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Releasing Addiction Memories Trapped in Perineuronal Nets

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Abstract

Drug addiction can be conceptualized on a basic level as maladaptive learning and memory. Addictive substances elicit changes in brain circuitry involved in reward, cognition, and emotional state, leading to the formation and persistence of strong drug-associated memories that lead to craving and relapse. Recently, perineuronal nets (PNNs), extracellular matrix structures surrounding neurons, have emerged as regulators of learning, memory, and addiction behaviors. PNNs do not just provide structural support to neurons, but are dynamically remodeled in an experience-dependent manner by metalloproteinases. They function in various brain regions through constituent proteins such as brevican that are implicated in neural plasticity. Understanding the function of PNN components in memory processes may lead to new therapeutic approaches to treating addiction.

Keywords

Addiction; Alcohol; Brevican; Memory; Metalloproteinase; Perineuronal Nets

Components and Regulators of Perineuronal Nets (PNNs)

PNNs are mesh-like, specialized **extracellular matrix** (see Glossary) structures that surround specific neurons in the central nervous system. They were first described by Camillo Golgi in 1882 but their functional importance in regulating **neural plasticity** only became appreciated in this century [1]. Over the past few years, it has become apparent that PNNs, and their individual constituents, are important regulators of memories associated with addiction-related behaviors in animals. Understanding the function of specific components that make up the structure of PNNs and the extracellular proteinases that remodel PNNs will provide potential new therapeutic avenues to reducing relapse to drug and alcohol addiction.

In the cerebral cortex, most neurons enclosed by PNNs produce the inhibitory neurotransmitter GABA and express the calcium-binding protein parvalbumin [2, 3], although PNNs also envelop a smaller number of neurons that produce the excitatory

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neurotransmitter glutamate in such regions as the cortex and specific areas of the hippocampus [3–6]. In the cerebellum, PNNs surround large excitatory deep cerebellar and inhibitory Golgi interneurons [7]. The structural composition of PNNs has recently been reviewed [1, 8] and will be briefly summarized here (Figure 1). Major constituents of PNNs are the **chondroitin sulfate** proteoglycans, proteins that are heavily modified by chondroitin sulfate glycosaminoglycan sugar chains. These proteins are aggrecan, brevican, neurocan and versican, and are encoded by the genes *ACAN*, *BCAN*, *NCAN,* and *VCAN*, respectively. They are collectively referred to as lecticans. Aggrecan is essential to the formation of PNNs, since cultured cortical neurons from *Acan* knockout mice, although able to differentiate into neurons that produce GABA and express parvalbumin, are unable to form PNNs [9]. *Acan* gene expression is induced by neuronal depolarization, indicating that it is regulated by neuronal activity [10]

The lecticans bind to **hyaluronan**, a non-sulfated glycosaminoglycan chain produced by hyaluronan synthases located on the plasma membrane of neurons. Hyaluronan is an important structural component of PNNs and also exists in a macromolecular complex with other PNN proteins known as link proteins and tenascins [8, 11]. A schematic of PNN structural components is shown in Figure 1. Different structural components of PNNs are produced by neurons and glia and secreted by these cell types into the extracellular space, where they assemble into PNNs [1, 10]. Link proteins (hyaluronan and proteoglycan link proteins, abbreviated HAPLNs) stabilize PNNs by binding to hyaluronan. Two link proteins that are known to localize to PNNs are HAPLN1 and HAPLN4, which are encoded by the *HAPLN1* and *HAPLN4* genes, respectively. Brain-specific *Hapln1* knockout mice form PNNs, but their structure is abnormal [12], while mice deficient in *Hapln4* have decreased brevican localization in PNNs in the cerebellum and brainstem [13]. Tenascin proteins are multimeric proteins that bind to lecticans. Tenascin-R (encoded by the *TNR* gene) and tenascin-C (encoded by the *TNC* gene) are expressed in the brain [14]. *Tnr* knockout mice do not form normal PNNs, demonstrating critical role for tenascin-R in PNN formation [11, 15]. Although tenascin-C is not necessary for PNN formation, it is co-localized with PNNs in the cerebellum and may play a functional role in PNNs [16, 17].

PNNs are formed during brain development at different times depending on the brain region. Their appearance in the cortex roughly correlates with the maturation of GABA neurons into parvalbumin-expressing neurons, with parvalbumin neurons in the somatosensory cortex appearing earlier than in the prefrontal cortex [18, 19]. PNNs are most often detected in the brain using immunohistochemistry (IHC) with labeled ***Wisteria floribunda* agglutinin** (WFA). In the mouse cortex, PNNs begin to appear in the somatosensory cortex at postnatal days 7–10 and continue to develop until adolescence at 5 weeks of age [18, 20], whereas in the mouse and rat prefrontal cortex, hippocampus, and amygdala, PNNs do not appear until postnatal day 14 and continue to develop until adulthood [19–21]. The developmental formation of PNNs is regulated by excitatory neural activity [22] and corresponds to the closure of periods of plasticity [23, 24].

PNNs are dynamically altered by experiences such as environmental enrichment, sensory deprivation, social isolation, epileptic neural activity, learning, and memory retrieval and as a result play a role in neural plasticity [6, 16, 23, 25–32]. Decreased intensity of PNNs and/or

numbers of neurons enveloped by PNNs as measured by WFA labeling occur in the cerebellum and cortex after environmental enrichment [16, 30–32], although PNN intensity has been demonstrated to increase in the hippocampal CA2 region after enrichment [6]. This may be due to the different neuronal types that have PNNs in these brain regions responding differently to environmental enrichment. Interestingly, the number of neurons with PNNs also decreased in the cortex after sensory deprivation and social isolation [23, 28, 29]. Brevican protein levels, as measured by Western blots, are decreased in the mouse hippocampus after environmental enrichment and in brain tissue from epileptic patients [26]. Decreased PNNs after different sensory experiences may enhance neural plasticity by allowing, for example, new synapse formation [33].

Remodeling of PNNs during an experience is controlled by extracellular proteases that cleave the structural components of PNNs. The **Matrix Metalloproteinase** (MMP) and **A Disintegrin and Metalloproteinase with Thrombospondin motifs** (ADAMTS) gene families encode proteins that enzymatically cleave extracellular matrix proteins, including those in PNNs. Each of these protein families comprises multiple members with overlapping and distinct protein substrates [34–36]. Like PNNs, the activity of MMP and ADAMTS proteins are regulated by neural activity, sensory experiences, and during learning and memory processes [16, 37–42]. For instance, MMP-9/2 activity as measured by an in situ enzymatic assay increased in the cerebellum after environmental enrichment, which correlated with decreased intensity of PNNs as measured by WFA staining [27], suggesting that high MMP activity triggered by environment enrichment was degrading the PNN components. MMP-9 co-localized with PNNs in the cerebellum and its activity was also associated with decreased PNNs after environmental enrichment, an effect that was absent in *Mmp9* knockout mice [16]. Likewise, *Mmp9* mRNA levels increased after fear learning, and *Mmp3* and *Mmp13* mRNA levels were elevated after an epileptic seizure [37, 38]. MMP-3 and MMP-13 both cleave aggrecan and MMP-13 was recently shown to co-localize with PNNs in the dentate gyrus of the hippocampus [37]. In the ADAMTS family, ADAMTS8 and 15 are expressed by parvalbumin-positive neurons [43, 44], whereas ADAMTS4 cleaves brevican and is localized to synapses [45]. Given the complexity of the MMP and ADAMTS protein families and their abilities to act on multiple substrates, it is likely that other members of these families are able to regulate the structure of PNNs in an experience- and activity-dependent manner.

Although decreased PNN intensity and numbers of cells enclosed by PNNs after different sensory experiences may be due to increased activity of MMP and ADAMTS proteins leading to degradation of PNN protein components, this is likely not the only mechanism for altering PNN structure in response to stimuli. Several studies have found that *Acan*, *Bcan* and *Ncan* mRNA expression is altered after different sensory experiences [23, 25–27]. These findings suggest that epigenetic mechanisms occurring at the promoters of genes encoding PNN structural components may also be involved in PNN remodeling in response to various experiences.

Involvement of PNNs in Learning and Memory

Evidence that PNNs contribute to memory storage was first demonstrated in experiments performed by Gogolla *et al* [46], who showed that long-term memories of a fearful event could be removed by injecting chondroitinase ABC (ChABC, an enzyme that degrades PNNs) into the amygdala of mice. In these experiments, mice treated with ChABC were tested for fear responding one day after conditioning and exhibited a normal fear response (freezing behavior) when presented with a stimulus associated with the foot shock. However, the ChABC-treated mice either more rapidly extinguished or forgot the fear memory. In addition, the fearful memory could be spontaneously reinstated in control mice by presentation of the foot shock-associated stimulus up to one month after **extinction** training, whereas the ChABC-treated mice did not reinstate the memory. These results indicate that PNNs in the amygdala prevent the removal of a stored fear memory.

In contrast, PNNs in the hippocampus and auditory cortex appear to be important for the **consolidation** of fear learning [25, 47]. Injection of ChABC into the auditory cortex of mice abolished freezing responses one day after fear conditioning, although ChABC-treated mice still learned the association between the auditory stimulus and the shock immediately after conditioning [25]. *Acan*, *Bcan*, and *Ncan* gene expression was increased four hours after fear conditioning (but not earlier) along with an increased intensity and number of PNN-containing neurons [25]. In the hippocampus, injection of hyaluronidase (another enzyme that destroys PNNs by digesting hyaluronan) attenuated fear responding one day after conditioning [47], and a combination of hyaluronidase plus ChABC injected into the hippocampus also attenuated long-term fear memory [48]. Together, these studies indicate that PNNs are important in the hippocampus, auditory cortex, and amygdala for consolidating or maintaining long-term fear memories.

Interestingly, other types of learning and memory controlled by different brain regions are differentially affected by PNN removal. Digestion of PNNs using ChABC in the perirhinal cortex, a brain region important for object recognition memory, resulted in enhanced memory for the object [49]. Similarly, removing PNNs in the auditory cortex using hyaluronidase led to an increased ability to relearn a task that requires **behavioral flexibility**, known as **reversal learning** [50]. Both of these studies indicate that PNNs may restrict the ability to learn new behaviors related to cognitive functioning. Although these results seem to contradict the fear conditioning studies, extinction of a memory is hypothesized to involve learning a new memory that suppresses the old memory, rather than the erasure of the old memory [51]. PNNs may play a role in preventing new learning and/or memory formation during both extinction and reversal learning by restricting plasticity. This is consistent with the well-known role of PNNs in inhibiting plasticity of visual cortex neurons during a critical period of brain development [24].

The studies described above used enzymes to digest PNNs and therefore do not provide information on the specific components of PNNs that might regulate learning and memory. Four genes encoding PNN components are known to modulate learning and memory: *Hapln1*, *Tnr*, *Tnc*, and *Bcan*. Mice deficient in *Hapln1* in the central nervous system exhibited enhanced object recognition, similar to what is observed in mice infused with

ChABC in the perirhinal cortex [49]. Mice deficient in *Tnr* also showed enhanced object recognition and faster reversal learning in several tests [52], although they had subtle deficits in associative and spatial learning [53]. The increased ability of *Tnr* knockout mice to learn a new behavioral response is reminiscent of mice treated with hyaluronidase in the auditory cortex [50], suggesting that *Tnr* may be one component of PNNs that restricts flexibility in learning a new behavior. Interestingly, *Tnr*-deficient mice do not show altered fear learning, although fear extinction and long-term fear memory have not been tested [52]. *Tnc*-deficient mice learn fear normally, but exhibit impaired extinction of a fear memory [54]. *Tnc* knockout mice also have deficits in spatial learning [55]. Finally, *Bcan* knockout mice have impaired short-term working memory, but normal long-term memory when tested one day after conditioning in the novelty preference test [26]. The short-term memory deficit in *Bcan*-deficient mice is recapitulated by knocking down *Bcan* in parvalbumin-expressing neurons in the hippocampus using a viral-delivered short-hairpin RNA, indicating that *Bcan* expression in these specific neurons in the hippocampus is necessary for short-term memory [26]. Interestingly, in another hippocampal-dependent learning and memory test, novel object recognition, *Bcan* knockout mice exhibit defective short-term memory, but enhanced long-term memory, similar to *Hapln1* and *Tnr* knockout mice [26].

As mentioned above, PNNs are altered by experience, including during learning and memory retrieval and reconsolidation [6, 16, 25–31, 56]. Activity-dependent modulation of PNN structure by extracellular proteases is likely important for the ability of PNNs to modulate learning and memory. MMP-9 is the most-well studied member of the MMP family in the brain and regulates several types of learning and memory [39–42, 57]. It is likely that ADAMTS proteins will play a similar role as MMPs in regulating PNN structure and learning and memory processes, but much remains to be discovered with regard to the remodeling of PNNs by specific MMP and ADAMTS proteins and where these act in the brain during learning and memory processes.

Effects of Drugs of Abuse on PNNs

Exposure to several drugs of abuse (cocaine, heroin, nicotine, and alcohol) alters PNNs in specific brain regions, similar to what is observed with other types of experience-dependent learning (Table 1). Several studies have focused on the **conditioned place preference** (CPP) test. In a study by Slaker *et al* [58], the medial prefrontal cortex was examined for PNNs after the rats had been tested for cocaine CPP and then confined in the cocaine- or non-cocaine (saline)-paired context. The number of cells labeled with WFA in the prelimbic area of the prefrontal cortex was decreased independently of the context. This indicates that PNNs decrease after learning CPP, but not in an environment-specific manner [58]. However, the number of cells that were double-labeled for WFA and c-fos, a marker of neuronal activity, were increased in the prelimbic cortex only in rats that were confined to the cocaine-paired chamber, suggesting that the reactivation of the cocaine-associated memory preferentially increased the activity of neurons surrounded by PNNs in this brain region [58].

Cocaine also alters PNNs in the cerebellum in a temporally- and spatially- specific pattern. Mice injected with six cocaine injections followed by a one-week withdrawal period and a

single cocaine challenge had increased intensity of PNNs around glutamatergic medial projection neurons of the cerebellum one day after the cocaine challenge [59]. In contrast, in mice treated with six cocaine injections followed by a one-month withdrawal and cocaine challenge, PNN intensity was decreased around these neurons [60], demonstrating a dynamic regulation of PNNs during withdrawal from chronic cocaine. After cocaine CPP, PNNs surrounding the medial projection neurons were also reduced in intensity independently of whether the mice developed preference for the drug-paired chamber [61]. In contrast, PNN intensity surrounding a different type of cerebellar neuron, the GABAergic Golgi interneuron, was increased following cocaine CPP, but only in mice that developed preference for the cocaine-paired chamber [61]. Increased PNN intensity on Golgi interneurons was also observed in mice that showed preference for the cocaine-paired chamber and were re-exposed to the cocaine-paired context [61], suggesting that the Golgi interneurons encoded the cocaine-associated memory. Together, these results indicate that PNNs in both the prelimbic cortex and the cerebellum are preferentially modified by the activation of a cocaine-associated memory.

Heroin and nicotine can also alter PNNs in another animal model of addiction, known as drug **self-administration**. Twenty-one days of abstinence from heroin self-administration reduced protein levels of brevican, tenascin-R, and HAPLN1 in synaptic membranes from the medial prefrontal cortex [62]. This reduction was normalized after cue-induced **reinstatement** of heroin self-administration. For nicotine, the number and intensity of PNNs surrounding parvalbumin-expressing neurons were decreased in the ventral tegmental area (VTA) and the orbitofrontal cortex (a region of the prefrontal cortex) forty-five minutes after a nicotine self-administration session [63]. The reduction in the number of PNNs in the VTA was maintained at seventy-two hours after the last self-administration session [63]. Nicotine also increased protein levels of MMP-2, MMP-3 and MMP-9 in the hippocampus and prefrontal cortex during CPP training [64], suggesting that PNNs could potentially be remodeled in these regions through elevated MMP activity during the learning of a nicotine-environment association.

Finally, alcohol (ethanol) exposure has also been shown to alter the intensity of PNNs in various regions of the brain, although ethanol has generally been shown to increase, rather than decrease, PNN intensity. Neonatal ethanol exposure in rats increased levels of chondroitin sulfate glycosaminoglycans and expression of neurocan in the hippocampus [65]. In adolescent rats, exposure to binge ethanol administration increased the intensity of PNNs and levels of brevican and neurocan immunoreactivity in the orbitofrontal cortex observed in adulthood, changes associated with deficits in reversal learning [66]. In adult mice, six weeks of binge-like drinking resulted in increased intensity of PNNs, expression of aggrecan mRNA and protein, and brevican protein in the insular cortex, a region of the brain involved in compulsive drinking [67, 68]. Ethanol injections decreased MMP-9 activity in the rat prefrontal cortex and hippocampus [69]. This decrease in MMP-9 activity is consistent with increased intensity of PNNs after ethanol exposure, since increased MMP activity is predicted to proteolytically degrade PNN components. In summary, PNNs are modified in various brain regions in response to multiple drugs of abuse (Figure 2A). Of

particular interest are changes in PNNs in the context of learning and memory of drug-associated environments that would implicate PNNs in addiction-related behaviors.

Effects of PNN Digestion on Addiction-Related Behaviors

The ability of drug exposure to regulate the structure of PNNs suggests that PNNs may, in turn, be involved in the adaptive response of the brain to drugs and the learning and/or memory of the drug-associated experience. This is important because strong memories for the environment in which the drug was used can promote relapse to drug use. Evidence that PNNs are involved in the learning and memory of a drug-associated environment has been provided using the CPP and drug self-administration tests.

When ChABC was injected into the prelimbic cortex or a specific region of the hypothalamus of rats prior to conditioning, the development of cocaine CPP was attenuated, indicating that PNNs are important for the learning of the drug-environment association [58, 70]. Interestingly, injection of ChABC into the prelimbic cortex after testing for CPP did not affect the extinction or reinstatement of CPP [58]. However, when ChABC was injected after extinction, just prior to reactivation of the memory, reinstatement to CPP was attenuated compared with control animals, suggesting that PNNs are important for the reconsolidation of the memory [58]. In comparison, ChABC injection into the amygdala after conditioning, but prior to extinction, attenuated the reinstatement of CPP [71]. This is comparable to what has been observed in the fear conditioning experiments, in which reinstatement of a fear memory is reduced after ChABC injection into the amygdala [46]. PNNs in the amygdala are also important for the reinstatement of morphine CPP [71]. Together, these results indicate that PNNs in brain regions involved in addiction are important for the learning and memory of a drug-associated environment (Figure 2B), and indicate that disruption of PNNs might be a novel way to suppress these memories and prevent relapse.

Another behavioral test that models addiction-like behavior in animals is drug self-administration. Digestion of PNNs in a specific region of the hypothalamus (anterior dorsal lateral hypothalamic area) prior to self-administration training decreased acquisition of cocaine self-administration [70]. ChABC injection into the amygdala after self-administration training but prior to extinction enhanced the extinction of cocaine and heroin self-administration, and attenuated reinstatement [71]. Again, this is similar to what has been observed with fear conditioning, in which extinction was enhanced but reactivation of a fear memory was reduced upon disruption of PNNs in the amygdala, and further supports the concept that disruption of PNNs might be a strategy to prevent relapse to drug use.

Brevican Regulates Addiction-Related Behaviors

Currently brevican is the only PNN structural component that has been investigated for its role in addiction-like behavior. Heterozygous *Bcan* knockout mice, which express ~50% of normal brevican levels, exhibited enhanced cocaine CPP at 21 days, but not one day, after conditioning compared with wild-type *Bcan* mice [72]. This is analogous to the enhanced long-term novel object recognition memory observed in homozygous *Bcan* knockout mice [26]. Interestingly, heterozygous *Bcan* knockout mice did not differ from wild-type mice in

cocaine CPP when tested one day after conditioning. Overexpression of *Bcan* in the hippocampus, but not prefrontal cortex, was able to normalize the enhanced cocaine CPP observed after 21 days [72], suggesting that brevican functions in the hippocampus to suppress a persistent cocaine-associated memory and that enhancing brevican function might allow for a cocaine-associated memory to be forgotten.

MMPs Regulate Addiction-Related Behaviors

The MMPs are also important for the reinstatement of drug-seeking behavior. In an elegant set of experiments by Smith et al [73], microinjection of an MMP-9 or MMP-2 inhibitor into the nucleus accumbens core region of the brain reduced cue-induced reinstatement of cocaine-seeking behavior and injection of an MMP-9 inhibitor decreased cocaine-induced reinstatement. Consistent with a role for MMPs in promoting drug-seeking, MMP activity increased in the nucleus accumbens core after cue-induced reinstatement of cocaine, nicotine, and heroin seeking [73]. MMP-9, in particular, appears to contribute to this increase in activity after reinstatement, because a selective MMP-9 inhibitor attenuated this increase [73]. MMP-9 in the rat medial prefrontal cortex was also higher after cocaine-induced reinstatement of CPP, and inhibition of MMPs in rats with the non-selective MMP inhibitor, FN-439, decreased cocaine-primed reinstatement of CPP [74, 75]. Although MMP-9 has multiple protein substrates and is likely degrading extracellular matrix proteins other than those found in PNNs, it is possible that changes in activity of MMP-9 (or other MMPs) may be responsible for PNN remodeling after drug exposure and subsequent behavioral outcomes.

MMPs also play a role in behaviors related to alcohol, nicotine, and amphetamine addiction. Injection of FN-439 into rat cerebral ventricles attenuated the escalation in lever pressing for ethanol that occurs during ethanol withdrawal [76], suggesting the MMPs are important for the neuroadaptive changes that occur during withdrawal that promote excessive drinking. In addition, *Mmp9* knockout mice exhibited decreased motivation to obtain alcohol [77]. MMP-9 activity in the central nucleus of the amygdala promotes alcohol-seeking behavior, since overexpression of MMP-9 in this brain region in *Mmp9* knockout mice eliminated the deficit in alcohol seeking [77]. *Mmp2* and *Mmp9* knockout mice have attenuated amphetamine CPP and administration of a non-specific MMP inhibitor into rat cerebral ventricles decreased amphetamine CPP [78, 79]. Finally, infusion of FN-439 into rat cerebral ventricles attenuated nicotine CPP [64].

Concluding Remarks and Future Perspectives

Experiments in rodents have provided evidence that PNNs and MMPs may regulate drug-seeking behavior and the long-term persistence of drug-associated memories that promote relapse to drug abuse. Manipulating PNNs during abstinence might be a novel strategy to reduce relapse, since administration of ChABC or MMP inhibitors prior to extinction or reinstatement attenuates drug-seeking behavior for several drugs of abuse. There is genetic evidence that PNNs may be involved in drug abuse in humans. A polymorphism in the human *MMP9* gene that increases MMP9 expression was associated with alcoholism and increased motivation to obtain alcohol in alcoholics [77, 80], and a genetic locus containing

the *TNR* gene was associated with high risk for alcohol dependence [81]. In addition, MMP-9 activity was decreased in the hippocampus of cocaine abusers, and ratios of MMP-2 and MMP-9 to their endogenous inhibitors were increased in the serum of heroin abusers, indicating that dynamic alterations in MMP activity may be relevant to cocaine and heroin addiction in humans [82, 83]. Future studies should examine potential genetic associations of other genes encoding PNN components with addiction phenotypes.

It will also be important in the future to understand how specific MMP and ADAMTS proteins in the brain regulate the remodeling of PNNs in response to drug exposure or memory retrieval using genetic mouse models (see Outstanding Questions), because these enzymes could be useful pharmacological targets to treat addiction. Likewise, other components of PNNs such as HAPLN1 and tenascin-R have not been studied for their roles in addiction-like behaviors, despite the fact that they play a role in learning and memory and the gene knockout mice are viable. Region-specific and/or cell-type specific knockout or knockdown of different genes encoding PNN components and enzymatic regulators will allow for a more refined understanding of how PNNs function to regulate drug relapse, facilitating the identification of new target genes for drug discovery efforts for addiction treatment.

Outstanding Questions

- How do individual components of PNNs, such as aggrecan, brevican, tenascin-R, and HAPLN1 regulate reinstatement of drug-seeking behavior?
- Which types of neurons surrounded by PNNs in specific brain regions are important for controlling reinstatement to CPP or drug self-administration?
- Do PNNs and their individual constituents in particular brain regions regulate alcohol consumption?
- How do PNNs control the neuronal firing properties and circuit-level activity in different brain regions and in response to drugs of abuse?
- How do individual components of PNNs, the MMPs, and the ADAMTS proteins regulate synaptic plasticity?
- Which MMP or ADAMTS proteins are responsible for remodeling PNNs in different brain regions and in response to different drugs of abuse?
- Are there genetic associations in humans between genes encoding PNN components, MMPs, or ADAMTS proteins and increased risk for developing addiction?
- Are changes in PNNs after drug exposure adaptive (protective) or maladaptive (detrimental)?
- How do we explain the paradox that disrupting PNNs enzymatically with ChABC decreases a cocaine-associated memory, but that decreasing brevican function increases a cocaine-associated memory?

Why are some forms of memory disrupted by digesting PNNs while others are enhanced?

Finally, recent appreciation that the molecular composition of PNNs is not uniform throughout the brain indicates that PNNs with different structural components, such as brevican and aggrecan, may have different functions in regulating neuronal activity and, by extension, learning and memory [3, 21, 84, 85]. A greater understanding of the molecular differences in PNNs, the cell types they surround in particular neuroanatomical locations, and the roles of these specific molecules in drug-associated memories is essential for potentially manipulating these fascinating structures as a way to reduce addiction.

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Glossary

A Disintegrin and Metalloproteinase with a Thrombospondin motifs (ADAMTS)
Secreted proteases that contain thrombospondin motifs. ADAMTS1, 4, 5, 8, 9, 15, and 20 are also known as aggrecanases because they enzymatically cleave the lecticans such as aggrecan.

Behavioral flexibility
The ability of an animal to change behavior in response to altered environmental conditions. This adaptive response involves learning a new behavior and is often disrupted in psychiatric and neurological disorders.

Chondroitin sulfate
A disaccharide (sugar) unit that consists of sulfated glucuronic acid and *N*-acetylgalactosamine. Chondroitin sulfate is best known for its role in cartilage. Chondroitin sulfate glycosaminoglycans are long chains of repeating chondroitin sulfate units and are attached to chondroitin sulfate proteoglycans.

Conditioned place preference
An associative learning task in which a drug is administered to an animal in a specific environmental context during conditioning. The animal learns to associate the environment in which it received the drug with the internal state achieved while on the drug. A positive experience results in the animal spending more time in the drug-paired compartment in the absence of drug, known as place preference.

Extracellular matrix (ECM)
A three-dimensional structure that is present in all tissues on the outside of the cells. The ECM provides structural support to tissues but is also actively remodeled and controls many normal physiological and disease-related processes.

Hyaluronan
A linear non-sulfated glycosaminoglycan that contains repeating disaccharide units of *N*-acetylglucosamine. Hyaluronan is a major component of the extracellular matrix and is found throughout the body.

Consolidation
The process of converting a short-term memory into a long-term memory.

Extinction
A process by which an animal learns that a conditioned stimulus (CS), such as a light or tone, associated with an unconditioned stimulus (US), such as a foot shock, is no longer associated with the unconditioned stimulus and decreases its behavioral response to the conditioned stimulus. With regard to animal models of drug addiction, extinction occurs when the drug is no longer available and the animal reduces the behavioral response that was previously associated with drug availability, such as place preference or lever pressing for drug.

Matrix metalloproteinase (MMP)
Enzymes that that proteolytically degrade the extracellular matrix.

Neural Plasticity
The ability of neurons to change in response to alterations in their environment. Neural plasticity is essential to learning and memory.

Reinstatement
A behavioral response in which an animal performs an action that was previously associated with drug availability but was extinguished. Reinstatement is a model for relapse and can be elicited by a cue (a stimulus that was previously associated with drug availability) or by an injection of a drug (known as drug-priming).

Reversal learning
A test of behavioral or cognitive flexibility in which an animal must learn a new response when the outcome has changed.

Self-administration
A behavioral task in which an animal presses a lever or performs another operant procedure to obtain a drug, typically adminstered by intravenous infusion.

***Wisteria floribunda* agglutinin (WFA)**
A plant protein (lectin) that binds to sugar chains terminating in *N*-acetylgalactosamine. WFA is routinely used to visualize PNNs.

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Trends Box

Perineuronal nets (PNNs) are distinct extracellular matrix structures consisting of dense protein and sugar chains that condense around specific neurons in the brain.

PNNs are involved in storing long-term memories and also preventing an animal from learning new behaviors.

PNNs are modified in several brain regions by many types of experiences, including exposure to drugs of abuse such as cocaine, heroin, nicotine and alcohol.

MMP and ADAMTS proteins are extracellular proteases that are activated by exposure to drugs of abuse and remodel the extracellular matrix.

Disruption of PNNs after an animal has learned a drug-environment association can prevent reinstatement, or relapse, of the drug-associated behavior.

Brevican is one component of PNNs that regulates the storage of long-term cocaine-associated memories.

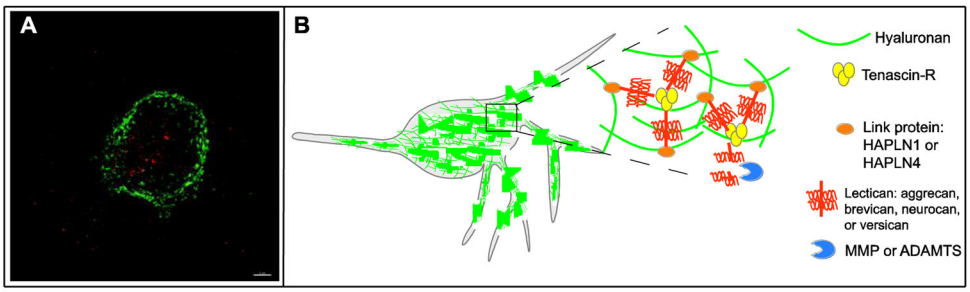

Figure 1. Fluorescent image and conceptual structure of PNNs

(**A**) A neuron expressing parvalbumin (red) covered by a PNN as visualized by WFA fluorescent staining. Scale bar, 3 μm. (**B**) Structural components and regulators of PNN structure: hyaluronan, tenascin-R, hyaluronan and proteoglycan link proteins (HAPLN1, HAPLN4), the lecticans (aggrecan, brevican, neurocan, and versican), and enzymes that degrade the extracellular matrix (MMP and ADAMTS proteins).

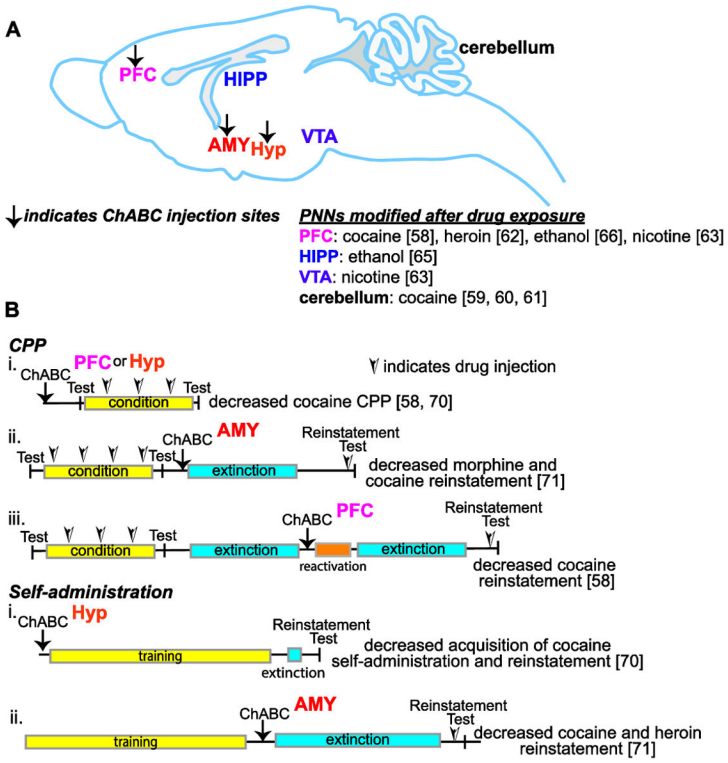

Figure 2. Brain regions in which PNNs are remodeled or regulate addiction behaviors

(**A**) Saggital view of rat or mouse brain showing areas in which PNNs have been demonstrated to change in response to drug exposure and/or in which PNNs have been degraded by microinjection of chondroitinase ABC (ChABC) and animals tested for CPP or drug self-administration. Black arrow shows locations in which ChABC has been injected. Note that the insula, a cortical area that shows an increase in PNNs after ethanol exposure, is not shown here. (**B**) Experimental design of CPP and drug self-administration experiments showing when ChABC was injected relative to testing animals for either CPP or reinstatement of drug-seeking behavior. Abbreviations: PFC, prefrontal cortex (includes prelimbic, medial prefrontal, and orbitofrontal); AMY, amygdala; HIPP, hippocampus; Hyp, hypothalamus; VTA, ventral tegmental area. References are indicated in brackets.

Table 1

Summary of changes in perineuronal nets induced by drugs of abuse.

Drug	Species/Brain Region	Alteration (Method Used)	Time Point	Reference
Cocaine	Rat/prelimbic cortex	Decreased number of WFA[a]- positive cells (IHC[b])	One hour after confinement to a previous cocaine- paired context (3 cocaine injections)	[58]
Cocaine	Mouse/cerebellum (glutamatergic medial projection neurons)	Increased intensity of WFA labeling (IHC)	One day after a single cocaine injection given one week after 6 prior injections	[59]
Cocaine	Mouse/cerebellum (glutamatergic medial projection neurons)	Decreased intensity of WFA labeling (IHC)	One day after a single cocaine injection, given one month after 6 prior injections	[60]
Cocaine	Mouse/cerebellum (glutamatergic medial projection neurons)	Decreased intensity of WFA labeling (IHC)	Seventy minutes after a cocaine CPP[c] test (8 cocaine injections)	[61]
Cocaine	Mouse/cerebellum (Golgi neurons)	Increased intensity of WFA labeling (IHC)	Seventy minutes after a cocaine CPP test (8 cocaine injections)	[61]
Ethanol	Mouse/orbitofront al cortex	Increased intensity of WFA, brevican, and neurocan labeling (IHC)	Two months after a binge ethanol procedure (6 injections)	[66]
Ethanol	Rat/hippocampus	Increased total chondroitin sulfate glycosamin oglycans (sulfated GAG assay) and neurocan (IHC)	Two hours after last ethanol intubation (10 ethanol exposures) in neonates	[65]
Ethanol	Mouse/insular cortex	Increased intensity of WFA labeling (IHC)	One day after last ethanol drinking session (24 drinking sessions)	[67]
Heroin	Rat/medial prefrontal cortex	Decreased synaptic tenascin-R and brevican protein (Western blots)	Twenty-one days after last heroin self-administration session (16 self administration sessions)	[62]
Heroin	Rat/medial prefrontal cortex	Normalized synaptic tenascin-R and brevican protein (Western blots)	After cue-induced reinstatement of heroin self-administration	[62]
Nicotine	Rat/orbitofrontal cortex	Decreased number of WFA- positive parvalbumin cells and WFA intensity (IHC)	Forty-five minutes after 21 days of nicotine self-administration	[63]
Nicotine	Rat/ventral tegmental area	Decreased number of WFA- positive parvalbumin cells and WFA intensity (IHC)	Forty-five minutes and seventy-two hours after 21 days of nicotine self- administration	[63]

[a] *Wisteria floribunda* agglutinin;

[b] Immunohistochemistry;

[c] Conditioned place preference