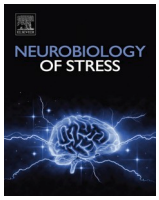
Neurobiology of Stress 33 (2024) 100692

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr



Modulation of stress-, pain-, and alcohol-related behaviors by perineuronal nets

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ARTICLE INFO

Handling Editor: Rita Valentino

Keywords:
Perineuronal nets
Depression
Alcohol
Ethanol
Pain
Stress

ABSTRACT

Perineuronal nets (PNNs) are a special form of central nervous system extracellular matrix enriched in hyaluronan, chondroitin sulfate proteoglycans, tenascins, and link proteins that regulate synaptic plasticity. Most PNNs in the brain surround parvalbumin-expressing inhibitory interneurons, which tightly regulate excitatory/inhibitory balance and brain activity associated with optimal cognitive functioning. Alterations in PNNs have been observed in neurological diseases and psychiatric disorders, suggesting that they may be key contributors to the neuropathological progression and behavioral changes in these diseases. Alcohol use disorder (AUD), major depressive disorder (MDD), and chronic pain are highly comorbid conditions, and changes in PNNs have been observed in animal models of these disorders, as well as postmortem tissue from individuals diagnosed with AUD and MDD. This review focuses on the literature describing stress-, alcohol-, and pain-induced adaptations in PNNs, potential cellular contributors to altered PNNs, and the role of PNNs in behaviors related to these disorders. Medicines that can restore PNNs to a non-pathological state may be a novel therapeutic approach to treating chronic pain, AUD, and MDD.

1. Introduction

The extracellular matrix (ECM) in the central nervous system has increasingly been recognized as an important contributor to behavior. The ECM is rapidly reorganized by stimuli and regulates synaptic plasticity, learning and memory, behavioral responses to stress, and addiction (Fawcett et al., 2019; Hodebourg et al., 2022). Perineuronal nets (PNNs) are a condensed, mesh-like form of ECM composed of hyaluronan, chondroitin sulfate proteoglycans (CSPGs: aggrecan, brevican, neurocan, versican, and phosphacan), link proteins (such as hyaluronan and proteoglycan link protein 1 [HAPLN1]), and tenascins (Fig. 1) (Fawcett et al., 2019). In most brain regions, the majority of PNNs envelop parvalbumin (PV)-expressing, GABAergic interneurons and are formed in an activity-dependent manner during the closure of critical periods of development, during which time plasticity is reduced (Carulli et al., 2010; Dityatev et al., 2007; Pizzorusso et al., 2002). In addition to restricting new synapse formation, PNNs stabilize mature synapses and cluster synaptic receptors (Favuzzi et al., 2017; Yuan et al., 2002). However, once formed, PNNs are not static structures. They can be rapidly remodeled through the action of the matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), and the cathepsins family of proteinases, all of which cleave proteoglycans, thus allowing for new synapse formation and heightened plasticity (Bonnans et al., 2014; Dubey et al., 2017; Stamenkovic et al., 2017; Tran et al., 2018; Valenzuela et al., 2014; Wen et al., 2018). Multiple cell types, including neurons and glia, are responsible for the production of the secreted structural components and the enzymes that degrade PNNs (Giamanco and Matthews, 2012; Ulbrich et al., 2021) (Fig. 1).

PNNs protect neurons from oxidative stress and are important for the ability of PV interneurons to rapidly generate action potentials (Cabungcal et al., 2013; Tewari et al., 2018). Cortical PV neurons and their PNNs tightly regulate the balance between excitation and inhibition in the brain and generate gamma rhythm, 30–100 Hz oscillations associated with optimal cognitive functioning (Cardin, 2018; Fernandez-Ruiz et al., 2023; Lensjo et al., 2017; Wingert and Sorg, 2021). Given that disturbance in gamma oscillations has been proposed to be a key contributor to neurological and psychiatric disorders (Guan et al., 2022), disruptions in PNNs and PV interneuron function could be a potential cellular mechanism underlying these disorders. PNNs are altered in neurological and psychiatric disorders such as Alzheimer's disease, schizophrenia, and substance use disorders (Berretta et al.,

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https://doi.org/10.1016/j.ynstr.2024.100692
Received 18 June 2024; Received in revised form 10 October 2024; Accepted 11 November 2024
Available online 14 November 2024

2015; Brandenburg and Blatt, 2022; Brown and Sorg, 2023; Crapser et al., 2020; Lasek et al., 2018; Logsdon et al., 2022; Pantazopoulos and Berretta, 2016; Pantazopoulos et al., 2015, 2021; Valeri et al., 2024).

Chronic stress is a common risk factor for many psychiatric disorders, including major depressive disorder (MDD) and alcohol use disorder (AUD), and changes in PNNs have been observed after both chronic stress and in AUD (Tables 1 and 3). More recently, evidence indicates that PNNs are also altered by chronic pain (Table 2), which, along with depression, is comorbid with AUD (Briere et al., 2014; De Aquino et al., 2024). Stress-induced adaptations in PNNs may contribute to PV interneuron dysfunction and excitatory/inhibitory imbalance in chronic pain states, AUD, and depression. Many groups have studied the effects of chronic stress on PNNs during distinct developmental periods (neonatal, juvenile, and adolescent) and several excellent recent reviews have covered this topic (Gore and Gould, 2024; Morphett et al., 2024; Perez-Rando et al., 2022). The goal of this review is to analyze the commonalities and differences in the adaptations and behavioral roles of PNNs in adult animals following chronic stress, alcohol exposure, and chronic pain, and discuss possible new avenues for treatment.

2. Tools to visualize and manipulate PNNs in the CNS

PNNs are typically visualized by staining brain sections with fluorescence-labeled *Wisteria floribunda* agglutinin (WFA)(Hartig et al., 1992), *Vicia villosa* agglutinin, or soybean agglutinin (Hartig et al., 2022), which are plant lectins that bind to glycosaminoglycans in the CSPG component of PNNs (Hartig et al., 2022), with WFA being the most commonly used lectin for PNN visualization. Antibodies targeting the CSPG core proteins aggrecan, brevican, tenascin R, and HAPLN1 have also been used (Giamanco and Matthews, 2012). The staining patterns of antibodies to these proteins overlap with WFA binding, but also label distinct cells, therefore WFA does not capture the full range of PNNs in the brain (Belliveau et al., 2024; Dauth et al., 2016; Ueno et al., 2018, 2019). PNNs are quantified by counting the number of cells enclosed by PNNs (PNN density) and/or the intensity of the fluorescence staining surrounding individual cells (PNN intensity). PNN intensity has historically been regarded as a measure of PNN maturity, with more intense WFA staining reflecting more mature PNNs (Slaker et al., 2016), based on studies showing that PNN intensity increases during brain maturation in mice and rats (Bruckner et al., 2000). Recently, two-photon imaging of fluorophore-labeled WFA has been used to measure changes in PNNs over time in the brains of awake mice (Benbenishty et al., 2023). This new method of longitudinal analysis of PNNs may be useful when examining PNNs over the trajectory of behavioral changes occurring with chronic stress, alcohol, and pain.

A common method to manipulate PNNs is microinjection of the bacterial enzyme chondroitinase ABC (ChABC) directly into the brain. ChABC breaks down the glycosaminoglycan chains on the CSPGs, thus disrupting PNNs (Muir et al., 2019). ChABC microinjection has the advantage that it is reversible, as PNNs will recover over time following acute disruption by ChABC, and the longitudinal effects of PNN dissociation on behavior can be assessed. A disadvantage of ChABC is that also breaks down chondroitin sulfate chains on CSPGs present in the interstitial ECM, so this approach is not specific to PNNs. PNNs can also be disrupted non-specifically by microinjection of hyaluronidase, an enzyme that degrades the hyaluronan backbone of PNNs and other hyaluronan-containing ECM (Frischknecht et al., 2009). More selective methods to disrupt PNNs include conditional gene knockout or viral-mediated knockdown using RNA interference to reduce levels of CSPGs such as aggrecan, brevican, tenascin R and HAPLN1, or expression of a dominant negative version of HAPLN1 (Bruckner et al., 2000; Favuzzi et al., 2017; Ramsaran et al., 2023). However, genes encoding the CSPGs are not only located in PNNs but are found in other ECM structures such as the perinodal ECM (Fawcett et al., 2019), so the knockout/knockdown approach is still not completely selective to PNNs.

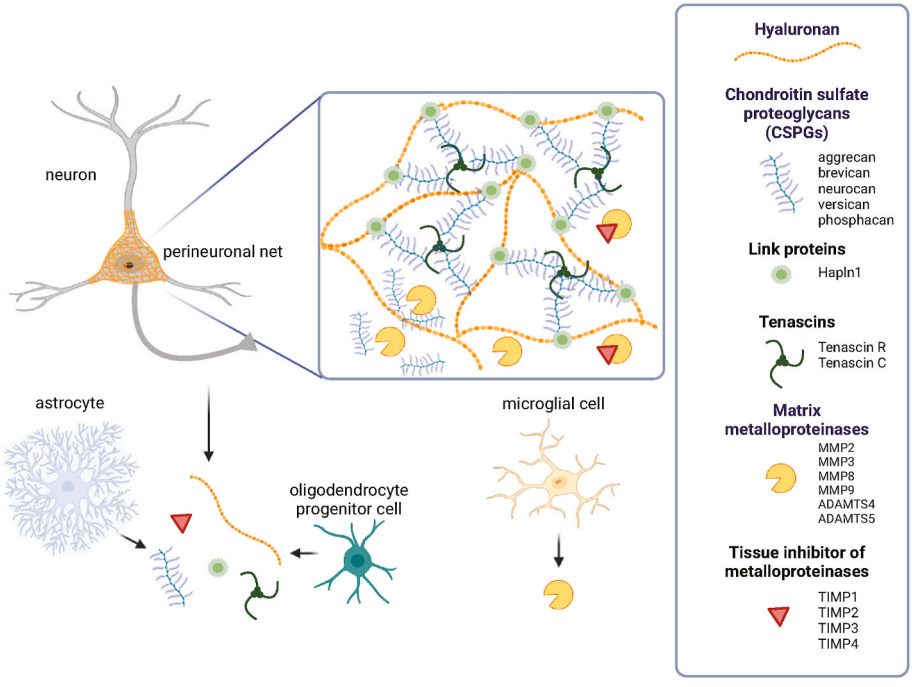

Fig. 1. Simplified schematic of perineuronal net (PNN) structure and cellular contributors to PNNs. PNNs typically, although not exclusively, surround parvalbumin-expressing interneurons in the cortex and hippocampus. They are enriched in hyaluronan, a glycosaminoglycan that forms the backbone of the PNN lattice, and chondroitin sulfate proteoglycans (CSPGs). The CSPGs and hyaluronan are crosslinked by link proteins such as HAPLN1 and tenascins. The matrix metalloproteinases (MMP and ADAMTS families) cleave CSPGs and alter PNN structure. The endogenous inhibitors of the MMPs and ADAMTSs are known as tissue inhibitors of metalloproteinases (TIMPs). Multiple cell types produce the different PNN structural components and their regulators, including the neurons themselves, astrocytes, oligodendrocyte progenitor cells, and microglia. This figure is not all-inclusive but is meant to highlight key components and known cellular contributors to PNNs discussed in this article. Created in BioRender. Lasek, A. (2024) BioRender.com/v37i567.

Table 1
Effects of chronic stress in adult animals on perineuronal nets (PNNs)[a].

Model	Species	Sex	Brain region	Time of PNNs assessment	Effect on PNNs[a]	Reference
Social defeat-induced persistent stress (5 daily episodes for 2–3 months), social isolation, and no enrichment	Rat	Male	dHPC (CA1)	2 months after last social defeat episode	**Increased** PNN numbers on PV + cells (assessed by CSPG antibody Cat-301)	**(Riga et al., 2017)**
Chronic variable stress (daily over 35 days)	Mice	Male	Cortex	3 days after last stress session	**Increased** PNN number	**(Simard et al., 2018)**
Chronic restraint stress (10 days)	Rats	Male	dHPC, vHPC, BLA, PFC, habenula, and TRN	1 day after last stress session	**Increased** PNN number in PFC and habenula; **decreased** PNN number in dHPC CA1; **increased** PNN intensity in TRN	(Pesarico et al., 2019)
Corticosterone treatment in drinking water (6 weeks)	Mice	Male	dHPC	While being treated with corticosterone	**Increased PNN** intensity in CA1	(Alaiyed et al., 2020)
Chronic unpredictable mild stress (twice per day for 30 days)	Rats	Male	PFC	7 days after last stress session	**Decreased** PNN number in prelimbic PFC	(Yu et al., 2020)
Social defeat stress (daily sessions for 5 days) plus social isolation	Rats	Male	dHPC	72 h and 2, 4 and 8 weeks after last social defeat stress session	**Decreased** PNN numbers in CA1 72 h after last social defeat session; **increased** PNN numbers in CA1 8 weeks after last social defeat session (assessed with Cat-301 antibody).	(Koskinen et al., 2020)
Restraint stress (24 h acute)	Mice	Male and female	BLA	35 days after stress session	**Increased** PNN numbers in males 35 days after acute stress.	**(Pesarico et al., 2022)**
Enrichment removal for two weeks	Rats	Male	BLA	During period of no enrichment	**Increased** PNN staining intensity and % PV cells with PNNs.	**(Smail et al., 2023)**
Chronic restraint stress, 2 h per day for 10 days	Mice	Male	Medial PFC	3 days after last stress session	**Decreased** PNN staining intensity	**(Li et al., 2024)**
Corticosterone treatment (2 weeks in drinking water)	Mice	Male	dHPC CA1	While being treated with corticosterone	**Increased** % of PV + cells with PNNs	**(Coutens et al., 2023)**
Chronic unpredictable mild stress (twice per day for 3–4 weeks)	Rats	Male	Medial PFC	5–7 days after last stress session	**Decreased** PNN number and % PV cells with PNNs in prelimbic and infralimbic areas	**(Zhang et al., 2023)**
Inescapable foot shock (twice per day for 6 days)	Rats	Male	dHPC CA1	Not indicated	**Decreased** PV cell number, but **increased** % of PV cells with PNNs	(Fan et al., 2024)
Intermittent social defeat (once every 3 days for 10 days)	Rats	Male	Medial PFC	2 weeks after last stress session	**Increased** PNN number	(Martinez et al., 2024)

Abbreviations: BLA, basolateral amygdala; CSPG, chondroitin sulfate proteoglycan; PFC, prefrontal cortex; dHPC, dorsal hippocampus; vHPC, ventral hippocampus, CA, cornu ammonis; TRN, thalamic reticular nucleus; PNN, perineuronal nets; PV, parvalbumin. **Bold text** indicates studies that demonstrated disruption of PNNs alters behavior.

[a] PNNs detected using *Wisteria floribunda* agglutinin (WFA) unless otherwise indicated.

Table 2
Effects of chronic pain on perineuronal nets (PNNs).

Pain model (pain assessment)	Species	Sex	Region	Time of PNN assessment	Effect on PNNs (marker used)	Reference
Experimental autoimmune encephalomyelitis [EAE] (mechanical	Mice	Female	Primary somatosensory cortex	7 and 21 days post EAE induction	**Decreased** PNN number at both time points (WFA)	(Potter et al., 2016)
Tibia fracture and cast immobilization (mechanical)	Mice	Male	dHPC (DG)	9 weeks post tibia fracture and cast immobilization; 6 weeks after cast removed	**Decreased** number of PV neurons with PNNs (WFA)	**(Tajerian et al., 2018)**
Spinal cord injury [SCI] (thermal)	Mice	Female	Dorsal column L5 motoneurons	5 and 11 weeks post SCI	**Decreased** PNN intensity 5 weeks post SCI (aggrecan antibody)	(Sanchez-Ventura et al., 2021)
Complete Freud's Adjuvant (mechanical and thermal)	Mice	Male	Somatosensory cortex, medial PFC, reticular thalamic nucleus (RTN)	3 and 7 days in somatosensory cortex, 7 days in PFC, and RTN	**Increased** PNN intensity in somatosensory cortex at 3 and 7 days and in medial PFC at 7 days. **Increased** PNN number in all three regions (WFA)	**(Mascio et al., 2022)**
Spared Nerve Injury [SNI] (mechanical, thermal)	Mice	Male and female	Dorsal horn of the spinal cord (lamina I)	3, 7 and 14 days after SNI	**Reduced** PNN intensity at all time points. (WFA)	**(Tansley et al., 2022)**

Abbreviations: PNN, perineuronal net; dHPC, dorsal hippocampus; DG, dentate gyrus; PFC, prefrontal cortex. **Bold text** indicates studies that demonstrated disruption of PNNs alters behavior.

3. Effects of chronic stress in adulthood on PNNs and the role of PNNs in behavioral responses to stress

3.1. Effects of chronic stress on prefrontal cortex (PFC) PNNs

The PFC is a key neuroanatomical site involved in stress-induced depression (Bittar and Labonte, 2021). PFC PNNs have been examined following chronic stress in both rats and mice and have yielded mixed results. Although the preponderance of evidence demonstrates increased PNN density following chronic stress, some have observed decreased PNN density or intensity following chronic stress (Table 1). This may be due to differences in the chronic stress model used, the time points at which PNNs were analyzed following chronic stress, or behavioral testing prior to measuring PNNs. Details of the chronic stress models used are provided in Table 1. For a discussion of differences between models of chronic stress, see (Tran and Gellner, 2023). Male mice exposed to chronic variable stress had increased PNN numbers throughout the cortex (Simard et al., 2018) and similarly, male rats exposed to chronic restraint stress had increased PNN numbers in the medial PFC, which included the infralimbic, prelimbic, and cingulate

Table 3
Effects of alcohol on perineuronal nets (PNNs).

Alcohol exposure procedure	Species	Sex	Age of alcohol exposure	Brain region	Age of PNNs analysis	Effect on PNNs[a]	Reference
5 g/kg ethanol gavage on PND 28, 29, 32, 33, 36, 37	Mice	Male	Adolescent (PND 28–37)	OFC	Adult (PND 110)	**Increased** PNN staining intensity	Coleman et al. (2014)
Drinking in the dark (4 consecutive days per week for 1 or 6 weeks)	Mice	Male	Adult	Insular and motor cortex	Adult (1 day after last drinking session)	**Increased** PNN staining intensity after 6 weeks alcohol drinking in insular cortex	Chen et al. (2015)
5 g/kg ethanol gavage, two days on, two days off from PND 25-54	Rats	Male	Adolescent (PND 25–54)	OFC, medial PFC	Adult (>PND 80)	**Increased** PNN numbers in OFC and medial PFC	Dannenhoffer et al. (2022)
Substance use disorder (including alcohol)	Humans	Male and female	N/A	HPC	Adult	**Increased** PNN number in CA1 stratum oriens, CA2 stratum oriens and stratum pyrimidale, CA3 stratum oriens, and CA4	Valeri et al. (2024)
Chronic alcohol self-administration	Rhesus macaques	Male	Adult	HPC	Adult (time after last drinking session not provided)	**Increased** PNN number in CA1 stratum oriens, CA3 stratum oriens and stratum pyrimidale, and CA2 stratum pyrimidale	Valeri et al. (2024)
Two-bottle choice ethanol consumption	Mice	Male and female	Adolescent (5–6 weeks old)	HPC	Young adult (9–10 weeks old; immediately after last drinking session)	**Decreased** PNN intensity in males	Galan-Llario et al. (2024)

[a] PNNs detected using *Wisteria floribunda* agglutinin (WFA) unless otherwise indicated. Abbreviations: OFC, orbitofrontal cortex; PV, parvalbumin; HPC, hippocampus; DG, dentate gyrus; CA, cornu ammonis; PND, postnatal day.

regions (Pesarico et al., 2019). Another more recent study in rats found increased PNN numbers in the superficial layers (layers 2 & 3) of the medial PFC following intermittent social defeat stress (Martinez et al., 2024). In contrast, two groups have found decreased PNN numbers and protein levels of aggrecan in the PFC of male rats following chronic unpredictable mild stress (Yu et al., 2020; Zhang et al., 2023). A pertinent difference among these studies (other than the stress procedures) was that in Yu et al. the rats underwent the stress procedures in a reversed light/dark cycle room rather than the standard light cycle. PNNs undergo diurnal fluctuations (Harkness et al., 2021; Pantazopoulos et al., 2020), so it is possible that the changes elicited following chronic stress could interact with the phase of the light/dark cycle. Another difference among these studies is that both Yu et al. and Zhang et al. euthanized rats several days following the final stressor, rather than one day later (Yu et al., 2020; Zhang et al., 2023). Finally, regarding WFA fluorescence staining intensity, one group found decreased WFA intensity and aggrecan protein levels by Western blot in the medial PFC of post-adolescent, young adult male mice after chronic stress (Li et al., 2024).

Changes in PFC PNNs have been linked to the depressive- and anxiety-like behaviors that develop following chronic stress exposure. Importantly, a study of postmortem human ventromedial PFC samples from depressed individuals who died by suicide and had a history of child abuse had increased PNN numbers compared to depressed suicides without a history of child abuse (Tanti et al., 2022). In mice, the increased PFC PNN number observed following chronic variable stress correlated with an "emotionality score" that comprised several different depressive- and anxiety-like behaviors (Simard et al., 2018). Disruption of the ECM in the mouse PFC by ChABC injection reversed the stress-induced increase in immobility time in both the forced swim test and open field and the emotionality score without affecting the behavior of control, non-stressed mice (Simard et al., 2018), demonstrating a role for the ECM in promoting depressive-like behavior. However, another study showed that ChABC injection into the medial PFC of control (non-stressed) mice resulted in anxiety-like behavior (Li et al., 2024). This same group found that PNN staining intensity in the PFC was decreased (rather than increased) following chronic restraint stress, and that chronic treatment with the antidepressant fluoxetine throughout the stress paradigm could alleviate anxiety-like behavior and increase PNN staining intensity to control levels (Li et al., 2024). Although the results of these studies differed in the effects of chronic stress on PNNs, both demonstrated that the ECM in the PFC can modulate depressive- and anxiety-like behaviors in mice.

3.2. *Effects of chronic stress on hippocampal and amygdala PNNs*

Given the importance of the hippocampus in both learning and memory and affective states, several groups have examined the effect of chronic stress on PNNs in the hippocampus (Table 1). As in the PFC, results in the hippocampus have been mixed. Social defeat-induced persistent stress in male rats resulted in impaired hippocampal-dependent spatial memory associated with higher levels of PNN-associated proteins (brevican, neurocan, phosphacan, HAPLN1, and tenascin-R), along with increased PNN numbers in the dorsal CA1 region of the hippocampus (Riga et al., 2017). The increase in dorsal CA1 PNNs was associated with increased inhibitory transmission. Increased inhibitory transmission is consistent with literature demonstrating that PNNs decrease membrane capacitance of PV neurons, resulting in increased excitability (Balmer, 2016; Tewari et al., 2018). Treating rats with the antidepressant imipramine reversed the social defeat stress-induced increases in the ECM proteins and PNNs (Riga et al., 2017). ChABC injection into the dorsal hippocampus also normalized social defeat stress-induced changes in PNNs, inhibitory neurotransmission, and restored spatial memory recall, demonstrating a role for the stress-induced increases in CSPGs in behavioral impairment (Riga et al., 2017).

In a corticosterone-induced model of depression in male mice (corticosterone in drinking water), hippocampal brevican protein was elevated and the PNN staining intensity around dorsal hippocampal CA1 neurons was increased, which was reversed by treatment with the antidepressant venlafaxine (Alaiyed et al., 2020). In a similar study, the percentage of PV neurons enclosed by PNNs was increased in the dorsal hippocampal CA1 region of male mice in the corticosterone model of depression, which was prevented by treatment with venlafaxine plus environmental enrichment (Coutens et al., 2023). ChABC injection into the dorsal hippocampus following chronic corticosterone administration reduced PNNs and improved the emotionality index, and even more so when combined with venlafaxine treatment (Coutens et al., 2023). Fan et al. also observed an increase in the percentage of PV neurons surrounded by PNNs in the rat dorsal hippocampal CA1 region following inescapable foot-shock stress (Fan et al., 2024). In contrast, Pesarico et al. found that chronic restraint stress resulted in reduced PNN

numbers in the dorsal hippocampal CA1 region (Pesarico et al., 2019), and Valeri et al. described decreased PNN numbers in the postmortem hippocampus CA1 of individuals with MDD (Valeri et al., 2024).

The disparity in the effects of chronic stress on hippocampal PNNs might, in part, be explained by an elegant study that examined temporal changes in the ECM for two months following social defeat stress (Koskinen et al., 2020). They found that CA1 PNN numbers and dorsal hippocampal CSPGs were decreased 72 h following the last social defeat episode, whereas they increased at 8 weeks following the last social defeat episode. Increased PNNs and CSPGs coincided with memory impairment at 8 weeks post-stress (Koskinen et al., 2020). Of note, one study found no effect of chronic stress in adulthood on ventral hippocampal PNNs in rats (Gomes et al., 2020).

In addition to the PFC and hippocampus, the amygdala is an important interconnected region also involved in depression (Pandya et al., 2012). Two studies have examined PNNs in the amygdala following stress (Pesarico et al., 2022; Smail et al., 2023). Thirty-five days after a single 24 h restraint stress episode, PNN numbers were increased in the basolateral amygdala (BLA) (Pesarico et al., 2022). A novel approach to chronic stress was used by Smail et al., who removed environmental enrichment from the housing cages of rats to create an impoverished environment and observed an increase in the percentage of PV neurons surrounded by PNNs and increased PNN fluorescence staining intensity in the BLA (Smail et al., 2023). In summary, accumulating evidence largely indicates a role for increased PNNs in the PFC, dorsal hippocampus, and amygdala in the persistent cognitive and mood disturbances following chronic stress in adulthood.

4. Effects of chronic pain on PNNs and their role in hyperalgesia

4.1. Effects of chronic pain on spinal cord PNNs

Research on the effects of chronic pain states on PNNs and the role of these ECM structures in the modulation of pain is a newly emerging area. The limited work in this area has revealed an involvement of PNNs in pain development. PNNs have been studied in the spinal cord and in the brain in models of chronic pain (Table 2). Two groups have examined PNNs in the spinal cord in different chronic pain models (Sanchez-Ventura et al., 2021; Tansley et al., 2022). Sanchez-Ventura et al. found that PNN intensity was decreased surrounding lumbar motoneurons 5 weeks following T11 spinal cord contusion in female mice, a procedure that induces long-lasting thermal hyperalgesia (Sanchez-Ventura et al., 2021). Activity (voluntary wheel running or forced treadmill running) initiated 7 days after injury prevented the decrease in PNN intensity and thermal hyperalgesia (Sanchez-Ventura et al., 2021). Tansley et al. used a spared nerve injury (SNI) model for neuropathic pain and found decreased PNN intensity surrounding dorsal horn lamina I projection neurons (Tansley et al., 2022). Disruption of PNNs in lamina I projection neurons of non-injured mice, either through aggrecan (*Acan*) gene knockout or expression of ChABC selectively in these neurons, induced thermal and mechanical pain-like behaviors (Tansley et al., 2022). These results demonstrate that PNNs in the spinal cord can regulate chronic pain states.

4.2. Effects of chronic pain on brain PNNs

PNNs have been examined in the somatosensory cortex, medial PFC, and dorsal hippocampus in models of chronic pain and results have been mixed (Table 2). Multiple sclerosis is a disease that frequently results in chronic pain (Osterberg et al., 2005). This disease is modeled in mice using experimental autoimmune encephalomyelitis (EAE). Potter et al. found in female mice that PNN numbers and PV immunostaining were reduced in the somatosensory cortex at early, pre-symptomatic stages of EAE 7 days post injection, and decreased PNN numbers 21 days post injection (Potter et al., 2016). However, in a chronic inflammatory pain model in which complete Freund's adjuvant was injected into the hind paw, PNN numbers in the contralateral (but not ipsilateral) somatosensory cortex, medial PFC, and reticular thalamic nucleus of male mice progressively increased over 7 days (Mascio et al., 2022). Microinjection of ChABC into the somatosensory cortex reduced mechanical hypersensitivity in the CFA-injected hind paw, demonstrating a role for PNNs in the somatosensory cortex in regulating inflammatory pain response in the periphery. This study also found increased inhibitory neurotransmission onto layer 5 pyramidal neurons in the contralateral cortex of CFA-injected mice that was reversed after ChABC degradation of PNNs (Mascio et al., 2022), thus adding to the literature indicating that increasing PNNs facilitates greater PV neuron inhibitory neurotransmission, whereas removing PNNs decreases PV neuron activity (Wingert and Sorg, 2021).

In the hippocampus, chronic pain was associated with a decrease in the number of PNNs specifically surrounding PV neurons in the dentate gyrus (Tajerian et al., 2018). In this study, tibia fracture and cast immobilization was used as a model of neuropathic pain in male mice. Mechanical sensitivity of the hind paw was measured 7 weeks following injury (4 weeks after the cast was removed) to demonstrate long-lasting allodynia even after the tibia had healed; hippocampal-dependent memory impairment was also observed at this time point, indicating cognitive impairment in a chronic pain state. In addition, protein levels of aggrecan, HAPLN1, and hyaluronan synthase (which produces hyaluronan, the PNN scaffolding) were reduced, along with overall ECM rigidity in the hippocampus as measured by atomic force microscopy (Tajerian et al., 2018). A potential mechanism leading to decreased PNNs was an increase in MMP8, an aggrecan-degrading metalloproteinase (Arner et al., 1997), and a decrease in the endogenous MMP inhibitor TIMP2, the net effect being a shift towards degradation of PNNs. Reducing MMP8 levels in the hippocampus with a viral-delivered short hairpin RNA normalized PNNs, reduced allodynia, and improved memory (Tajerian et al., 2018). Collectively, these findings indicate that chronic pain is associated with changes in PNNs in different CNS locations and that manipulating PNNs can alter chronic pain states. Understanding the interplay between PNNs and pain offers novel insights into the mechanisms underlying chronic pain and opens potential avenues for targeted therapeutic interventions aimed at modulating PNN-related processes to alleviate pain.

5. Effects of adolescent and adult alcohol exposure on PNNs and their role in alcohol-related behaviors

5.1. Effects of adolescent and adult alcohol exposure on cortical PNNs

Several studies have demonstrated that PNN intensity and/or density increases in cortical regions following chronic alcohol exposure either in adolescence or adulthood (Table 3). Following adolescent alcohol exposure, the intensity of WFA staining was increased in the orbitofrontal cortex (OFC) of adult male mice (Coleman et al., 2014) and the OFC and medial PFC of adult male rats (Dannenhoffer et al., 2022). The number of PNNs was also increased in the mPFC and OFC of adult male rats following adolescent intermittent ethanol exposure (Dannenhoffer et al., 2022). These cortical regions regulate behavioral flexibility, thus increased PNNs after chronic ethanol exposure might contribute to restricted plasticity of PV neurons and behavioral inflexibility, leading to continued alcohol misuse (Dannenhoffer et al., 2021).

WFA staining intensity also increased in the insular cortex of male mice following six weeks of binge ethanol consumption (Chen et al., 2015). Interestingly, mechanical allodynia in the hind paw also developed in male mice using this same protocol (Aguilar et al., 2024), possibly providing a link between insular cortex PNNs, alcohol consumption, and the development of chronic pain. The insula is involved in interoception, pain and compulsive alcohol drinking, another type of inflexible behavior (Centanni et al., 2021; McBenedict et al., 2024). Support for the role of insular cortex PNNs in compulsive alcohol drinking was provided in a study in which ChABC was microinjected

into the insula of adult male mice. Mice underwent a compulsive-like drinking test wherein ethanol was adulterated with bitter tasting quinine. Ablation of PNNs/ECM in the insula resulted in mice consuming less of the quinine-adulterated ethanol solution, but not the ethanol-only solution, suggesting that PNNs in the insula specifically contribute to aversion-resistant ethanol consumption (Chen and Lasek, 2020). This effect was only observed in males, as ECM disruption in the insula of female mice had no effect on aversion-resistant ethanol consumption (Martins de Carvalho et al., 2023). Interestingly, ChABC injection into the BLA of male mice reduced regular (*i.e.*, non-quinine adulterated) ethanol consumption (Maiya et al., 2021), indicating anatomical specificity in the role of the ECM in regulating different aspects of ethanol drinking.

5.2. Effects of adolescent alcohol exposure on hippocampal PNNs

In the hippocampus, adolescent alcohol exposure resulted in decreased WFA intensity specifically on PV neurons in male mice (Galan-Llario et al., 2024), which differed from the observed increase in PFC PNNs following adolescent alcohol exposure (Coleman et al., 2014; Dannenhoffer et al., 2022). This discrepancy might be due to either the brain region examined, the ethanol exposure procedure (intragastric binge *vs.* two-bottle choice drinking), or the time post-exposure that PNNs were analyzed (~30 days after last binge vs. immediately after last drinking session). This is analogous to the effects of chronic stress, in which PNNs in the hippocampus were decreased 72 h post-stress but increased many weeks after the last stressful episode (Koskinen et al., 2020). Importantly, the number of PNNs in the hippocampus was increased in monkeys that had been chronically self-administering ethanol and in postmortem hippocampus of adult human subjects with substance use disorder (SUD), including alcohol (Valeri et al., 2024). The increase in PNNs in the hippocampus of humans with SUD was associated with increased expression of the genes encoding PV (*Pvalb*) and chondroitin sulfate synthase 1 (*Chsy1*), and decreased expression of matrix metalloproteinase 9 (*Mmp9*) and cathepsin S (*Ctss*) transcripts, which is expected to shift the balance towards increased PNN accumulation (Valeri et al., 2024). Overall, most evidence suggests that after chronic alcohol use, PNNs increase in the cortex and hippocampus, regions involved in cognitive control and behavioral flexibility.

6. Cellular contributors to PNN alterations following chronic stress, pain and alcohol exposure

As it has become increasingly recognized that there are long-lasting alterations in PNNs following chronic stress, pain, and alcohol use that contribute to behavioral changes, current efforts aim to identify the cellular mechanisms that drive the shift in balance between PNN accumulation and degradation. Neurons and glia produce the structural components of PNNs and the metalloproteinases that degrade PNNs (Giamanco and Matthews, 2012; Ulbrich et al., 2021). Under pathological states, glia may play a particularly important role in PNN alterations (Giamanco and Matthews, 2012; Tansley et al., 2022; Tewari et al., 2022). For example, cortical astrocytes increased the expression of genes encoding ECM proteins following chronic variable stress (Simard et al., 2018), oligodendrocyte progenitor cells (OPCs) from human postmortem dorsolateral PFC of depressed individuals with a history of child abuse had higher transcript levels of versican (*VCAN*) and phosphacan/protein tyrosine phosphatase receptor β/ζ (*PTPRZ1*) (Tanti et al., 2022), and the WFA-positive glia morphologically resembling astrocytes were increased in the postmortem hippocampus of humans diagnosed with SUD (Valeri et al., 2024). These results suggest that astrocytes and oligodendrocyte progenitor cells may respond to chronic stress and alcohol by producing and secreting ECM proteins that are incorporated into PNNs.

Conversely, microglia appear to be involved in the breakdown of PNNs in chronic pain states. In the EAE model, an increase in cortical microglia was associated with decreased PNNs (Potter et al., 2016). In the SNI model of chronic pain, the authors demonstrated that microglia were responsible for the decreased PNN intensity following spinal cord injury, as depleting microglia prevented the decrease in PNNs as well as mechanical hyperalgesia following spinal cord injury (Tansley et al., 2022). This is comparable to a study that found that microglial depletion in adult mice resulted in increased density and intensity of PNNs (Liu et al., 2021). In addition, microglia appear to endocytose PNN components, because WFA and CD68 (an endosomal marker) colocalized within microglia acutely after spinal cord injury (Tansley et al., 2022). This is consistent with findings in mouse models of Alzheimer's disease and in postmortem brain tissue from individuals with Alzheimer's disease, in which inclusions of PNN material were seen within microglia near amyloid plaques (Crapser et al., 2020). PV neurons themselves may also be involved in the breakdown and accumulation of PNN material. PV neurons express tissue-type plasminogen activator, which converts plasminogen into plasmin, a proteinase that was recently shown to degrade aggrecan (Lepine et al., 2022). Tissue-type plasminogen activator (*Plat*) gene knockout mice exhibit increased PNN intensity in the cortex (Lepine et al., 2022). At this point, we have only glimpsed the complexities of PNN assembly and disassembly in normal and pathological states. Further studies are needed to disentangle the neuronal and glial cell type contributions to altered PNNs following chronic stress, pain, and alcohol exposure.

Several studies described in **section 3** demonstrated that treatment with the antidepressants venlafaxine or imipramine reduced the stress-induced increase in PNN number or intensity in the hippocampus (Alaiyed et al., 2020; Coutens et al., 2023; Riga et al., 2017). One mechanism of action for venlafaxine's effect on PNNs is an increase in MMP9 activity, as chronic treatment with venlafaxine increased protein levels of MMP9 in the cortex and hippocampus and also promoted cleavage of brevican; venlafaxine-induced cleavage of brevican and reduction of PNNs was eliminated in *Mmp9* knockout mice (Alaiyed et al., 2019, 2020).

Decreased MMP activity may be partially responsible for the increase in PNNs observed after chronic stress or alcohol exposure. For example, Koskinen et al. found that MMP2 activity was reduced in a chronic depressive-like state in which PNNs and CSPG proteins were increased in the hippocampus (Koskinen et al., 2020). MMP9 has been shown to promote alcohol consumption, craving, and reward (Go et al., 2020; Smith et al., 2011; Stefaniuk et al., 2017; Yin et al., 2019, 2020), and *Mmp9* transcript is reduced in the hippocampus of humans with SUD (Valeri et al., 2024), but it is not yet known whether MMP9 activity or that of other numerous MMPs, ADAMTSs, cathepsins, or tPA involved in PNN remodeling are reduced in brain regions in which PNNs are increased following chronic ethanol exposure. Levels of the TIMPS, the endogenous inhibitors of MMPs, may also increase following chronic alcohol exposure and contribute to increased PNNs.

Microglia and astrocytes are important mediators of the brain immune response, and chronic alcohol exposure, stress, and pain induce a CNS immune response (Crews and Vetreno, 2014; Erickson et al., 2019; Wohleb et al., 2016). Alcohol also increases oxidative stress (Crews et al., 2015), so the increase in cortical PNNs following chronic alcohol exposure may be a compensatory mechanism to protect PV neurons following oxidative stress through increased production of the CSPGs (Cabungcal et al., 2013; Harkness et al., 2021). Further work is needed to elucidate complex interplay between the neuroimmune system and oxidative stress in the modulation of PNN structure under these pathological conditions.

7. Could PNNs represent a new target for treatment of chronic pain, AUD, and major depressive disorder?

Development of therapeutic approaches to correct PNN alterations and PV neuron dysfunction in pathological states represents a new and exciting research area. In situations where PNNs are decreased (such as

in the spinal cord or brain in *distinct* chronic pain states), MMP inhibitors could represent a useful approach (Dai et al., 2024). One MMP2/9 inhibitor showing promise in the treatment of neuropathic pain is AQU-118 (Henry et al., 2015). Other broad spectrum MMP inhibitors, such as marimastat, have been developed for clinical use, but they are problematic because of side effects, the most severe being musculoskeletal syndrome involving joint pain, stiffness, and inflammation (Winer et al., 2018), so understanding which MMPs are activated during chronic pain states and choosing an appropriate selective MMP inhibitor is crucial. In addition, treatment timing will be important, as PNNs may initially be reduced following trauma because of inflammation and increased MMP activity, but then increase over time from compensation. Differences between brain regions in PNN responses in chronic pain states (*e.g.*, hippocampus vs. somatosensory cortex, see Table 2) are also important to consider.

MMP inhibitors would likely not be useful for treatment of MDD and/or AUD because most studies have demonstrated that PNNs accumulate in the brain following chronic stress and alcohol exposure (Tables 1 and 3). Instead, a drug that can reduce PNN density and/or intensity would be optimal. Proof of concept for this approach was provided in studies that used a hyaluronan synthesis inhibitor, 4-methylumbelliferone (4-MU) (Dubisova et al., 2022; Stepankova et al., 2023), also known as hymechromone, which is used clinically as an anti-spasmodic. Mice treated for six months with 4-MU had decreased PNN intensity in the hippocampus and enhanced memory retention (Dubisova et al., 2022). The effect of 4-MU was long-lasting, as PNN intensity was still reduced after a one-month washout period (Dubisova et al., 2022).

8. Conclusions and future directions

A common theme that has emerged following both chronic stress and extensive alcohol exposure in adults is that PNNs are increased in PFC and hippocampus, regions important for learning and memory and emotional regulation. Increased accumulation of PNNs in these regions is associated with increased overall inhibition by PV neurons and decreased behavioral flexibility. Stress- and alcohol-induced disturbances in glia function are likely to contribute to PNN accumulation, but the role of PV neurons themselves cannot yet be ruled out. In the case of chronic pain, many studies have shown decreased PNNs in the spinal cord and brain, likely due to microglia engulfment of ECM and increased activity of MMPs. However, there are still contradictory and non-generalizable findings that need to be reconciled. This may be related to both timing and brain region-specific effects. Although much has been done in the past decade to understand the involvement of PNNs in normal brain function and neuropathology, more studies are needed, especially considering that PNNs represent novel targets for therapies to treat psychiatric disorders.

Finally, an important consideration is sex differences in PNNs and whether modulating PNNs would have the same behavioral outcome in males and females. Most studies have examined PNNs only male animals, but emerging evidence indicates sex differences in PNNs and the response to PNN manipulation (Galan-Llario et al., 2023, 2024; Gore and Gould, 2024; Griffiths et al., 2019; Martins de Carvalho et al., 2023; Mayne et al., 2024). The biological factors contributing to sex differences in PNNs are not well understood, although ovarian hormones could play an important role (Hernandez-Vivanco et al., 2022; Laham et al., 2022). Future studies need to include both male and female subjects and determine the factors that contribute to sex differences in PNNs.

CRediT authorship contribution statement

Jhoan S. Aguilar: Writing – review & editing, Writing – original draft. **Amy W. Lasek:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the United States National Institutes of Health (grants R01 AA027231 and U01 AA020912 to AWL).

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