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Casting a Wide Net: Role of Perineuronal Nets in Neural Plasticity

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Abstract

Perineuronal nets (PNNs) are unique extracellular matrix (ECM) structures that wrap around certain neurons in the central nervous system (CNS) during development and control plasticity in the adult CNS. They appear to contribute to a wide range of diseases/disorders of the brain, are involved in recovery from spinal cord injury, and are altered during ageing, learning and memory, and after exposure to drugs of abuse. Here we focus on how a major component of PNNs, chondroitin sulfate proteoglycans (CSPGs), control plasticity, and on the role of PNNs in memory in normal ageing, in a tauopathy model of Alzheimer's disease, and in drug addiction. We discuss how altered ECM/PNN formation during development may produce synaptic pathology associated with schizophrenia, bipolar disorder, major depression, and autism spectrum disorders. Understanding the molecular underpinnings of how PNNs are altered in normal physiology and disease will offer insights into new treatment approaches for these diseases.

Abbreviations: **Ch-ABC,** chondroitinase-ABC; **C4S,** chondroitin sulfate with 4-*O*-sulfation; **C6S,** chondroitin sulfate with 6-*O*-sulfation; **C4ST-1,** chondroitin 4-*O*-sulfotransferase-1; **C6ST-1,** chondroitin 6-*O*-sulfotransferase-1; **Crtl-1,** cartilage link protein-1; **CS,** chondroitin sulfate; **CSE,** chondroitin 4,6-disulfate; **CSPG,** chondroitin sulfate proteoglycan; **DMN,** deep medial nucleus; **ECM,** extracellular matrix; **GalNAc,** N-acetylgalactosamine; **GWAS,** genome-wide association studies; **mPFC,** medial prefrontal cortex; **PFC,** prefrontal cortex; **PNN,** perineuronal net; **PrC,** perirhinal cortex; **PV,** parvalbumin

Running title: Perineuronal nets and plasticity

Introduction

An emerging concept in neuroscience is that brain plasticity is dependent not only on neurons and glial cells, but also what is present on the *outside* of these cells, the extracellular matrix (ECM). This matrix comprises approximately 20% of the brain's volume (Nicholson and Sykova, 1998), and critically contributes to communication between neurons and glia. Advances in our understanding of the ECM has led to progression from the tripartite theory of synaptic signaling (Araque et al., 1999) to the tetrapartite theory (Dityatev and Rusakov, 2011). If we are to understand normal physiological functioning of the brain such as learning and memory as well as pathologies underlying brain disorders, we must integrate the contribution by ECM molecules into our understanding of brain signaling processes.

There are three major types of ECM: 1) the "loose" ECM that is present throughout the brain and spinal cord; 2) the membrane-bound molecules on cells; and 3) the unique, lattice-like structures that wrap around specific neurons in the brain and spinal cord called perineuronal nets (PNNs) that tightly interdigitate with synaptic contacts on the soma and proximal dendrites of neurons (Celio et al., 1998; Deepa et al., 2006; Soleman et al., 2013). *The primary focus of this review is on PNNs*: their basic structure, function, and roles in normal physiological function and brain disorders. PNNs were first described as reticular structures by Golgi in the late 1800s (Spreafico et al., 1999), but only recently has there been intense focus on the role of PNNs in normal brain function, such as learning and

memory, and in many disorders or pathologies, such as schizophrenia, Alzheimer's stroke, epilepsy, autism, and drug addiction.

PNNs form during development, completed by about 40 days in the cortex of rodents (Bruckner et al., 2000). The developmental time window for PNN formation is significant because it marks the period when plasticity is greatly reduced and when the critical period ends (Pizzorusso et al., 2002a). A centralizing concept is that PNNs *limit plasticity* in adulthood and that they can be degraded to *reinstate* juvenile-like states of plasticity to produce axon sprouting and regeneration of function in damaged neurons. As such, PNNs play key roles in neural development, synaptogenesis, neuroprotection, and experience-dependent synaptic plasticity (Celio et al., 1998; Dityatev and Schachner, 2003; McRae and Porter, 2012; Soleman et al., 2013; Suttkus et al., 2016).

Composition and Function of PNNs

PNNs are formed by four families of ECM molecules. **(1)** Hyaluronan and its synthesizing enzymes hyaluronan synthases (HASs; HAS1 and 3 are found in the CNS); hyaluronin is extruded extracellularly and forms a backbone onto which other PNN molecules bind. **(2)** Chondroitin sulfate proteoglycans (CSPGs; more than 15 isoforms are identified in the CNS; see below for greater detail on the role of CSPGs). Among CSPGs, lectican family members including aggrecan, versican, neurocan, and brevican are principal constituents of PNNs (Galtrey and Fawcett, 2007; Kwok et al., 2011). Whereas mice deficient for versican, neurocan, or brevican have largely normal PNNs (Dours-Zimmermann et al., 2009), cortical

primary neurons derived from aggrecan-deficient mice are abnormal in that they are not stained by the lectin *Wisteria floribunda* agglutinin (WFA), a broad PNN marker, indicating an essential role of aggrecan in PNN formation (Giamanco et al., 2010). **(3)** Tenascins (Tns; Tn-R is a key component in the PNNs). **(4)** Hyaluronan and proteoglycan link proteins (HAPLNs; HAPLN1, 3 and 4 are found in the CNS), or simply, "link proteins", which bind to both the hyaluronin backbone and CSPGs to stabilize PNNs (Koppe et al., 1997; Carulli et al., 2007; Carulli et al., 2010; Kwok et al., 2010). The combination of these molecules creates PNNs of large variety and confers them with diverse biochemical properties. The complexity is further stratified by other modifications, such as sulfation in the chondroitin sulfate (CS) chains (Wang et al., 2008; Lin et al., 2011; Miyata et al., 2012) (see below for detailed role of CS chains).

PNNs are found primarily around fast-spiking, parvalbumin (PV)-containing GABAergic interneurons in many brain regions (Hartig et al., 1992; Schuppel et al., 2002; Dityatev et al., 2007), but not all regions (e.g., cerebellum; see below). Given their location surrounding fast-spiking interneurons, PNNs are in a prime position to alter the excitatory/inhibitory balance and thus regulate output of these regions. PNNs are believed to protect neurons from oxidative stress (Morawski et al., 2004; Cabungcal et al., 2013), perhaps by limiting GABAergic interneuron excitability. It is hypothesized that PNNs play a role in regulating neural plasticity via three mechanisms (**Figure 1**) (Wang and Fawcett, 2012): 1) altering the formation of new neuronal contacts (Corvetti and Rossi, 2005; Barritt et al., 2006); 2) acting as a scaffold for molecules that can inhibit synaptic formation (Deepa et al., 2002); and 3) limiting receptor motility at synapses (Frischknecht et al., 2009).

Role of Chondroitin Sulfate Proteoglycans (CSPGs) during Development

CSPGs consist of core proteins with one or more covalently attached CS chains. Studies from the Kitagawa laboratory have focused on the role of sulfation patterns of CSPGs in neural development. The importance of sulfation patterns of CS chains in such plasticity has been overlooked in previous studies because Ch-ABC destroys all CS chains, irrespective of CS sulfation status. CS chains are long linear polysaccharides composed of repeating disaccharide units; each unit comprises a glucuronic acid and an *N*-acetylgalactosamine (GalNAc) residue. During biosynthesis, individual GalNAc residues of the repeated disaccharide units can be sulfated by chondroitin 6-*O*-sulfotransferase-1 (C6ST-1) or chondroitin 4-*O*-sulfotransferase-1 (C4ST-1), thereby generating 6-sulfation or 4-sulfation, respectively (Mikami and Kitagawa, 2013; Miyata and Kitagawa, 2015).

Notably, there are drastic changes in the sulfation patterns of CS chains during the formation of PNNs. Specifically, 6-*O*-sulfation is dominant in the juvenile brain to produce C6S, which is more permissive (Lin et al., 2011; Miyata et al., 2012), whereas 4-*O*-sulfation becomes dominant in the adult brain to produce C4S, which is the most inhibitory form of CS: it inhibits the growth of cerebellar granular neurons in culture and is upregulated in regions that do not support axonal growth after spinal cord injury (Deepa et al., 2006; Wang et al., 2008). Overall then, there is a substantial increase in the 4-sulfation/6-sulfation (C4S/C6S) ratio during brain development (Kitagawa et al., 1997; Miyata et al., 2012). The percentages of both C6S and another isoform, chondroitin 4,6-disulfate (CS-E),

decrease drastically after birth and remain at a low level in adults. (However, there is an enrichment of C6S and CS-E in the PNNs when compared to the CSs isolated from the loose brain ECM (Deepa et al., 2006; Dick et al., 2013). The shift in sulfation patterns is essential for PNN formation: transgenic mice with reduced C6S show poor regeneration after a lesion in the CNS (Lin et al., 2011), and transgenic mice overexpressing C6ST-1 retain juvenile-like CS sulfation and show impaired PNN formation (Miyata et al., 2012). In addition, overexpression of C6ST-1 prevents the maturation of electrophysiological properties of PV-expressing interneurons and reduces the inhibitory effects these of PV cells because of impaired PNN formation. As a result, transgenic mice overexpressing C6ST-1 retain a *juvenile* level of ocular dominance plasticity *even in adulthood* (Miyata et al., 2012). Interestingly, overexpression of C6ST-1 selectively decreases aggrecan in the aged brain without affecting other PNN components. In addition, the increased 6-sulfation accelerates proteolysis of aggrecan by a disintegrin and metalloproteinase domain with thrombospondin motif (ADAMTS) protease (Miyata and Kitagawa, 2016). These results indicate that sulfation patterns of CS chains on aggrecan influence the stability of the CSPG, thereby regulating formation of PNNs and neural plasticity, and overall, the CS chains regulate the plasticity characteristic of the critical period.

Alteration of C6ST-1 expression and CS sulfation patterns are found in brains of human patients with bipolar disorder or schizophrenia and mice with cortical brain injury (Yi et al., 2012; Okuda et al., 2014; Pantazopoulos et al., 2015) (see also below). Notably, chondroitin 6-sulfation and chondroitin 6-sulfation-enriched PNNs increase in the mouse cerebral cortex after kainic acid treatment; simultaneously, chondroitin 4-sulfation-

enriched PNNs and the 4S/6S ratio decrease. Furthermore, C6ST-1 TG mice are more susceptible to kainic acid-induced seizures than wild-type mice (Yutsudo and Kitagawa, 2015). These results suggest that chondroitin 6-sulfation is relevant to epilepsy most likely because of dysregulated PNN formation and PV cell maturation, and that an abnormal balance of 4-sulfation and 6-sulfation produced by both neurons and astrocytes may contribute to the disease.

Role of PNNs in Memory, Ageing, and an Alzheimer's Disease Model

Memory is a form of plasticity, so it is reasonable to ask whether PNN interventions affect memory. The first memory model to be explored was fear conditioning, which involves the amygdala. Chondroitinase ABC treatment does not affect fear conditioning, but it restores the ability to reverse or unlearn the conditioning (Gogolla et al., 2009). This enzyme treatment also enhances reversal learning in the auditory cortex (Happel et al., 2014). In contrast, PNN removal has also been shown to *prevent* plasticity induced by fear conditioning (Hylin et al., 2013) and impairs certain aspects of learning/memory in animal models of addiction (see Addiction Models below).

The Fawcett laboratory has recently focused on object recognition memory, which relies on the tendency of rodents to investigate novel objects in preference to familiar ones, and it relies on the perirhinal cortex (PrC). Digestion of CSPGs in PrC or transgenic attenuation of PNNs had the effect of greatly extending object memory, from 12 to 96 hours (Romberg et al., 2013). This was unexpected; greater plasticity might mean more rapid turnover. A

possible explanation came from the work of the Caroni laboratory, looking at synaptic changes during memory. In the hippocampus, a memory task leads to an increased number of inhibitory synapses on PV interneurons, reducing their GABA production and thereby promoting cortical excitability (Donato et al., 2013). Ch-ABC treatment has exactly the same effect on this late-born population of PV neurons in both the hippocampus and PrC, providing a possible link to the effect of PNN removal on memory.

Prolongation of object memory is probably not very useful. However, in situations where memory is defective, restoration would be valuable. Transgenic mice that overexpress a mutant form of tau that gives tauopathy and dementia in humans provide a model for Alzheimer's disease and related conditions (Allen et al., 2002). These mice develop neurofibrillary tangles and hyperphosphorylation of tau, with obviously dystrophic neurons by 3 months and neuronal loss after 4 months. This translates to a profound loss of object memory by 3 months. Treating these animals with Ch-ABC to the PrC restored object memory to normal levels (Yang et al., 2015), and transgenic attenuation of PNNs in tauopathy mice delays by several weeks the onset of memory loss. How might these interventions act to restore memory? Two mechanisms are likely. First, Ch-ABC treatment enables sprouting of axons to create bypass circuits, and this may enable the CNS to bypass dysfunctional neurons affected by tau pathology. Second, removal of PNNs may make it easier for memories to form, based on easier access for new inhibitory synapses onto PV neurons, leading to reduced GABA inhibition of cortical circuits.

Memory loss is a feature of ageing even in the absence of Alzheimer's disease. This can be seen in aged mice, which have a marked deficit in memory retention at 18 months of age. Again, Ch-ABC injections to the PrC can restore object memory, or injections to the hippocampus restore object place memory (Yang, unpublished results). The deterioration of memory with age has usually been assumed to be caused by a decrease in the number of synapses with age. However, there is a possible alternative PNN-based mechanism. The findings that CS sulfation patterns are different across development together with the idea that mice with enhanced C6S production have increased plasticity prompted the Kwok and Fawcett laboratories to ask the question: do PNNs in ageing brains, where plasticity has been drastically reduced, show different sulfation composition than young brains? Biochemical analysis of isolated brain glycans from 3-month to 18-month old brains shows that there is a three-fold reduction of C6S in the PNNs from 12- and 18-month old brains. This reduction is specific to the PNNs and is not observed in young brains or in the general brain ECM (Foscarin et al., unpublished results). This change almost eliminates the permissive C6S, leaving only 4-sulfated forms (Carulli, unpublished results). This might be expected to make PNNs yet more inhibitory and to block the formation of new synapses on PV neurons that underlies memory.

These changes could explain the loss of plasticity in aged animals. In addition to acting directly on neuronal growth, CSs also modulate growth and plasticity by binding to different molecules in the ECM. The chemorepulsive molecule semaphorin 3A binds specifically to PNNs via CS-E (found in adults), and this binding exerts an additional level of inhibition of PNN matrix to the growth of neurons (Dick et al., 2013; Vo et al., 2013). The

transcription factor Otx-2 also binds to the CS-E in the PNNs and thus regulates the maturation of neurons and the duration of the critical period, a time period when the CNS remains plastic during visual cortex development (Beurdeley et al., 2012; Spatazza et al., 2013). These studies suggest that the functions of PNNs are heavily dependent on the composition of PNN components and their assembly. They present a promising avenue for plasticity enhancement to improve CNS pathologies through PNN manipulation.

In summary, PNNs have many potential sites for therapeutic action. Compounds acting on the PNN will not slow the progression of the pathology of Alzheimer's disease or prevent ageing. However, based on the current rodent results, there is a strong possibility that PNN interventions will enable the brain to keep working *despite* the underlying pathology.

Role of PNNs in Psychiatric Disorders

Rapidly emerging evidence points to ECM abnormalities as a key component of the pathophysiology of psychiatric disorders, including schizophrenia (Berretta laboratory), bipolar disorder, major depression, autism, and addiction (see Addiction Models section below) (Berretta, 2012; Folsom and Fatemi, 2013; Berretta et al., 2015). Disruption of PNNs has been particularly well documented in schizophrenia, with marked decreases of CSPG-labeled PNNs in the amygdala, entorhinal cortex and PFC (Pantazopoulos et al., 2010a; Mauney et al., 2013; Pantazopoulos et al., 2015). These interconnected brain regions are involved in emotion-related learning and associative sensory information processing and in the pathophysiology of this disorder (Prasad et al., 2004; Berretta et al.,

2007; Pantazopoulos et al., 2015). PNN decreases are accompanied by altered CSPG expression in glial cells (Pantazopoulos et al., 2010b; Pantazopoulos et al., 2015), a significant finding because these cells represent the main contributors to the ECM/PNNs molecular building blocks (Faissner et al., 2010) (see also above). Additional support comes from human genetic and postmortem studies pointing to the involvement of key ECM/PNN molecules, including CSPGs, Reelin, semaphorin 3A, integrins, and remodeling enzymes such as metalloproteinases in schizophrenia (Guidotti et al., 2000b; Eastwood et al., 2003; Ripke and Schizophrenia Working Group of the Psychiatric Genomics, 2014).

Similar findings have been reported in bipolar disorder and major depression. For instance, decreased Reelin expression has been observed in the PFC, hippocampus, and cerebellum, as well as in blood of subjects with bipolar disorder or major depression (Guidotti et al., 2000a; Fatemi, 2005). Postmortem studies in the Berretta laboratory on bipolar disorder show marked decreases of PNNs across several nuclei in the amygdala (Pantazopoulos et al., 2015).

Multiple lines of evidence implicate ECM abnormalities in autism spectrum disorders. Genome-wide association studies (GWAS) on autism implicate a number of ECM and PNN regulating molecules, including the ECM remodeling enzymes, ECM molecules Reelin, semaphorins 3A and 4D, the hyaluronan surface receptor CD44, and Otx-2, a transcription factor involved in PNN formation (e.g.Weiss et al., 2009; Hussman et al., 2011). By far the strongest evidence for ECM involvement in the pathophysiology of autism comes from investigations on Reelin. Consistent with these findings, altered expression of Reelin and its

signaling pathways has been observed in the frontal, parietal, and cerebellar cortices of subjects with autism (Fatemi et al., 2005). Similarly, involvement of ECM/PNN molecules has been reported in Fragile X syndrome and Rett Syndrome, this latter also shown to have PNN abnormalities (Belichenko et al., 1997; Dziembowska et al., 2013).

During development and in adulthood, ECM/PNN molecules and their cell surface receptors mediate a broad range of synaptic regulatory functions impacting dendritic spine and synapse structure and plasticity as well as glutamatergic and GABAergic transmission (Faissner et al., 2010; Dityatev and Rusakov, 2011; Frischknecht and Gundelfinger, 2012). Evolving in parallel with our understanding of these functions, evidence for ECM/PNN pathology in psychiatric disorders supports the intriguing hypothesis that ECM/PNN abnormalities may contribute to a critical pathological component shared by psychiatric disorders, i.e., disruption of synaptic functions (e.g. Penzes et al., 2013; Duman, 2014; Xu et al., 2014). These may include well-documented synaptic pathology in these disorders, including loss of dendritic spines, pre- and postsynaptic regulatory elements, and disruption of glutamatergic synaptic signaling and GABAergic inhibitory neuron functions. In addition to synaptic dysregulation, critical functions performed by the ECM during brain development and adulthood (Bandtlow and Zimmermann, 2000; Tissir and Goffinet, 2003; Maeda et al., 2010; Kwok et al., 2011) suggest that the consequences of brain ECM abnormalities in psychiatric disorders may be complex and far-reaching, affecting several aspects of neural connectivity (Rhodes and Fawcett, 2004; Sykova, 2004; Berretta, 2008; Fatemi, 2010; Berretta, 2012; McRae and Porter, 2012; Lubbers et al., 2014; Berretta et al., 2015; Fawcett, 2015).

Potentially integral to disruption of glutamatergic/GABAergic function in psychiatric disorders (including addiction) is the possibility that PNNs contribute substantially to the excitatory/inhibitory balance because they surround PV-containing fast-spiking GABAergic interneurons in the PFC. These interneurons are central for generating gamma oscillations (30-120 Hz), and their removal alters these oscillations (Steullet et al., 2014). Gamma oscillations underlie synchronous network activity that mediates information processing and cognitive flexibility that is impaired in schizophrenia (Cho et al., 2006; Minzenberg et al., 2010; Cho et al., 2015), consistent with the observation that PV neurons do not develop normally in schizophrenia (Lewis et al., 2005) or in autism (Orekhova et al., 2007).

Role of PNNs in Addiction Models

Addiction is a psychiatric disease whose aberrant strength and persistence of drug-induced memories are believed to have a primary role in drug seeking and relapse (Everitt and Robbins, 2005; Kalivas and Volkow, 2005; Hyman et al., 2006). Despite the emerging significance of PNNs in plasticity, only a handful of studies in rats and mice have thus far examined the role of PNNs in addiction models, with a focus on the amygdala, the cerebellum (Miquel laboratory; see below), and the PFC (Sorg laboratory; see below). Proteins from the ECM, including those in PNNs, are decreased in the PFC after heroin self-administration but rapidly elevated after re-exposure to heroin-associated cues (Van den Oever et al., 2010). In the amygdala, PNN degradation by Ch-ABC following drug exposure (morphine, cocaine, and heroin) but before extinction training can augment extinction and

inhibit subsequent reinstatement (relapse) of drug-seeking behavior (Xue et al., 2014). Both cocaine and ethanol exposure upregulate PNNs in the cerebellum and PFC (see below for more detail) and insular cortex, respectively (Chen et al., 2015; Vazquez-Sanroman et al., 2015a).

Studies in the Miquel laboratory have focused on the role of the cerebellum in cocaine addiction models. The cerebellum may be a key region in the transition to the automatized (habit) responding observed in addiction (Miquel et al., 2009; Miquel et al., 2016). Animal models of cocaine addiction indicate that local circuits in the apex of the cerebellar cortex might be an important and largely overlooked part of the networks involved in forming, maintaining and/or retrieving drug memories that underlie relapse. Further, cerebellar PNNs play an integral role in cocaine-associated memories. Using an olfactory preference conditioning paradigm with cocaine exposure, the Miquel laboratory observed that PNNs surrounding Golgi inhibitory interneurons in the apex of the cerebellar cortex are up-regulated (more intensely labeled), but only in those animals that prefer the odor cue associated with cocaine (unpublished data). Aside from more intensely stained PNNs around Golgi neurons, neighboring granule cells show elevated levels of activity (estimated by cFos expression) that correlates with preference toward the cocaine-related cue (Carbo-Gas et al., 2014a; Carbo-Gas et al., 2014b). Remarkably, neither of these distinctive cerebellar signatures occurs when animals do not express cocaine-induced preference conditioning.

Golgi neurons play a crucial role in modulating the activity and plasticity of local circuits in the cerebellar cortex (D'Angelo et al., 2013). Golgi neuron-mediated inhibition appears to be experience dependent. When a sensory input arrives to the cerebellum through mossy fibers, it activates Golgi and granule cells, triggering a feedforward inhibitory loop (D'Angelo and De Zeeuw, 2009). Moreover, Golgi neurons are key elements to controlling synaptic plasticity at the level of the granule cell layer. When Golgi neuron inhibition is blocked, long-term potentiation is induced *in vitro* (Mapelli and D'Angelo, 2007), and *in vivo* (Roggeri et al., 2008). On the contrary, when inhibition is higher than excitation, long-term depression is developed in granule cells (D'Angelo and De Zeeuw, 2009). It is now clear that PNNs restrict the capacity of their enwrapped neurons for experience-dependent plasticity (Pizzorusso et al., 2002b). Consequently, one could speculate that a fully condensed PNN surrounding Golgi neurons, which is found only in mice that have acquired conditioned preference for cocaine, might "stamp in" synaptic changes related to cue-drug associations, thereby preventing posterior synaptic rearrangements in the local circuits of the granule cell layer.

Cocaine-induced changes in PNN expression in the cerebellum show anatomical specificity and different functional regulation. Indeed, PNNs that surround large glutamatergic projection neurons in the deep medial nucleus (DMN) are not changed after acquisition of cocaine-induced preference memory but appear to be regulated during the withdrawal period. These PNNs may be regulated by plasticity in Purkinje neurons during a prolonged experience with cocaine and subsequent withdrawal time. After a short-withdrawal period, the expression of PNNs increases around these DMN neurons (Vazquez-Sanroman et al.,

2015a). These changes are associated with an accumulation of pro brain-derived neurotrophic factor (proBDNF) and higher levels of its receptor p75NGFR, the pruning of dendritic spines, and a reduction in the size and density of Purkinje synaptic terminals. As a consequence, Purkinje cells reduce their activity and thus their potential to inhibit DMN neurons that appear to be more active. Following a longer withdrawal time, Purkinje neurons develop opposite plasticity changes, including an increase in pro- and mature-BDNF mechanisms, dendritic sprouting, and enlarged terminal size (Vazquez-Sanroman et al., 2015b). In this case, PNNs are down-regulated in DMN neurons. More lightly stained PNNs (i.e., less PNN material around the cell) might facilitate the subsequent remodeling of Purkinje-DMN synapses (Vazquez-Sanroman et al., 2015b). Together, these findings point towards different functions for cerebellar PNNs in drug-related plasticity. The PNNs around Golgi neurons would act as "brain tattoos" (Hustvedt, 2014) for stabilizing long-term drug memory encoded in local circuits of the cerebellar cortex. However, those that enwrap DMN projection neurons would serve as "temporary stickers" to dynamically control the cerebellar output by promoting or restricting plasticity in Purkinje-DMN synapses.

Consistent with the idea that cocaine alters the intensity of PNNs and associated plasticity, the Sorg laboratory focused on PNNs in the medial PFC (mPFC), and found that a single injection of cocaine rapidly decreased PNN intensity 2 hr later, whereas five daily injections increased PNN intensity 2 hr later (unpublished). The potential significance of this finding is that decreased PNN staining intensity appears to correspond to an immature PNN with increased capacity for plasticity, whereas increased PNN intensity corresponds a mature

PNN with decreased capacity for plasticity (Wang and Fawcett, 2012). Our findings are consistent with the idea that initial learning (1 day cocaine) decreases PNN intensity and may allow for greater cocaine-induced plasticity, whereas repeated cocaine (5 days cocaine) may "stamp in" synaptic changes, as discussed above for the cerebellum, rendering the circuitry more impervious to plasticity induced by other stimuli such as natural rewards. We also found that PV staining mirrored PNN staining after cocaine, but the changes lagged behind those of PNNs, suggesting that PNNs and PV may be co-regulated in some way, and that cocaine-induced changes may significantly alter GABAergic output from these interneurons due to altered PV content (Donato et al., 2013). Overall, cocaine-induced metaplasticity appears to restrict the formation of new plasticity (Moussawi et al., 2009; Kasanetz et al., 2010), setting in place neural connectivity underlying addictive behaviors, and PNNs may play a role in this restriction of plasticity. Interestingly, some of the effects of cocaine on PV/PNN changes may be related to oxidative stress. Cocaine produces oxidative stress in neurons (Dietrich et al., 2005; Numa et al., 2008; Jang et al., 2014; Sordi et al., 2014). PNNs protect against oxidative stress (Morawski et al., 2004; Cabungcal et al., 2013), and consistent with this protection, unpublished findings in the Sorg laboratory found that the antioxidant N-acetyl cysteine reverses relatively small increases in an oxidative stress marker in the mPFC after cocaine in PV neurons that are surrounded by PNNs, but not the larger increases in this marker in PV neurons devoid of PNNs.

The results that cocaine-induced plasticity restricts further plasticity is in accordance with recent work demonstrating that degrading PNNs with Ch-ABC in the mPFC reduced the

acquisition and/or maintenance (reconsolidation) of cocaine memory in a conditioned place preference model of addiction in rats (Slaker et al., 2015) and blunted the ability of rats to learn cocaine self-administration (unpublished findings). In addition, PNNs in another brain area contribute to cocaine-induced memories: a region of the anterior dorsal lateral hypothalamic area was recently discovered to exhibit a small patch of robust PNN and loose ECM expression; degradation of this patch with Ch-ABC abolished the acquisition of cocaine- but not sucrose-induced cocaine conditioned place preference and also blocked the acquisition of cocaine self-administration (Blacktop; unpublished findings).

In summary, changes in PNNs are rapid and regulated by both drug exposure and its associated memory. Although the contribution of PNNs to both drug-induced neuroplasticity and behavior is in its infancy, emerging evidence supports strong functional interactions among drugs of abuse, changes in PNN expression and function, and drug-seeking behavior.

Limitations, Future Directions, and Conclusions

One of the current limitations in understanding the contribution by PNNs in brain and spinal cord plasticity is that the enzyme Ch-ABC has been used almost exclusively to degrade PNNs. However, Ch-ABC also destroys the loose ECM, and therefore the contribution of PNNs is not entirely clear. However, strong evidence supports a key contribution by PNNs to critical period closure for ocular dominance plasticity, because knockout mice that lack a key link protein demonstrate reduced formation of PNNs, but no changes in the loose ECM, and they maintain juvenile levels of ocular dominance plasticity

(Carulli et al., 2010). One potential future direction is to specifically knock down cartilage link protein -1 (Crtl-1) to reduce PNN formation (Carulli et al., 2010), since this protein is found only in PNNs but not in loose ECM (Galtrey et al., 2008). Unpublished findings (Sorg laboratory) demonstrate that a morpholino that interferes with Crtl-1 expression reduces PNN intensity and number, but future studies will need to determine the functional consequences of this knockdown strategy. Other strategies are to target local expression of Otx-2, which maintains PNNs (Beurdeley et al., 2012; Bernard and Prochiantz, 2016), as well as other molecules such as semaphorin 3A to regulate synaptic inputs (Dick et al., 2013; Vo et al., 2013; de Winter et al., 2016) or neuronal pentraxin-2 (NARP) (Gu et al., 2013), which regulates PV neuron excitation through recruitment of glutamate (AMPA) receptors (Chang et al., 2010; Pelkey et al., 2015)

In conclusion, recent discoveries show that PNN formation contributes to a loss of brain plasticity in adults, and that brain and spinal cord plasticity can be re-established in adults after removal of PNNs. Dynamic changes in PNNs appear after environmental manipulations, and these changes, in addition to interference with normal development of PNNs, may contribute to a wide range of diseases and disorders of the brain, including Alzheimer's, autism, epilepsy, schizophrenia, bipolar disorder, ageing, brain injury, and learning and memory, including that associated with drug abuse. Understanding the molecular underpinnings of how PNNs are altered in normal physiology and disease will offer insights into new treatment approaches for these diseases.

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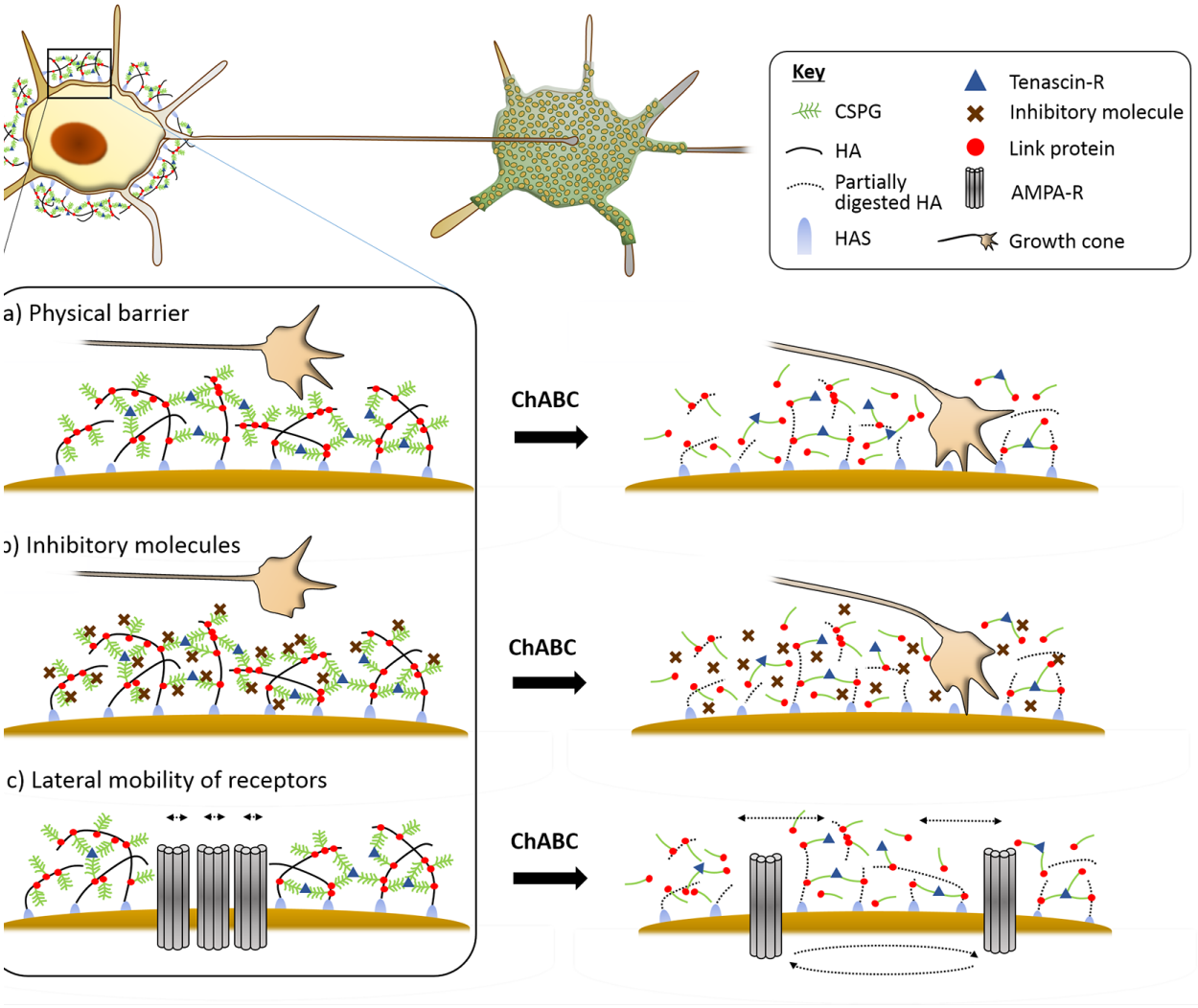

Figure 1. Limitation of plasticity by PNNs *via* three mechanisms and reinstatement of plasticity by treatment with Ch-ABC. Plasticity involving PNN-surrounded neurons is limited by: a) a physical barrier by PNNs to incoming synaptic inputs; b) binding of molecules via specific sites on CSPGs of PNNs; molecules such as semaphorin 3A inhibit new synaptic inputs; and c) prevention of lateral diffusion of AMPA receptors, limiting the ability to exchange desensitized receptors in the synapse for new receptors from extrasynaptic sites. Treatment with Ch-ABC disrupts PNNs, reinstating juvenile-like states of plasticity. *Ch-ABC*, chondroitinase-ABC; *CSPG,* chondroitin sulfate proteoglycan; *HA*, hyaluronic acid; *HAS*, hyaluronic acid synthase. Figure courtesy of J.C.F. Kwok, modified from (Wang and Fawcett, 2012).