Rodriguez E, Rotarska-Jagiela A, Singer W. Neural synchrony and the development of cortical networks. *Trends Cogn Sci* 2010; **14**(2)**:** 72-80.

91. Buzsaki G, Wang XJ. Mechanisms of gamma oscillations. *Annu Rev Neurosci* 2012; **35:** 203-225.

92. Stark E, Eichler R, Roux L, Fujisawa S, Rotstein HG, Buzsaki G. Inhibition-induced theta resonance in cortical circuits. *Neuron* 2013; **80**(5)**:** 1263-1276.

93. Sacco T, Sacchetti B. Role of secondary sensory cortices in emotional memory storage and retrieval in rats. *Science* 2010; **329**(5992)**:** 649-656.

94. Thompson EH, Lensjo KK, Wigestrand MB, Malthe-Sorenssen A, Hafting T, Fyhn M. Removal of perineuronal nets disrupts recall of a remote fear memory. *Proc Natl Acad Sci U S A* 2018; **115**(3)**:** 607-612.

95. Yang S, Cacquevel M, Saksida LM, Bussey TJ, Schneider BL, Aebischer P *et al.* Perineuronal net digestion with chondroitinase restores memory in mice with tau pathology. *Exp Neurol* 2015; **265:** 48-58.

96. Yang S, Hilton S, Alves JN, Saksida LM, Bussey T, Matthews RT *et al.* Antibody recognizing 4-sulfated chondroitin sulfate proteoglycans restores memory in tauopathy-induced neurodegeneration. *Neurobiol Aging* 2017; **59:** 197-209.

97. Aggleton JP, Brown MW, Albasser MM. Contrasting brain activity patterns for item recognition memory and associative recognition memory: insights from immediate-early gene functional imaging. *Neuropsychologia* 2012; **50**(13)**:** 3141-3155.

98. Cinalli DA, Cohen SJ, Guthrie K, Stackman RW. Object Recognition Memory: Distinct Yet Complementary Roles of the Mouse CA1 and Perirhinal Cortex. *Front Mol Neurosci* 2020; **13:** 527543.

99. Aggleton JP, Nelson AJD. Distributed interactive brain circuits for object-in-place memory: A place for time? *Brain Neurosci Adv* 2020; **4:** 2398212820933471.

100. Tanimizu T, Kono K, Kida S. Brain networks activated to form object recognition memory. *Brain Res Bull* 2018; **141:** 27-34.

101. Lensjo KK, Christensen AC, Tennoe S, Fyhn M, Hafting T. Differential Expression and Cell-Type Specificity of Perineuronal Nets in Hippocampus, Medial Entorhinal Cortex, and Visual Cortex Examined in the Rat and Mouse. *eNeuro* 2017; **4**(3).

102. Carstens KE, Lustberg DJ, Shaughnessy EK, McCann KE, Alexander GM, Dudek SM. Perineuronal net degradation rescues CA2 plasticity in a mouse model of Rett syndrome. *J Clin Invest* 2021; **131**(16).

103. O'Keefe J, Dostrovsky J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 1971; **34**(1)**:** 171-175.

104. Fyhn M, Molden S, Witter MP, Moser EI, Moser MB. Spatial representation in the entorhinal cortex. *Science* 2004; **305**(5688)**:** 1258-1264.

105. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. *Nature* 2005; **436**(7052)**:** 801-806.

106. Hoydal OA, Skytoen ER, Andersson SO, Moser MB, Moser EI. Object-vector coding in the medial entorhinal cortex. *Nature* 2019; **568**(7752)**:** 400-404.

107. Kropff E, Carmichael JE, Moser MB, Moser EI. Speed cells in the medial entorhinal cortex. *Nature* 2015; **523**(7561)**:** 419-424.

108. Avigan PD, Cammack K, Shapiro ML. Flexible spatial learning requires both the dorsal and ventral hippocampus and their functional interactions with the prefrontal cortex. *Hippocampus* 2020; **30**(7)**:** 733-744.

109. Poitreau J, Buttet M, Manrique C, Poucet B, Sargolini F, Save E. Navigation using global or local reference frames in rats with medial and lateral entorhinal cortex lesions. *Behav Brain Res* 2021; **413:** 113448.

110. Bertocchi I, Mele P, Ferrero G, Oberto A, Carulli D, Eva C. NPY-Y1 receptor signaling controls spatial learning and perineuronal net expression. *Neuropharmacology* 2021; **184:** 108425.

111. Yoshino Y, Shimazawa M, Nakamura S, Inoue S, Yoshida H, Shimoda M *et al.* Targeted deletion of HYBID (hyaluronan binding protein involved in hyaluronan depolymerization/ KIAA1199/CEMIP) decreases dendritic spine density in the dentate gyrus through hyaluronan accumulation. *Biochem Biophys Res Commun* 2018; **503**(3)**:** 1934-1940.

112. Riga D, Kramvis I, Koskinen MK, van Bokhoven P, van der Harst JE, Heistek TS *et al.* Hippocampal extracellular matrix alterations contribute to cognitive impairment associated with a chronic depressive-like state in rats. *Sci Transl Med* 2017; **9**(421)**:** 8753.

113. Morellini F, Sivukhina E, Stoenica L, Oulianova E, Bukalo O, Jakovcevski I *et al.* Improved reversal learning and working memory and enhanced reactivity to novelty in mice with enhanced GABAergic innervation in the dentate gyrus. *Cereb Cortex* 2010; **20**(11)**:** 2712-2727.

114. Chao OY, de Souza Silva MA, Yang YM, Huston JP. The medial prefrontal cortex - hippocampus circuit that integrates information of object, place and time to construct episodic memory in rodents: Behavioral, anatomical and neurochemical properties. *Neurosci Biobehav Rev* 2020; **113:** 373-407.

115. Savalli G, Bashir ZI, Warburton EC. Regionally selective requirement for D1/D5 dopaminergic neurotransmission in the medial prefrontal cortex in object-in-place associative recognition memory. *Learn Mem* 2015; **22**(2)**:** 69-73.

116. Porter JT, Sepulveda-Orengo MT. Learning-induced intrinsic and synaptic plasticity in the rodent medial prefrontal cortex. *Neurobiol Learn Mem* 2020; **169:** 107117.

117. Slaker M, Churchill L, Todd RP, Blacktop JM, Zuloaga DG, Raber J *et al.* Removal of perineuronal nets in the medial prefrontal cortex impairs the acquisition and reconsolidation of a cocaine-induced conditioned place preference memory. *J Neurosci* 2015; **35**(10)**:** 4190-4202.

118. Talpos JC, Winters BD, Dias R, Saksida LM, Bussey TJ. A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the

hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease. *Psychopharmacology (Berl)* 2009; **205**(1)**:** 157-168.

119. Anderson MD, Paylor JW, Scott GA, Greba Q, Winship IR, Howland JG. ChABC infusions into medial prefrontal cortex, but not posterior parietal cortex, improve the performance of rats tested on a novel, challenging delay in the touchscreen TUNL task. *Learn Mem* 2020; **27**(6)**:** 222-235.

120. Choleris E, Clipperton-Allen AE, Phan A, Kavaliers M. Neuroendocrinology of social information processing in rats and mice. *Front Neuroendocrinol* 2009; **30**(4)**:** 442-459.

121. Engelmann M, Hädicke J, Noack J. Testing declarative memory in laboratory rats and mice using the nonconditioned social discrimination procedure. *Nat Protoc* 2011; **6**(8)**:** 1152-1162.

122. Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. *Nat Rev Neurosci* 2010; **11**(7)**:** 490-502.

123. Tanimizu T, Kenney JW, Okano E, Kadoma K, Frankland PW, Kida S. Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social Recognition Memory. *J Neurosci* 2017; **37**(15)**:** 4103-4116.

124. Reichelt AC, Gibson GD, Abbott KN, Hare DJ. A high-fat high-sugar diet in adolescent rats impairs social memory and alters chemical markers characteristic of atypical neuroplasticity and parvalbumin interneuron depletion in the medial prefrontal cortex. *Food Funct* 2019; **10**(4)**:** 1985-1998.

125. Hitti FL, Siegelbaum SA. The hippocampal CA2 region is essential for social memory. *Nature* 2014; **508**(7494)**:** 88-92.

126. Meira T, Leroy F, Buss EW, Oliva A, Park J, Siegelbaum SA. A hippocampal circuit linking dorsal CA2 to ventral CA1 critical for social memory dynamics. *Nat Commun* 2018; **9**(1)**:** 4163.

127. Oliva A, Fernández-Ruiz A, Leroy F, Siegelbaum SA. Hippocampal CA2 sharp-wave ripples reactivate and promote social memory. *Nature* 2020; **587**(7833)**:** 264-269.

128. Stevenson EL, Caldwell HK. Lesions to the CA2 region of the hippocampus impair social memory in mice. *Eur J Neurosci* 2014; **40**(9)**:** 3294-3301.

129. Leroy F, Park J, Asok A, Brann DH, Meira T, Boyle LM *et al.* A circuit from hippocampal CA2 to lateral septum disinhibits social aggression. *Nature* 2018; **564**(7735)**:** 213-218.

130. McDonald AJ, Hamilton PG, Barnstable CJ. Perineuronal nets labeled by monoclonal antibody VC1.1 ensheath interneurons expressing parvalbumin and calbindin in the rat amygdala. *Brain Struct Funct* 2018; **223**(3)**:** 1133-1148.

131. Steullet P, Cabungcal JH, Cuenod M, Do KQ. Fast oscillatory activity in the anterior cingulate cortex: dopaminergic modulation and effect of perineuronal net loss. *Frontiers in cellular neuroscience* 2014; **8:** 244.

132. Cope EC, Zych AD, Katchur NJ, Waters RC, Laham BJ, Diethorn EJ *et al.* Atypical perineuronal nets in the CA2 region interfere with social memory in a mouse model of social dysfunction. *Mol Psychiatry* 2021.

133. Dominguez S, Rey CC, Therreau L, Fanton A, Massotte D, Verret L *et al.* Maturation of PNN and ErbB4 Signaling in Area CA2 during Adolescence Underlies the Emergence of PV Interneuron Plasticity and Social Memory. *Cell Rep* 2019; **29**(5)**:** 1099-1112 e1094.

134. Tatsukawa T, Ogiwara I, Mazaki E, Shimohata A, Yamakawa K. Impairments in social novelty recognition and spatial memory in mice with conditional deletion of Scn1a in parvalbumin-expressing cells. *Neurobiol Dis* 2018; **112:** 24-34.

135. Lee HHC, Bernard C, Ye Z, Acampora D, Simeone A, Prochiantz A *et al.* Genetic Otx2 mis-localization delays critical period plasticity across brain regions. *Mol Psychiatry* 2017; **22**(5)**:** 785.

136. Reinhard SM, Abundez-Toledo M, Espinoza K, Razak KA. Effects of developmental noise exposure on inhibitory cell densities and perineuronal nets in A1 and AAF of mice. *Hear Res* 2019; **381:** 107781.

137. Myers AK, Ray J, Kulesza RJ, Jr. Neonatal conductive hearing loss disrupts the development of the Cat-315 epitope on perineuronal nets in the rat superior olivary complex. *Brain Res* 2012; **1465:** 34-47.

138. Sharma A, Dorman MF, Spahr AJ. A sensitive period for the development of the central auditory system in children with cochlear implants: implications for age of implantation. *Ear Hear* 2002; **23**(6)**:** 532-539.

139. Balmer TS, Carels VM, Frisch JL, Nick TA. Modulation of perineuronal nets and parvalbumin with developmental song learning. *J Neurosci* 2009; **29**(41)**:** 12878-12885.

140. Happel MF, Niekisch H, Castiblanco Rivera LL, Ohl FW, Deliano M, Frischknecht R. Enhanced cognitive flexibility in reversal learning induced by removal of the extracellular matrix in auditory cortex. *Proc Natl Acad Sci U S A* 2014; **111**(7)**:** 2800-2805.

141. Niekisch H, Steinhardt J, Berghauser J, Bertazzoni S, Kaschinski E, Kasper J *et al.* Learning Induces Transient Upregulation of Brevican in the Auditory Cortex during Consolidation of Long-Term Memories. *J Neurosci* 2019; **39**(36)**:** 7049-7060.

142. Saroja SR, Sase A, Kircher SG, Wan J, Berger J, Höger H *et al.* Hippocampal proteoglycans brevican and versican are linked to spatial memory of Sprague-Dawley rats in the morris water maze. *J Neurochem* 2014; **130**(6)**:** 797-804.

143. Bijata M, Labus J, Guseva D, Stawarski M, Butzlaff M, Dzwonek J *et al.* Synaptic Remodeling Depends on Signaling between Serotonin Receptors and the Extracellular Matrix. *Cell Rep* 2017; **19**(9)**:** 1767-1782.

144. Dankovich TM, Kaushik R, Olsthoorn LHM, Petersen GC, Giro PE, Kluever V *et al.* Extracellular matrix remodeling through endocytosis and resurfacing of Tenascin-R. *Nat Commun* 2021; **12**(1)**:** 7129.

145. Spijker S, Koskinen MK, Riga D. Incubation of depression: ECM assembly and parvalbumin interneurons after stress. *Neurosci Biobehav Rev* 2020; **118:** 65-79.

146. Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Matsumoto Y *et al.* Juvenile stress induces behavioral change and affects perineuronal net formation in juvenile mice. *BMC Neurosci* 2018; **19**(1)**:** 41.

147. Gildawie KR, Honeycutt JA, Brenhouse HC. Region-specific Effects of Maternal Separation on Perineuronal Net and Parvalbumin-expressing Interneuron Formation in Male and Female Rats. *Neuroscience* 2020; **428:** 23-37.

148. Murthy S, Kane GA, Katchur NJ, Lara Mejia PS, Obiofuma G, Buschman TJ *et al.* Perineuronal Nets, Inhibitory Interneurons, and Anxiety-Related Ventral Hippocampal Neuronal Oscillations Are Altered by Early Life Adversity. *Biol Psychiatry* 2019; **85**(12)**:** 1011-1020.

149. Guadagno A, Verlezza S, Long H, Wong TP, Walker CD. It Is All in the Right Amygdala: Increased Synaptic Plasticity and Perineuronal Nets in Male, But Not Female, Juvenile Rat Pups after Exposure to Early-Life Stress. *J Neurosci* 2020; **40**(43)**:** 8276-8291.

150. Pesarico AP, Bueno-Fernandez C, Guirado R, Gomez-Climent MA, Curto Y, Carceller H *et al.* Chronic Stress Modulates Interneuronal Plasticity: Effects on PSA-NCAM and Perineuronal Nets in Cortical and Extracortical Regions. *Frontiers in cellular neuroscience* 2019; **13:** 197.

151. Koskinen MK, van Mourik Y, Smit AB, Riga D, Spijker S. From stress to depression: development of extracellular matrix-dependent cognitive impairment following social stress. *Sci Rep* 2020; **10**(1)**:** 17308.

152. Mennesson M, Orav E, Gigliotta A, Kulesskaya N, Saarnio S, Kirjavainen A *et al.* Kainate Receptor Auxiliary Subunit NETO2-Related Cued Fear Conditioning Impairments Associate with Defects in Amygdala Development and Excitability. *eNeuro* 2020; **7**(4).

153. Lasek AW, Chen H, Chen WY. Releasing Addiction Memories Trapped in Perineuronal Nets. *Trends Genet* 2018; **34**(3)**:** 197-208.

154. Slaker ML, Jorgensen ET, Hegarty DM, Liu X, Kong Y, Zhang F *et al.* Cocaine Exposure Modulates Perineuronal Nets and Synaptic Excitability of Fast-Spiking Interneurons in the Medial Prefrontal Cortex. *eNeuro* 2018; **5**(5).

155. Gil-Miravet I, Guarque-Chabrera J, Carbo-Gas M, Olucha-Bordonau F, Miquel M. The role of the cerebellum in drug-cue associative memory: functional interactions with the medial prefrontal cortex. *Eur J Neurosci* 2019; **50**(3)**:** 2613-2622.

156. Vazquez-Sanroman DB, Monje RD, Bardo MT. Nicotine self-administration remodels perineuronal nets in ventral tegmental area and orbitofrontal cortex in adult male rats. *Addict Biol* 2017; **22**(6)**:** 1743-1755.

157. Saito M, Smiley JF, Hui M, Masiello K, Betz J, Ilina M *et al.* Neonatal Ethanol Disturbs the Normal Maturation of Parvalbumin Interneurons Surrounded by Subsets of Perineuronal Nets in the Cerebral Cortex: Partial Reversal by Lithium. *Cereb Cortex* 2019; **29**(4)**:** 1383-1397.

158. Chen H, He D, Lasek AW. Repeated Binge Drinking Increases Perineuronal Nets in the Insular Cortex. *Alcoholism, clinical and experimental research* 2015; **39**(10)**:** 1930-1938.

159. Blacktop JM, Todd RP, Sorg BA. Role of perineuronal nets in the anterior dorsal lateral hypothalamic area in the acquisition of cocaine-induced conditioned place preference and self-administration. *Neuropharmacology* 2017; **118:** 124-136.

160. Blacktop JM, Sorg BA. Perineuronal nets in the lateral hypothalamus area regulate cue-induced reinstatement of cocaine-seeking behavior. *Neuropsychopharmacology* 2019; **44**(5)**:** 850-858.

161. Vazquez-Sanroman D, Carbo-Gas M, Leto K, Cerezo-Garcia M, Gil-Miravet I, Sanchis-Segura C *et al.* Cocaine-induced plasticity in the cerebellum of sensitised mice. *Psychopharmacology (Berl)* 2015; **232**(24)**:** 4455-4467.

162. Sanchez-Hernandez A, Nicolas C, Gil-Miravet I, Guarque-Chabrera J, Solinas M, Miquel M. Time-dependent regulation of perineuronal nets in the cerebellar cortex during abstinence of cocaine-self administration. *Psychopharmacology (Berl)* 2021; **238**(4)**:** 1059-1068.

163. Carbo-Gas M, Moreno-Rius J, Guarque-Chabrera J, Vazquez-Sanroman D, Gil-Miravet I, Carulli D *et al.* Cerebellar perineuronal nets in cocaine-induced pavlovian memory: Site matters. *Neuropharmacology* 2017; **125:** 166-180.

164. Vazquez-Sanroman D, Leto K, Cerezo-Garcia M, Carbo-Gas M, Sanchis-Segura C, Carulli D *et al.* The cerebellum on cocaine: plasticity and metaplasticity. *Addict Biol* 2015; **20**(5)**:** 941-955.

165. Van den Oever MC, Lubbers BR, Goriounova NA, Li KW, Van der Schors RC, Loos M *et al.* Extracellular matrix plasticity and GABAergic inhibition of prefrontal cortex pyramidal cells facilitates relapse to heroin seeking. *Neuropsychopharmacology* 2010; **35**(10)**:** 2120-2133.

166. Roura-Martinez D, Diaz-Bejarano P, Ucha M, Paiva RR, Ambrosio E, Higuera-Matas A. Comparative analysis of the modulation of perineuronal nets in the prefrontal cortex of rats during protracted withdrawal from cocaine, heroin and sucrose self-administration. *Neuropharmacology* 2020; **180:** 108290.

167. Xue Y-X, Xue L-F, Liu J-F, He J, Deng J-H, Sun S-C *et al.* Depletion of Perineuronal Nets in the Amygdala to Enhance the Erasure of Drug Memories. *J Neurosci* 2014; **34**(19)**:** 6647-6658.

168. Xue Y-X, Xue L-F, Liu J-F, He J, Deng J-H, Sun S-C *et al.* Depletion of Perineuronal Nets in the Amygdala to Enhance the Erasure of Drug Memories. *The Journal of neuroscience* 2014; **34**(19)**:** 6647-6658.

169. Lubbers BR, Matos MR, Horn A, Visser E, Van der Loo RC, Gouwenberg Y *et al.* The Extracellular Matrix Protein Brevican Limits Time-Dependent Enhancement of Cocaine Conditioned Place Preference. *Neuropsychopharmacology* 2016; **41**(7)**:** 1907-1916.

170. Chen H, Lasek AW. Perineuronal nets in the insula regulate aversion-resistant alcohol drinking. *Addict Biol* 2019**:** e12821.

171. Dong Y, Nestler EJ. The neural rejuvenation hypothesis of cocaine addiction. *Trends Pharmacol Sci* 2014; **35**(8)**:** 374-383.

172. Bin Ibrahim MZ, Benoy A, Sajikumar S. Long-term plasticity in the hippocampus: maintaining within and 'tagging' between synapses. *The FEBS journal* 2021.

173. Harkness JH, Gonzalez AE, Bushana PN, Jorgensen ET, Hegarty DM, Di Nardo AA *et al.* Diurnal changes in perineuronal nets and parvalbumin neurons in the rat medial prefrontal cortex. *Brain Struct Funct* 2021; **226**(4)**:** 1135-1153.

174. Miyata S, Komatsu Y, Yoshimura Y, Taya C, Kitagawa H. Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. *Nat Neurosci* 2012; **15**(3)**:** 414-422, S411-412.

175. Lin R, Rosahl TW, Whiting PJ, Fawcett JW, Kwok JC. 6-Sulphated chondroitins have a positive influence on axonal regeneration. *PloS one* 2011; **6**(7)**:** e21499.

176. Foscarin S, Raha-Chowdhury R, Fawcett JW, Kwok JCF. Brain ageing changes proteoglycan sulfation, rendering perineuronal nets more inhibitory. *Aging* 2017; **9**(6)**:** 1607-1622.

177. D'Agostino A, Stellavato A, Corsuto L, Diana P, Filosa R, La Gatta A *et al.* Is molecular size a discriminating factor in hyaluronan interaction with human cells? *Carbohydrate polymers* 2017; **157:** 21-30.

178. Al'Qteishat A, Gaffney J, Krupinski J, Rubio F, West D, Kumar S *et al.* Changes in hyaluronan production and metabolism following ischaemic stroke in man. *Brain* 2006; **129**(8)**:** 2158-2176.

179. Cargill R, Kohama SG, Struve J, Su W, Banine F, Witkowski E *et al.* Astrocytes in aged nonhuman primate brain gray matter synthesize excess hyaluronan. *Neurobiol Aging* 2012; **33**(4)**:** 830.e813-824.

180. Sugitani K, Egorova D, Mizumoto S, Nishio S, Yamada S, Kitagawa H *et al.* Hyaluronan degradation and release of a hyaluronan-aggrecan complex from perineuronal nets in the aged mouse brain. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2021; **1865**(2)**:** 129804.

181. Patrizi A, Awad PN, Chattopadhyaya B, Li C, Di Cristo G, Fagiolini M. Accelerated Hyper-Maturation of Parvalbumin Circuits in the Absence of MeCP2. *Cereb Cortex* 2020; **30**(1)**:** 256-268.

182. Pirbhoy PS, Rais M, Lovelace JW, Woodard W, Razak KA, Binder DK *et al.* Acute pharmacological inhibition of matrix metalloproteinase-9 activity during development restores perineuronal net formation and normalizes auditory processing in Fmr1 KO mice. *J Neurochem* 2020; **155**(5)**:** 538-558.

183. Berretta S, Pantazopoulos H, Markota M, Brown C, Batzianouli ET. Losing the sugar coating: potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophr Res* 2015; **167**(1-3)**:** 18-27.

184. Cabungcal JH, Steullet P, Morishita H, Kraftsik R, Cuenod M, Hensch TK *et al.* Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc Natl Acad Sci U S A* 2013; **110**(22)**:** 9130-9135.

185. Scholefield Z, Yates EA, Wayne G, Amour A, McDowell W, Turnbull JE. Heparan sulfate regulates amyloid precursor protein processing by BACE1, the Alzheimer's beta-secretase. *J Cell Biol* 2003; **163**(1)**:** 97-107.

186. Liu CC, Zhao N, Yamaguchi Y, Cirrito JR, Kanekiyo T, Holtzman DM *et al.* Neuronal heparan sulfates promote amyloid pathology by modulating brain amyloid-beta clearance and aggregation in Alzheimer's disease. *Sci Transl Med* 2016; **8**(332)**:** 332ra344.

187. Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA. Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature* 1996; **383**(6600)**:** 550-553.

188. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K *et al.* Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci U S A* 2013; **110**(33)**:** E3138-3147.

189. Suttkus A, Holzer M, Morawski M, Arendt T. The neuronal extracellular matrix restricts distribution and internalization of aggregated Tau-protein. *Neuroscience* 2016; **313:** 225-235.

190. Baig S, Wilcock GK, Love S. Loss of perineuronal net N-acetylgalactosamine in Alzheimer's disease. *Acta Neuropathol* 2005; **110**(4)**:** 393-401.

191. Crapser JD, Ochaba J, Soni N, Reidling JC, Thompson LM, Green KN. Microglial depletion prevents extracellular matrix changes and striatal volume reduction in a model of Huntington's disease. *Brain* 2020; **143**(1)**:** 266-288.

192. Crapser JD, Spangenberg EE, Barahona RA, Arreola MA, Hohsfield LA, Green KN. Microglia facilitate loss of perineuronal nets in the Alzheimer's disease brain. *EBioMedicine* 2020; **58:** 102919.

193. Fu AK, Ip NY. Regulation of postsynaptic signaling in structural synaptic plasticity. *Curr Opin Neurobiol* 2017; **45:** 148-155.

194. Yang S, Cacquevel M, Saksida LM, Bussey TJ, Schneider BL, Aebischer P *et al.* Perineuronal net digestion with chondroitinase restores memory in mice with tau pathology. *Exp Neurol* 2014; **265CP:** 48-58.

195. Tsilibary E, Tzinia A, Radenovic L, Stamenkovic V, Lebitko T, Mucha M *et al.* Neural ECM proteases in learning and synaptic plasticity. *Prog Brain Res* 2014; **214:** 135-157.

196. Rossi D, Gruart A, Contreras-Murillo G, Muhaisen A, Avila J, Delgado-Garcia JM *et al.* Reelin reverts biochemical, physiological and cognitive alterations in mouse models of Tauopathy. *Prog Neurobiol* 2020; **186:** 101743.

197. Duncan JA, Foster R, Kwok JCF. The potential of memory enhancement through modulation of perineuronal nets. *Br J Pharmacol* 2019; **176**(18)**:** 3611-3621.

FIGURE LEGENDS

Figure 1

The CNS extracellular matrix. Synapses are tripartite structures involving pre- and postsynaptic structures and astrocytes. All synapses are embedded in interstitial extracellular matrix (iECM), **which regulates the extracellular volume**, but some synapses are also surrounded by a condensed form of ECM, the PNNs, consisting mainly of CSPGs attached to a hyaluronan backbone.

Figure 2.

Memory effects of chondroitinase digestion. A) Eyeblink conditioning learning is increased by ChABC to the cerebellar nuclei but persistence is decreased. In fear memory PNN digestion enables extinction. B) Spontaneous object recognition is assessed in a Y-maze, animals distinguishing between familiar and non-familiar objects in the arms. After 5 min exposure to objects, memory gradually decays and by 24 hr is mostly lost. ChABC treatment prolongs memory in young animals, restores it in models of Alzheimer's and ageing. C) The Morris water maze tests place learning: ChABC treatment increases reversal and short-term learning. Grid cells provide a map of the external world: the grid cell map is destabilized by ChABC treatment. D) The trial-unique nonmatching-to-location assay (TUNL) is a hippocampus-dependent automated test of location memory. Memory acquisition is enhanced by ChABC treatment. E) In normal animals, ChABC digestion impairs social memory, but in animals with defective social memory due to abnormal PNNs, digestion restores memory F) ChABC digestion increases the agility of auditory relearning and decreases firing of fast-spiking neurons.

Figure 3.

Effects of external events, neurodegeneration and ageing on the CNS extracellular matrix. A) Stressful early life events, social isolation, social defeat and fear conditioning all have effects on numbers and intensity of PNNs. B) Drugs of abuse have various and complex effects on PNN formation in different brain areas: please refer to the text. C) During ageing, the sulphation pattern of PNNs changes, with a loss of permissive 6-sulphated CSPGs, leaving a predominance of inhibitory 4-sulphated forms. In addition, hyaluronan chains, which form the backbone of PNNs, become degraded into shorter fragments with unknown effects on memory. D) The CNS ECM participates in

neurodegenerative conditions. Proteoglycans participate in formation of tau tangles and beta-amyloid aggregates. In Huntington's disease PNNs are engulfed by activated microglia.

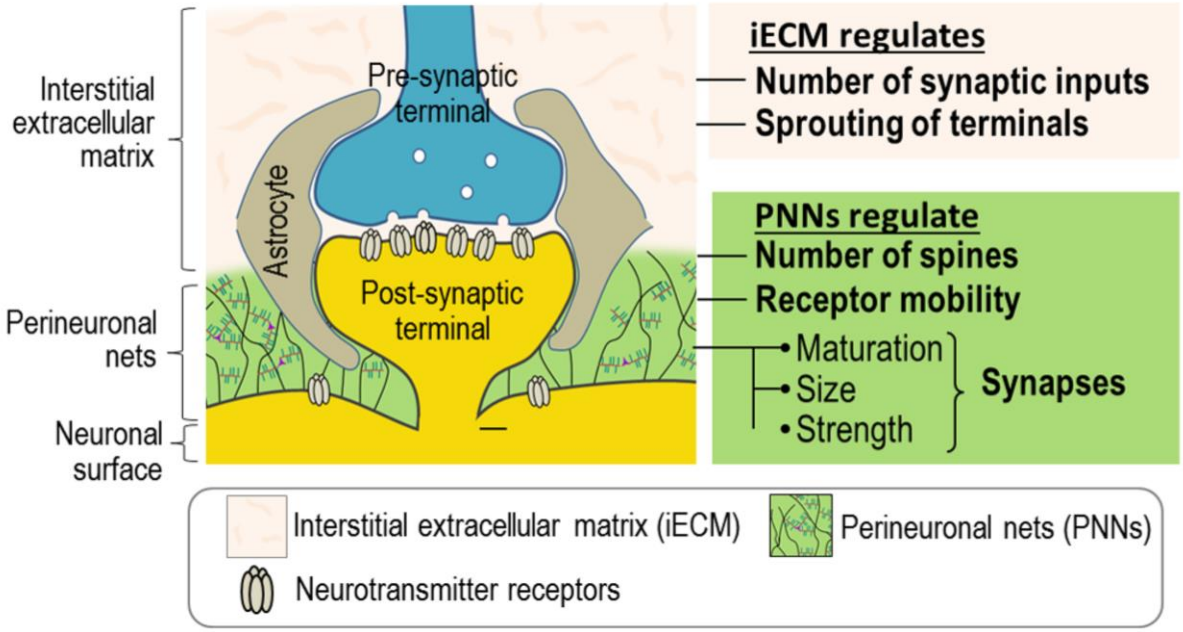


Figure 1

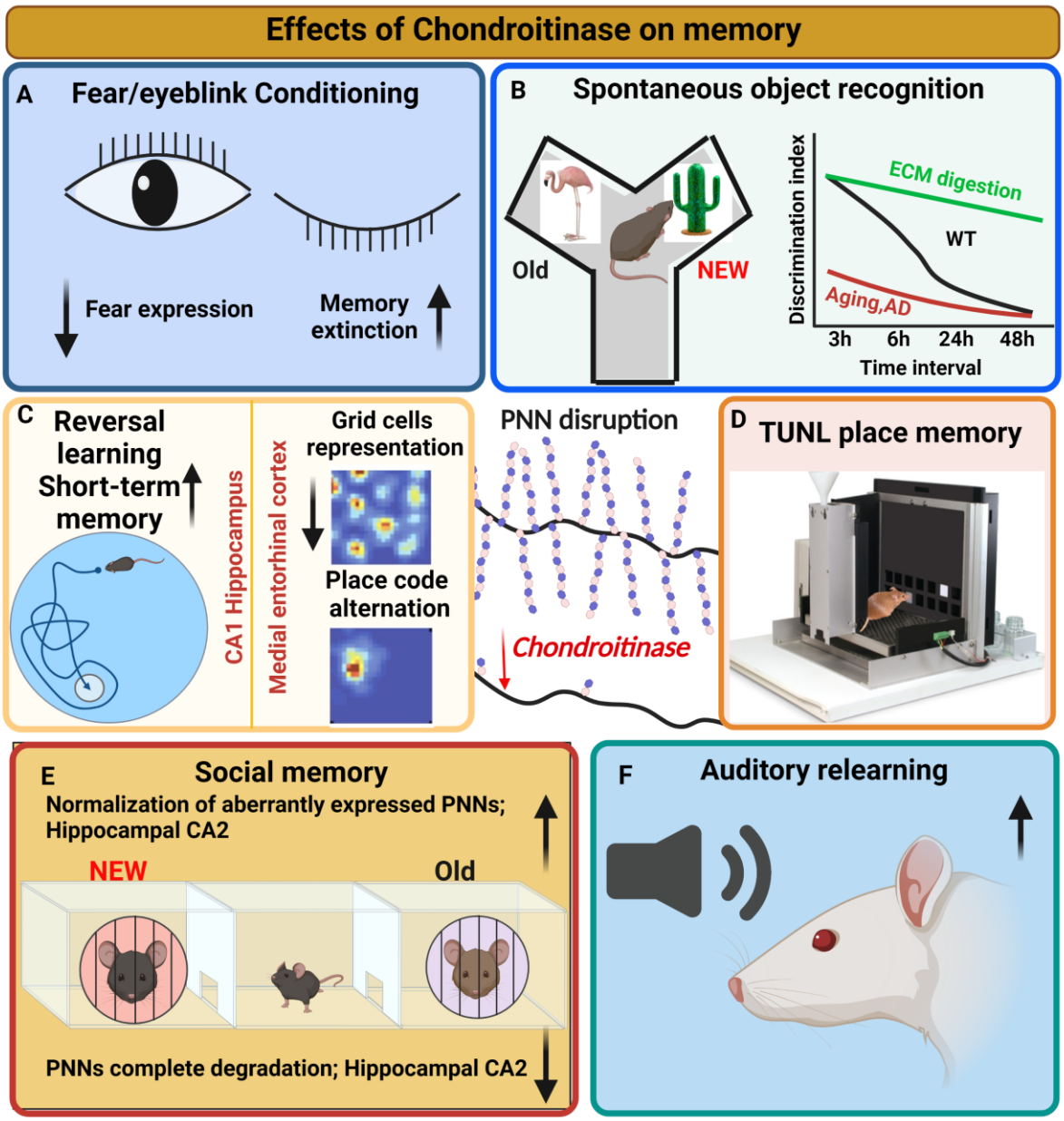
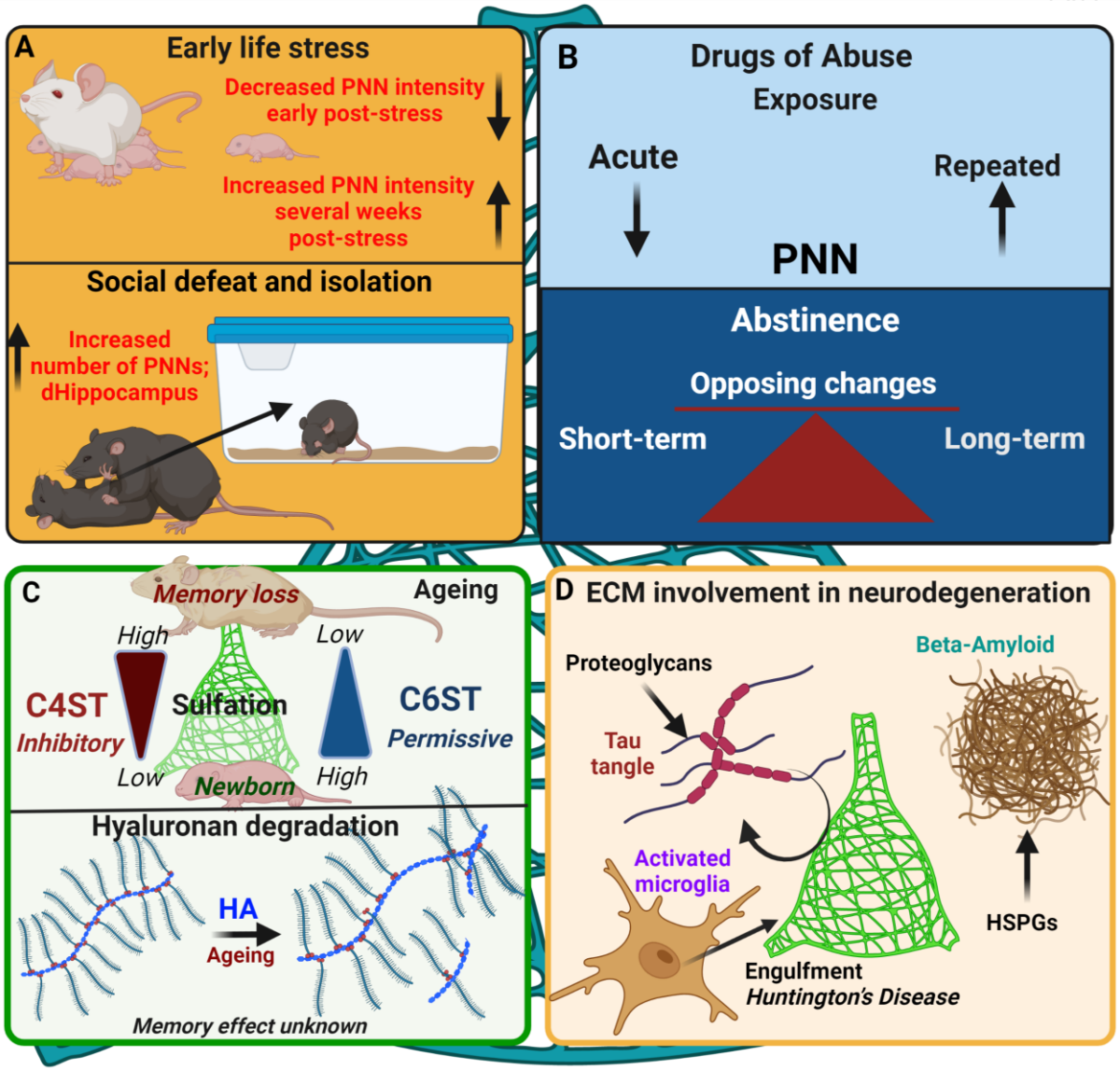

Effects of Chondroitinase on memory
A
Fear/eyeblink Conditioning
Fear expression
Memory extinction
B
Spontaneous object recognition
Old
NEW
Discrimination index
ECM digestion
WT
Aging,AD
3h
6h
24h
48h
Time interval
C
Reversal learning
Short-term memory
CA1 Hippocampus
Medial entorhinal cortex
Grid cells representation
Place code alternation
PNN disruption
Chondroitinase
D
TUNL place memory
E
Social memory
Normalization of aberrantly expressed PNNs; Hippocampal CA2
NEW
Old
PNNs complete degradation; Hippocampal CA2
F
Auditory relearning

Figure 2

1304 Figure 3



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