Perineuronal nets: Plasticity, protection, and therapeutic potential

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Keywords: critical periods; memory; plasticity; parvalbumin interneurons; perineuronal nets; neuroprotection

Abstract

The relationship between neurons and perineuronal nets (PNNs) is attracting attention as a central mechanism controlling brain plasticity. In the cortex, PNNs primarily surround inhibitory parvalbumin interneurons, playing roles as both a regulator of synaptic plasticity and a protective barrier. PNNs have a delayed developmental trajectory and are key components in the closure of critical periods of heightened neuroplasticity. In animal models, manipulating PNNs outside this critical window can enhance cognition, suggesting a potentially therapeutic approach for attenuating cognitive decline. However, the crucial role of PNNs in plasticity and protection means that such therapeutic modulation must strike a careful balance: manipulation of PNNs to promote plasticity may have unintended negative consequences resulting from excessive plasticity or from exposure of neurons to neurotoxins.

The extracellular matrix: Plasticity and protection

The **tetrapartite synapse** (see **Glossary**) represents the union of pre- and post-synaptic neuronal terminals, supporting glial cells, and the surrounding extracellular matrix (ECM). The ECM has emerged as an important participant in synaptic plasticity, and in particular the structures comprised of condensed ECM known as perineuronal nets (PNNs) [1]. PNNs are proposed to serve multiple functions including: 1) *regulating plasticity* by stabilizing synaptic connectivity, 2) *protecting neurons and synapses* by forming a physical barrier and an anionic shield to preserve the integrity of synaptic junctions and insulate them from potentially damaging neurochemical stimuli, 3) harboring molecules that are permissive or inhibitory of *synapse formation*, and 4) refining the *timing and precision* of signal processing [2-8].

PNNs surround various specific subgroups of neurons [9-12]. In many cortical regions, however, PNNs preferentially envelop parvalbumin-expressing (PV^+) inhibitory interneurons [9, 13] known to be vital for cognition [14, 15]. Cortical PNNs have delayed developmental trajectories [16] and are key components in the closure of **critical periods** of heightened neuroplasticity (see Text box 1). Defective PNNs have been implicated in a range of neurodegenerative and neuropsychiatric disorders linked to aberrant neuroplasticity including dementia [17], anxiety disorders [18] and drug addiction [19]. The role of PNNs in regulating plasticity makes them a particularly exciting therapeutic target for a range of disorders; including ones where enhanced plasticity is desirable, or ones where plasticity needs to be suppressed [20].

Therapeutic targeting of PNNs is not a new idea. For example, unlocking PNNs pharmacologically following spinal cord damage has been shown to promote neuroplasticity restoration through axonal regeneration [21, 22]. With the presence of PNNs within cortical regions essential for learning, memory and higher-order cognition, insights from spinal cord studies may be extrapolated to modification of brain plasticity [23]. However, caution is required: following manipulation of PNNs, neuronal circuits may become excessively plastic or neurons and synapses rendered vulnerable to neurotoxic stimuli, either of which could have a deleterious effect on

cognition. In this Review, we explore current thinking with regard to the role of PNNs in learning and memory. We also discuss the intimate involvement of these extracellular structures in neurodegenerative disorders, include ones involving memory decline, and the modification of PNNs as a possible therapeutic target.

Text Box 1 about here

Targeted modification of PNNs to uncover their role in memory

A scaffold for memories

Structurally, PNNs form mesh-like webs around neurons. Link proteins (Crtl1/Hapln1 and Bral2/Hapln4) stabilise the binding of various **chondroitin sulfate proteoglycans** (CSPGs) to a hyaluronan backbone, which attaches to the cell surface membrane by hyaluronic acid synthase (HAS) [24] (see Table I and Text box 2). It has been suggested that PNNs are part of a physical framework that controls long term memory storage by stabilizing synapses [25, 26], and some have even speculated that PNNs may physically represent a memory, or **engram** [26]. Further experimentation is required to test these ideas. Mature PNNs are thought to influence synaptic plasticity by establishing microenvironments around neurons and synapses that dictate where synapses can form [27]. The loosening of PNNs can therefore favor enable synaptic plasticity by destabilizing the inhibitory environment and releasing growth factors [27]. Experimental studies have demonstrated that PNNs are highly dynamic and modified through experiences and environmental influences, as we described in this review. Moreover, the location of PNNs around neurons makes them an accessible mechanism for memory modulation [28-30].

Text box 2 here

Making memories

Recognition Memory

Removal of major structural components of PNNs in the perirhinal cortex via the enzyme **chondroitinase-ABC** (chABC), which degrades CSPGs, substantially prolongs long-term object recognition memory measured by **spontaneous object recognition** in mice [31]. This assay explores whether an animal recognizes that an object has been experienced previously through whether they choose to explore a novel or previously encountered object after a delay period, and is reliant upon plasticity within medial temporal lobe structures [32].

An alternative approach to disrupting structural components of PNNs is to manipulate specific genes associated with PNNs. Postnatal neuronal expression of Crtl1 is among the key events triggering formation of PNNs [33]. Mice lacking the Crtl1/Hapln1 gene specifically in the central nervous system (CNS) have disrupted CSPG organization and attenuated structural integrity of PNNs [34]. These mice also demonstrate substantially prolonged long-term recognition memory compared to wild-type littermate controls [31]. Learning and memory deficits have also been observed in mice deficient for the glycoprotein tenascin-R, another key element of PNN structure [35]. However, no cognitive deficits were observed in mice lacking the lectin brevican, although changes were shown in synaptic transmission [36].

Genetic or enzymatic reduction of PNNs can increase basal synaptic transmission and long-term depression (LTD) in the temporal lobe [37], thought to be among the principal plasticity mechanisms underlying recognition memory [38]. Intriguingly, enzymatic degradation of PNNs throughout the hippocampus reduced long-term potentiation (LTP) in slice preparations, but restricted degradation within the CA2 region of the hippocampus increased LTP [10]. These studies indicate that removal of PNNs can modify plasticity and functionally enhance memory, suggesting that unlocking PNNs in brain regions involved in memory may provide an approach to memory enhancement and modulation.

Event Memory

Negative or damaging experiences become bound in memory alongside some of the corresponding environmental stimuli – locations, sights and sounds – present during

the event. These stimulus elements can serve as reminders of the original traumatic episode. **Pavlovian fear conditioning** is used to explore how animals learn about stimuli that predict aversive events. Pairing an initially neutral stimulus such a tone (conditioned stimulus; CS) or environment (context) with an aversive stimulus such as a footshock (unconditioned stimulus; US) forms a robust fear memory that is retrieved upon reintroduction of the CS [39].

A network of multiple brain regions is involved in encoding memories for events, including the hippocampus [40], basolateral amygdaloid complex (BLA) [41] and medial prefrontal cortex (mPFC) [42]. The hippocampus in particular is integral in encoding contextual representations of where an event occurred [40], and the mPFC has key roles in learning, maintaining and updating associations between a CS and events [42]. Degradation of PNNs by infusion of a combination of chABC and hyaluronidase in either the hippocampus or the mPFC impaired the formation of conditioned fear memories in rats [43]. Removal of hippocampal PNNs impaired the contextual specificity of a fear memory [43], demonstrating that PNNs in these cortical regions involved in cognition are key in controlling the plasticity required for learning about aversive events.

PNNs are found in abundance within key sensory areas, including the auditory and visual cortex, and these regions are involved in encoding components of fear memories. Following auditory fear conditioning - where the presentation of a sound precedes a footshock–PNNs transiently increase in the auditory cortex, which may represent a general physiological mechanism supporting the ‘locking in’ of a long-term memory [44]. Removing PNNs from the auditory cortex prior to conditioning prevents auditory fear learning, potentially due to increased interference from subsequent sound experiences [45].

Fear memories formed following visual fear conditioning–where the presentation of a light precedes a footshock–are disrupted by removal of PNNs from visual cortical regions. Removal of PNNs in the secondary visual cortex (V2) of rats disrupted the recall of a 35-day old (remote) visual fear memory, but not a recent (4-day old) fear

memory [46]. These studies bear significance for the function of PNNs in the integration of the sensory features of memories derived from experiences, holding particular importance for anxiety disorders where fear becomes generalized, whereby visual cues or noises trigger memories of trauma.

It should be noted that PNNs surround functionally distinct neurons with regional specificity and distribution, and may underpin differential behavioral effects [15]. For example, in the hippocampus, PNNs surround PV+ basket cells [47] and also excitatory pyramidal cells in the CA2 region [10]. In the amygdala, PNNs surround PV and calbindin positive neurons [48] and also CaMKII positive neurons [49].

Updating fear memories

Memories can last across the lifespan, yet their relevance must be updated over time. One example of this, known as **extinction** learning, occurs when individuals are exposed to repeated reminders of a traumatic event, but without ensuing harm, resulting in the formation of a new memory. Fear extinction reduces anxiety by learning a competing association between a new memory of safety and the original fear memory, forming the basis for exposure-based therapies for anxiety disorders. Memories of fear in older subjects are much more resistant to extinction than in the young, including in humans [50]. The rapid extinction of memories in young animals may be due to the enduring critical period of plasticity (see Text box 1), including in the BLA, which extends through to adolescence [51]. In the BLA of juvenile animals, PNNs are essentially absent, due to their late development [52], raising the possibility that the presence of BLA-associated PNNs in adults is among the factors that prevent extinction from erasing memories of fear.

To functionally demonstrate the importance of PNNs in extinction learning, Gogolla and colleagues [53] used chABC degradation of PNNs within the BLA in adult mice to simulate a juvenile state prior to fear conditioning. As a result, fear memories extinguished faster and mice showed low levels of freezing when retention of the extinction learning was tested (Figure 1) [53]. This effect was specific for fear

memories acquired in the absence of PNNs; degrading BLA PNNs in mice that acquired fear memories *prior to* chABC infusion did not affect extinction. This suggests that acquiring fear memories in the presence of PNNs may protect conditioned fear memories from extinction-induced updating [53]. Translation implications of this approach, however, need to be considered with much caution, partly because in reality, many traumatic memories are formed during adulthood, when PNNs are mature. Moreover, the differential findings between observations that memories can be enhanced or impaired following the removal of PNNs, suggests a highly complex role in the balance of plasticity and activity within neural networks responsible for learning and memory.

Figure 1 about here

Updating drug addiction-related memories

Environmental cues, such as people or situations, can become associated with drug use and can trigger drug-seeking behaviors. PNNs have gained prominence in addiction research from studies demonstrating that the ECM regulates drug-associated memories that contribute to relapse, and removal of PNNs has been discussed as a possible pathway for treating addiction [20]. Removal of PNNs within the prelimbic mPFC, a key region involved in the formation of drug-associated memories, disrupted the acquisition of cocaine-induced **conditioned place preference** (CPP), suggesting that PNNs are important for the formation of a cocaine-induced memory [54]. Removal of mPFC PNNs did not alter the rate of extinction of the place preference [54]. In contrast to the fear conditioning and extinction studies discussed previously [53], depletion of PNNs within the amygdala enhanced the extinction of both CPP and drug self-administration following the acquisition of the drug memory [55]. This finding indicates that an absence of PNNs in the amygdala may make addiction-like memories easier to form, but also more susceptible to modification. This evidence indicates that PNNs may be necessary for both creating

and maintaining drug-related memories, and thus may serve as targets for weakening memories that drive relapse.

Prospects for modulation of PNNs as a therapeutic target

The considerable evidence indicating that PNNs are integral to the formation, storage, and extinction of memories offers compelling justification for investigating PNNs as a potential therapeutic target. With careful modulation, it may eventually be possible to break maladaptive memories, such as those observed in post-traumatic stress disorder or drug addiction. For age-related cognitive decline and dementia, PNNs may also hold a key to enhancing the protection of memories, or even restoring impaired memory.

Restoring memory in dementia

Mechanisms to boost neuroplasticity provide an approach to attenuate age-related cognitive decline and memory loss caused by neurodegenerative disorders including dementias. Modifying PNNs to promote neuroplasticity has the potential to mitigate effects of damage and degeneration by facilitating new synaptic connections to restore aspects of normal cognition. This idea has been explored in mouse models of dementia, including mice overexpressing the P301S variant of human tau protein (*MAPT*) that progressively develop neurofibrillary tangles in the neocortex, hippocampus and amygdala, leading to neuronal loss and memory impairments [56]. Digestion of PNNs in the perirhinal cortex with chABC normalized object recognition memory performance in *MAPT* mice, indicating that this boost in plasticity attenuated memory impairments [57]. In the APP/PS1 mouse model of Alzheimer's disease-like amyloid pathology, degradation of hippocampal PNNs in young (3-month old) mice rescued deficits in contextual fear memory, as well as normalizing LTP [58].

Modulating cortical PNNs: A double-edged sword

In experimental mouse models, the genetic or pharmacological removal of PNNs can facilitate the formation of enduring memories by increasing plasticity and increasing encoding of newly learned information, either by preventing the brain from forgetting, or making newly learned information easier to encode [31, 57, 59]. However, this is not without important potential drawbacks.

Boosting plasticity at the expense of stability

Optimal conditions for memory encoding and retention involve a tradeoff between plasticity and stabilizing synaptic changes: the brain needs to maintain sufficient levels of plasticity to encode memories whilst remaining sufficiently stable to support lifelong memory storage. This is known as the **stability-plasticity tradeoff.** While increasing plasticity levels may seem like a good idea, excessive plasticity also has the potential for unintended negative consequences. For example, irrelevant or competing information can disrupt efficient recall due to interference between existent memory traces [60]. In rats, recognition memory impairments following damage to the perirhinal cortex can manifest as false memories–whereby novel experiences were treated as familiar [61]. Such errors arise from interfering memory traces encoded during the retention period. Exposure to a visually deprived environment during the delay, which minimizes memory trace encoding interference, can rescue the memory impairment in lesioned animals [61]. In a state of heightened plasticity, the rapid encoding of multiple interfering memory traces may emerge as false memories. Too much plasticity associated with absent PNNs may impede memory, as information being encoded may become subject to greater interference, causing multiple competing memory traces to accrue from external information.

Boosting plasticity at the expense of protection

The neurobiological mechanisms that restrict plasticity are also known to provide a protective environment encasing synapses and highly plastic neurons. The neuroprotective function of PNNs is likely provided by their presence as a barrier, for instance against the oxidative stress and inflammatory cytokine activity common to

neurodegenerative diseases [2]. Transgenic mice that lack specific components of PNNs, including aggrecan, *Crtl1,* and tenascin-R have increased susceptibility to neuronal damage induced by infusion of pro-oxidant iron [5], accumulation of which is associated with cortical neuron loss in Alzheimer's disease [6, 62]. Cultured neurons surrounded by a PNN were protected from the accumulation of neurofibrillary tangles, phosphorylated tau and lipofuscin [17], whereas cortical neurons without PNNs were more susceptible to $A\beta_{1\text{-}42}$ toxicity [63]. These studies indicate that while compromising the integrity of PNNs may transiently evoke improvements in cognition and normalize certain aspects of plasticity, these manipulations can also degrade the neuroprotective barrier, rendering neurons vulnerable to long-term damage from endogenous and environmental neurotoxins. This picture may be conceptualized as a '*plasticity-protection'* tradeoff.

In sum, research presents two major potential pitfalls in the ablation of PNNs that limit therapeutic application via these strategies, 1) too much plasticity leading to memory interference and 2) increasing neurons' vulnerability to damage. It is conceivable, however, that more refined PNN modulation either by less invasive, lifestyle-based interventions or by more subtle molecular manipulations may have therapeutic potential. If PNNs prove to be viable targets, therapies must strike the right balance to restore relevant levels of plasticity to avoid memory interference. Furthermore, reverting PNNs to a less condensed state, rather than removing them completely, may shift the balance of neuroplasticity whilst maintaining a sufficient barrier to protect synaptic integrity. The blunt approach of enzymatic digestion of PNNs from cortical regions has provided a rationale for PNNs as a target to influence memory, but more refined PNN modulators need to be developed and tested in animal models before considering possible use in humans.

How lifestyle can impact PNN structure

The dynamic nature of PNN integrity across the lifespan and its key role in protecting neurons and synapses from environmental insults presents not only a possible target for manipulation, but also a mechanism contributing to cognitive decline. As detailed

above, neurons without a surrounding PNN are highly vulnerable to insults like oxidative stress [64]. This emphasizes the importance of regulating the neuronal environment, particularly during sensitive neurodevelopmental periods when PNNs are yet to fully form. Recent research shows that subtle shifts in PNN integrity can evoke functional alterations in plasticity, which can impact cognition. Lifestyle factors modelled in laboratory rodents such as diet, drug exposure, exercise and cognitive enrichment may influence both PNN structure and the environment surrounding neurons.

PNNs as a key mediator of diet-evoked cognitive decline

Consumption of poor diets have been shown to negatively impact memory formation in both humans [65] and rodents [66-68]. Moreover, recent studies have suggested that alterations in diet evoke modifications to PNNs [69-71], which also could contribute to the development of neuropsychiatric disorders such as schizophrenia [16, 64, 71]. In particular, chronic vitamin D deficiency might contribute to defects in PNNs, and it has been further argued that these alterations could contribute to cognitive deficits typical of schizophrenia [69-71].

In rodents, diets high in saturated fat and refined sugars can induce neuroinflammation [72] and cognitive decline-particularly in memory domains [66, 73], and recent research in rodents demonstrates that these cognitive effects are most pronounced when poor diets are consumed during early life, including the juvenile-to-adolescent period [68, 74, 75]. A recent study showed a high-fat diet in adult rats decreases PNN numbers and staining intensity in the PFC [76], and hypercaloric diet consumption in young rats with immature PNNs can reduce numbers of mPFC and HPC PV^+ interneurons [77-80], and altered numbers of colocalized mPFC PV^+PNN neurons [79] with resulting behavioral dysfunction. This suggests a mechanism for the enhanced vulnerability of the developing brain to the detrimental effects of hypercaloric diets, acting through the effects of diet on PNNs and thereby neural circuitry, particularly PV^+ interneurons. Further studies are needed to deepen our mechanistic understanding of

how poor diets impact cognition in rodent models, including the effects of diet on PNN-mediated neuroplasticity and neuroinflammation.

Influence of psychoactive drugs on PNN structure

Degrading PNNs may augment the process of breaking maladaptive memories associated with drug addiction and relapse [55, 81]. Additionally, drugs can modify PNNs. In the PFC of rats PNNs decrease after heroin self-administration but rapidly increased in density after re-exposure to heroin-associated cues, potentially "locking in" addiction-like memories [82]. These changes in PNNs are not limited to drugs of abuse. Chronic treatment with the antidepressant fluoxetine can reactivate critical period-like plasticity in the visual cortex of rats [83]. Moreover, memory deficits in rats evoked by a depression-like state following chronic social stress were linked to increased PNN numbers in the hippocampus [84]. Treatment with the tricyclic antidepressant imipramine normalized hippocampal PNN numbers to that of non-stressed rats, and restored memory deficits, indicating that monoamine manipulations may neurochemically alter PNN structure and function [84].

Exercising the body and brain

Certain lifestyle factors that are known to enhance neuroplasticity, including aerobic exercise and environmental enrichment, have also been observed to modify PNNs [30, 85]. Rats with free access to running wheels showed decreased hippocampal PNN density compared to sedentary animals, and a general reduction in number of PNNs suggesting enhanced plasticity [85]. Similarly, environmentally enriched housing conditions that facilitate enhanced sensory, cognitive, motor and social stimulation also decreases the number and staining intensity of PNNs within the brain [86]. Lifestyle interventions, such as aerobic exercise, might prove beneficial in cognitive restoration through modulation of PNNs whilst concurrently increasing neurotrophic factors and reducing circulating oxidative species, promoting optimal plasticity as well as protection. This emphasizes how dynamic PNNs are in response to environmental stimuli, and why coarse modifications to these structures pose

substantial risks that must be well understood before any possible translation to a clinical environment.

Refining PNN manipulations for cognitive restoration

Targeted approaches

Selectively manipulating specific components of PNNs, compared to the coarser approach of enzymatic degradation, provides a possible avenue for modifying neuroplasticity to attenuate cognitive decline, whilst minimizing the exposure of neurons to damaging neurotoxins. Manipulation of specific PNN elements, such as the glycosaminoglycan chains of chondroitin sulfate (CS) proteoglycans are potential targets for restoring PNNs to a less condensed state, and therefore more permissive of plasticity. Sulfonation of CS proteoglycans can occur in the 4 (C4S) or 6 (C6S) positions. While C4S is inhibitory, C6S is more permissive to axon growth, regeneration and plasticity. C6S decreases during critical period closure and aging, and is associated with memory loss in aged rats, which exhibit an increased C4S/C6S ratio [87].

Recently, a novel antibody specific to C4S, which blocks the inhibitory CSPG and attenuates PNN formation, has been used in *MAPT* mice [59]. Infusion of this antibody into the perirhinal cortex normalized object recognition memory performance in *MAPT* mice [59] and enhanced object memory in wild-type mice, demonstrating efficacy in both a deficit state and in enhancing memory under normal physiologic conditions. Furthermore, the proteoglycan aggrecan is a core component of condensed PNNs. Selective in vivo deletion of the *Acan* gene in the visual cortex of adult mice ablated PNN structures and reactivated a critical-period like state of plasticity seen in juveniles typical of ocular dominance [88].

Matrix metalloproteinases: An endogenous mechanism of PNN-degradation?

The biomolecular mechanisms underpinning endogenous and/or environmentally-stimulated modulation of PNN structure and integrity are yet to be fully characterized. One possible mediator are matrix metalloproteinases (MMPs), a family of extracellular proteolytic enzymes that have the ability to remodel the ECM and have recently emerged as possible controllers of neuroplasticity that can be influenced by environment (i.e. diet), pharmacological compounds [82] or gene expression [89]. Regulation of PNN formation is influenced by secretion of the matrix metalloproteinase-9 (MMP-9), a protease that is able to cleave multiple components of a mature PNN [90]. Particular interest has been paid to links between the abnormal cognitive phenotype of Fragile X Syndrome caused by repeat expansion of the *Fmr1* gene, elevated MMP-9 levels and a loss of PNN integrity. *Fmr1*-null mice show decreased PNN development, reduced PV^+ interneurons and increased MMP-9 expression [91]. Increased MMP-9 has also been associated with obesity in humans [92] and in obese Zucker rats [93]. This suggests that high concentrations of circulating MMP-9 is a potential biomarker of PNN loss and cognitive decline, though this link has not yet been confirmed in a clinical setting. Expression of MMP-9 in the brain is suppressed by aerobic exercise, which is neuroprotective in experimental ischemic stroke models [94], and elevated within the hippocampus of rats in enriched environments [95] providing a further exogenous mechanism underpinning the modification of PNNs. Nevertheless, MMPs represent a potential physiological mechanism to modulate PNN integrity in a more refined manner than enzymatic degradation.

Figure 2 about here

Concluding remarks

Modulation of the structural integrity of PNNs has added a new layer to our understanding of neuroplasticity and memory in both the healthy brain and neurodegenerative and neuropsychiatric states. Animal studies suggest that harnessing the structural integrity of PNNs could provide a path for breaking

maladaptive memories, such as those that underpin drug addiction and anxiety disorders, and boost plasticity in neurodegenerative diseases allowing memories to be encoded more readily. Although degrading PNNs provides a strategy to enhance aspects of memory, any such approach must be mindful of both '*plasticity-stability'* and *'plasticity-protection'* tradeoffs. In particular, artificial removal of PNNs in neurodegenerative diseases may provide only limited long-term therapeutic efficacy and compromise long-term synapse integrity and stability by increasing exposure to damaging neurotoxic molecules. It is even possible that non-specific removal of PNNs may *exacerbate* rather than attenuate long-term cognitive decline. One solution may be to use lifestyle changes in diet and exercise, or combine redox-regulators/antioxidants with PNN modulation (see Outstanding Questions). Furthermore, designing more precise and selective manipulations of PNN structure, for instance by genetically targeting specific components of PNNs, such as the *Acan* gene encoding the CSPG aggrecan [88] or immunotherapies targeting specific glycosaminoglycan chains of proteoglycan structures [87] could address the plasticity-stability and plasticity-protection tradeoffs associated with PNN degradation. Whether some of these approaches could lead to therapeutic approaches for the revival of damaged synaptic connections in neurodegeneration and dementia remain an exciting question to be addressed in future research.

Text box 1. PNNs as regulators of neurodevelopment and neuronal signaling

During early life, neurocircuitry is readily modified by experiences – from perceptual to emotional – that shape neuronal networks as they mature. Cortical PNNs have a protracted maturational trajectory, becoming fully formed in mid-to-late adolescence in both rodents and humans [16]. In the developing brain, the formation and condensation of PNNs coincides with the closure of **critical periods** of postnatal development. PNNs develop around specific populations of highly-active neurons, preserving structural connectivity whilst controlling experience-dependent synaptic pruning processes that are essential during certain neurodevelopmental stages [96] (Figure I). In adult rats, degrading the PNNs that encase neurons either by enzymatic ablation or decondensing them to a 'looser' state can result in heightened cortical plasticity akin to that seen during critical periods [88, 97].

While PNNs surround a variety of neuronal cells, PNNs have heightened affinity for inhibitory PV^+ GABAergic interneurons within the cortex. PV^+ neurons are involved in shaping critical period plasticity during neurodevelopment, as well as in stabilization and synchronization of synaptic networks [98]. The majority of cortical PNNs support fast-spiking PV^+ cells [3, 96], which generate and maintain cortical gamma oscillations necessary for the consolidation and retrieval of memories [99, 100]. Loss of PNNs around PV^+ interneurons is associated with atypical critical period plasticity and decreased neuronal excitability [29, 101], as well as degradation of the protective physical barrier surrounding neurons and synapses [6, 102]. Without regulation of cortical PV^+ neuron activity, the excitatory–inhibitory balance and signal transmission within key neural networks involved in cognition becomes disrupted [7, 8]. This could contribute to the altered inhibitory signaling and gamma oscillations that are implicated in a range of psychiatric sequelae involving cognitive deviations including autism spectrum disorder (ASD), schizophrenia and bipolar disorders [103], conditions that emerge during childhood and early adulthood.

Text box 2. Perineuronal nets: Lattice-like structures with a chequered past

Perineuronal nets (PNNs) are specialized, mature ECM structures that surround the somata, dendrites and proximal axons of neurons. These lattice-like extracellular formations were initially described by Golgi in the late 1800s as 'neurokeratin' structures, which have had a conflicting history (reviewed by Celio et al. [104]). Initially, PNNs were used to support the now obsolete **reticular theory** of nervous system organization, while other prominent contemporary neuroanatomists and neuropathologists, including Ramón y Cajal disputed any functional role, and claimed they were merely artifacts of histochemical fixation.

Driven by increasingly-sophisticated tools for probing the structure and function of neurological milieu in the brain, the last half-century has seen the emergence and refinement of a fundamental role for PNNs to a point where they are now considered a central pillar of neuroplasticity. Structurally, PNNs have a hyaluronic acid backbone, to which **chondroitin sulfate proteoglycans** (CSPGs) are bound *via* glycosaminoglycan sidechains (see Table I). This binding is stabilized by glycoproteins such as tenascins (primarily tenascin-R) and link proteins that maintain structural stability, guide neuron growth and support synaptic development [105, 106]. Differential sulfonation patterns in CSPGs create specific binding sites for molecules and receptors that can control neuronal plasticity [87]. For example, CSPGs can interact with orthodenticle homeobox 2 protein, Otx2 - a transcription factor involved in neuronal differentiation and signaling. Diminishing CSPG binding of Oxt2 in visual cortex neurons reduces the intensity of PNN assembly and reinstated critical period-like plasticity to recover binocular vision in amblyopic mice [107]. Moreover, composition of PNNs varies depending on neuroanatomical location and type of neuron [108, 109], stages of neural differentiation [110], and age of the subject – with PNNs becoming more intense or 'condensed' with age (Figure I), which may have further effects on learning and updating more hard-wired behaviors [87]. The function and structure of PNNs in diverse brain regions and neuronal subtypes has recently been reviewed [15].

PNNs can be labelled using the lectin Wisteria floribunda agglutinin (WFA), which binds specifically to the N-acetyl-D-galactosamine on terminal ends of chrondroitin sulfate chains. Immunostaining for a number of lectican-family components listed in Table I can also be used to image PNNs. As such, innovative anatomical imaging and molecular biology techniques are revealing that PNNs are much more than a coating of WFA-positive ECM around certain PV+ interneurons, and are found widely within other regions of the central nervous system [11, 12, 111].

Glossary

Chondroitin sulfate proteoglycans (CSPGs): Secreted proteoglycans consisting of a protein core and a chondroitin sulfate side chain, and contain the lecticans aggrecan, versican, brevican, and neurocan that form structural components of a variety of tissues, including cartilage and extracellular matrix.

Chondroitinase-ABC: Enzyme that cleaves chondroitin sulfate side-chains of CSPGs, breaking the link to the hyaluronic acid and disrupting PNN structure.

Conditioned place preference (CPP): A form of Pavlovian conditioning used to measure the amount of time an animal spends in a controlled environment that has been associated with a stimulus, such as a drug reinforcer, and the memory that associates the drug with the environment

Critical periods: Temporal windows of heightened plasticity. Critical periods are dependent on the multiple factors, including brain regions, neuron type and developmental process being involved.

Engram: The enduring physical and/or chemical changes within the brain that are elicited by learning and underlie the newly formed memory associations.

Extinction learning: When a conditioned stimulus is presented repeatedly alone, so that it no longer predicts the coming of the unconditioned stimulus, the conditioned responding gradually abates as a new memory is formed that competes with the original combined stimuli memory

Matrix metalloproteinases (MMPs): Zinc- and calcium-dependent enzymes that have the ability to remodel ECMs by cleaving covalently-linked components, including CPSGs

Memory consolidation: General term for a range of cellular and systems-level processes that collectively strengthen or modify encoded memories.

Pavlovian fear conditioning: A form of learning in which an aversive (unconditional) stimulus, (*e.g.* foot shock) is associated with a neutral context, or conditional stimulus, such as a room context or aural stimulus. When paired together they result in expression of a fear, or 'conditional' response.

Perineuronal net: Elements of specialized extracellular matrix, which surround neurons allowing the regulation of neuronal excitability and plasticity

Reticular theory: A now-obsolete theory in neurobiology proposing that the nervous system operates as a network of continuous nerve fibers, without clear-cut separation between nerve cells.

Stability-plasticity tradeoff: Tradeoff between efficiently learning associations to form enduring memories, without being forced to forget previously learned, but still useful, memories.

Tetrapartite synapse: The concept that four cellular units contribute to synaptic signalling – the presynaptic neuron, postsynaptic neuron, glia, and the extracellular matrix.

Acknowledgments

A.C.R receives funding from a Canada First Research Excellent Fund BrainsCAN Fellowship and the Australian Research Council (DP180101974). D.J.H is supported by a National Health and Medical Research Council Career Development (Industry) Fellowship (1122981) in partnership with Agilent Technologies. T.J.B. holds a Western Research Chair in Cognitive Science. L.M.S. holds a Tier 1 Canada Research Chair and receives funding from Canadian Institute for Advanced Research (CIFAR)

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Table I (for Text Box 2). Major structural components of cortical PNNs

Glycosaminoglycan	Chondroitin sulfate proteoglycans (CSPGs)			Matrix glycoprotein	Link proteins	Secreted / bound proteins
	Lecticans	Matrix-associated proteoglycans	Cell surface proteoglycans			
Hyaluronan / hyaluronic acid	Aggrecan Brevican Neurocan Versican	Phosphacan	Neuroglycan-C Neuroglycan-G	Tenascin-R	Hyaluronan and proteoglycan link protein 1 (Hapln1/Crtl1) Hyaluronan and proteoglycan link protein 4 (Hapln4/Bral2)	Semaphorin 3A Orthodenticle homeobox 2 protein

Figure legends

Figure 1. The role of PNNs in fear memory updating. A. Removal of PNNs prior to fear conditioning facilitated later fear memory extinction in comparison to vehicle-treated mice, where fear extinction training was unsuccessful. B. Removal of PNNs following fear memory acquisition had no impact on the subsequent rate of fear extinction. Thus, the formation of PNNs around PV^+ interneurons may alter the function of local inhibitory circuits to promote the formation of an erasure-resistant memory trace during fear conditioning. Figure adapted from data reported by Gogolla et al [53]. Figure components include material from Adobe Stock Services (adapted from original works per terms of the Standard License Agreement and Terms of Use), *Brain Explorer 2* and the Allen Mouse Brain Atlas (Allen Institute) [112], and original illustrations by Maija Karala, used with express written consent.

Figure 2. Potential mechanism by which diet induced obesity may disrupt PNN integrity and enhance vulnerability to oxidative stress. Animal studies indicate that hypercaloric diets during adolescence increase neuroinflammation, which activates the endogenous PNN-degrading enzyme MMP-9 and raises levels of oxidative stress. As PNNs are stripped away, the encased PV^+ neurons become susceptible to free radicals in the inflamed brain region. This would lead to altered balance of excitation/inhibition in the cortex that can manifest as cognitive deficits. Figure components include material from Adobe Stock Services (adapted from original works per terms of the Standard License Agreement and Terms of Use).

Figure I (for Text Box 1). Plasticity trajectory of neurons and glia in the human prefrontal cortex. Arrangement and development of inhibitory interneuron circuitry in

the cortex, including the PFC, commences during early life, though synaptogenesis and other features of neuroplasticity involved in higher-order cognitive functions fall within a critical period spanning adolescence. Through development into adulthood the proportion of PV^+ interneurons surrounded by PNNs, and the 'intensity' of PNNs (i.e. how condensed the PNN structures are, which is linked to plasticity levels) steadily increases as the brain reaches maturity.

Figure 1.

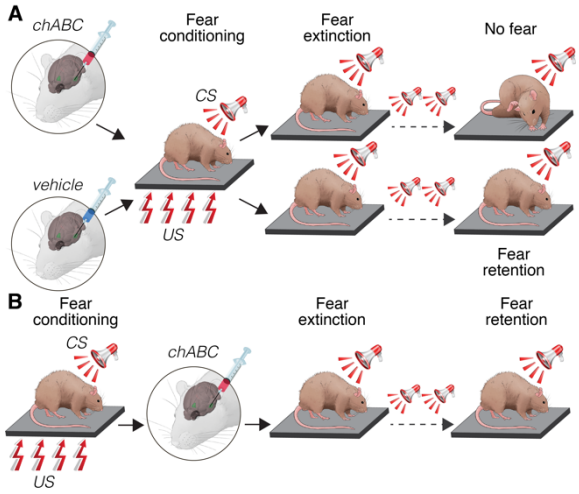

Figure 2.

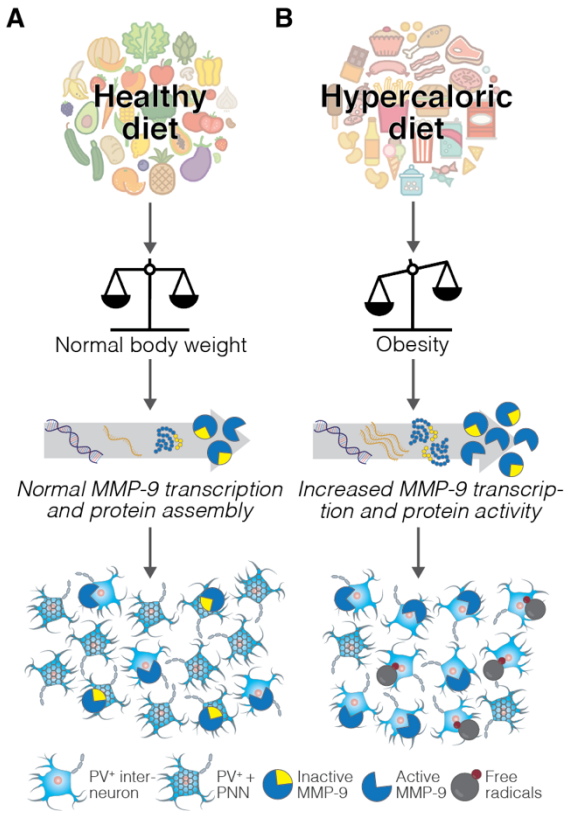

Figure i. (In text box 1)