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Distinct Roles for Hyaluronan in Neural Stem Cell Niches and Perineuronal Nets

Weiping Su^a, Steven Matsumoto^{a,b}, Barbara Sorg^c, and Larry S. Sherman^{a,d}

^aDivision of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, Oregon, 97006, USA

^bIntegrative Biosciences Department, School of Dentistry, Oregon Health & Science University, Portland, OR 97239, USA

^cDepartment of Integrative Physiology and Neuroscience, Washington State University, Vancouver, Washington, 98686, USA

^dDepartment of Cell, Developmental, and Cancer Biology, Oregon Health & Science University, Portland, OR 97239

Abstract

Adult neurogenesis in mammals is a tightly regulated process where neural stem cells (NSCs), especially in the subgranular zone (SGZ) of the hippocampal dentate gyrus, proliferate and differentiate into new neurons that form new circuits or integrate into old circuits involved in episodic memory, pattern discrimination, and emotional responses. Recent evidence suggests that changes in the hyaluronan (HA)-based extracellular matrix of the SGZ may regulate neurogenesis by controlling NSC proliferation and early steps in neuronal differentiation. These studies raise the intriguing possibility that perturbations in this matrix, including HA accumulation with aging, could impact adult neurogenesis and cognitive functions, and that alterations to this matrix could be beneficial following insults to the central nervous system that impact hippocampal functions.

Keywords

neurogenesis; neural stem cell; perineuronal net; hyaluronan

Introduction

Neural stem/progenitor cells (NSCs) in both the developing and adult brain are multipotent self-renewing cell populations that give rise to neurons and glial cells in the mammalian central nervous system (CNS). During early development, NSCs reside in the neural tube and undergo numerous rounds of self-renewal before dividing asymmetrically, giving rise to

Correspondence to: Larry S. Sherman: Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, 505 NW 185th Avenue, Beaverton, Oregon, 97006, USA. shermanl@ohsu.edu.

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early populations of neurons and radial glial cells [1]. Later in development, NSCs that reside in the ventricular zone and subventricular zone differentiate predominantly into astrocytes, then oligodendrocytes during perinatal periods. In the adult CNS, NSCs reside in NSC niches including the subventricular zone lining the lateral ventricles and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus [2]. These NSCs are heterogeneous with regards to their localization and their patterns of differentiation [3], and are capable of neurogenesis and gliogenesis throughout life in response to a variety of stimuli ranging from learning to injury.

A variety of extracellular signals in NSC niches, including the ligands of receptor tyrosine kinases, have been implicated in regulating NSC proliferation, differentiation and survival [4, 5]. Components of the extracellular matrix (ECM) have also been implicated in dynamically regulating NSC behaviors during both embryonic development and in adults. For example, NSC niches include a basal lamina that is enriched in collagen and laminin [6–8]. In adult NSC niches, the basal lamina regulates the adhesion of NSCs to the ECM. Extracellular glycoproteins such as tenascin C also play roles in regulating NSC within NSC niches. Disruption of tenascin C results in increased generation of astrocytes due to altered responsiveness to fibroblast growth factor-2 and epidermal growth factor [9]. Finally, proteoglycans have been implicated in regulating a number of NSC behaviors. Proteoglycans consist of a core protein and covalently attached glycosaminoglycan chains. While they have signaling properties of their own, they also can bind other extracellular factors, including signaling molecules, membrane proteins, and components of the ECM to influence NSC migration, differentiation, and survival [10].

Another major constituent of the CNS ECM is hyaluronan (or hyaluronic acid, HA). HA is a large unbranched, non-sulfated glycosaminoglycan composed of repeating disaccharide units of N-glucuronic acid and N-acetylglucosamine. HA acts as a backbone for numerous proteoglycans in the CNS including neurocan, aggrecan, and versican [11, 12]. However, HA is also found at cell surfaces independent of proteoglycans where it binds to various transmembrane and extracellular receptors and HA-binding proteins. HA is synthesized at the inner face of the plasma membrane and secreted into the extracellular space by a family of transmembrane proteins known as HA synthases (HASs). In some instances, HA can remain tethered to synthases or to transmembrane HA receptors. Mammals have three such synthase genes, *HAS1*, *HAS2* and *HAS3*. HA catabolism, on the other hand, is carried out by hyaluronidases (HYALs) and HA depolymerizing proteins that differ in their cellular localization and pH optima. Mammals possess multiple hyaluronidase and HA depolymerizing genes, including HYAL-1 through HYAL-5, PH20/SPAM1, transmembrane protein 2 (TMEM2), and Cell Migration-Inducing hyaluronan binding Protein (CEMIP) (also called HYBID and KIAA1199) [13–15]. In addition, humans carry a hyaluronidase pseudogene designated PHYAL-1 [13].

Changes in HA synthesis and catabolism play numerous roles in CNS development and homeostasis (e.g. [16–19]). In this review, we discuss recent data supporting distinct roles for HA in adult neurogenesis and, subsequently, in regulating neuronal function within perineuronal nets. We further explore the possibility that changes in the HA matrix with

aging or following a variety of insults to the CNS can significantly impact neuronal differentiation as well as learning, memory, and behavior.

Disruption of HA signaling influences hippocampal function

The hippocampal dentate gyrus contributes to the formation of episodic memories (e.g. the times, places, emotions and contexts associated with specific events) as well as pattern separation and emotional control. Each of these functions have been linked to alterations in adult neurogenesis in the SGZ [20]. A role for the HA-based ECM in regulating adult neurogenesis in the dentate gyrus is supported by the finding that HA is enriched in the SGZ [21, 22]. Interestingly, HA is synthesized by NSCs themselves, which express all three mammalian HA synthases [22] (Fig. 1). Furthermore, a major transmembrane HA receptor, the CD44 glycoprotein, is also expressed in the dentate gyrus especially by NSCs [22–24] (Fig. 1).

A functional role for HA in hippocampal activity was first demonstrated by Kochlamazashvili and co-workers [25] who found that disruption of HA in the hippocampus by intra-hippocampal injection of a hyaluronidase impaired hippocampus-dependent contextual fear conditioning. We subsequently found that mice lacking CD44 show hippocampus-dependent memory impairments [26]. Specifically, CD44 null mice demonstrated impaired hippocampus-dependent spatial memory retention in the probe trial following the first hidden-platform training day in the Morris water maze. There were no genotype differences in swim speeds during the water maze training sessions with the visible or hidden platform. However, some sensorimotor impairments were seen in other behavioral tests. In the inclined screen and balance beam tests, CD44 null animals moved less than wild type mice, while in the wire hang test, CD44 null mice fell off of the wire faster than wild type mice. No genotype differences were observed in tests of emotional learning and memory [26]. These data support an important role for CD44 in hippocampal spatial memory retention and in locomotor and sensorimotor functions.

More recently, Barzilay and co-workers [27] demonstrated that CD44 null mice have increased susceptibility to stress-induced anxiety and demonstrate increased anhedonia and despair compared to wild type mice. These authors also reported that CD44 null mice display reduced cortical serotonergic and striatal dopaminergic turnover, and that the hippocampal expression of another HA receptor, the receptor for HA-mediated motility (RHAMM) is reduced in non-stressed CD44 null mice compared with wild type animals [27]. Although this study did not directly examine changes in adult neurogenesis in CD44 null mice under these conditions, these findings, in conjunction with the previous studies discussed above, collectively support a role for HA and HA receptors in hippocampal neuron function and neurogenesis.

HA regulates NSC proliferation and neuronal differentiation in the SGZ

A study of NSCs on scaffolds made of HA and collagen indicated that HA could play a role in potentiating NSC differentiation [28]. Another study, using HA-laminin hydrogels, demonstrated that the HA-based ECM can regulate NSC migration in response to stromal

cell-derived factor-1-alpha (SDF1 α) via the CXCR4 receptor [29]. To address the specific roles of HA and CD44 in hippocampal neurogenesis, we recently examined the phenotypes of CD44 null SGZ-derived NSCs and of NSCs treated with a hyaluronidase both *in vitro* and *in vivo*. We found that CD44-null NSCs or hyaluronidase-treated wild type NSCs demonstrate increased proliferation rates and delayed neuronal maturation *in vitro* [22]. Treatment of NSCs with high molecular weight HA (>2 MDa) correspondingly inhibited NSC proliferation in wild type but not CD44-null NSCs [22]. Consistent with these *in vitro* results, mice lacking CD44 and wild type mice stereotactically injected with hyaluronidase into the SGZ demonstrated increased NSC proliferation, as assessed by Ki-67 and bromodeoxyuridine labeling, and delayed neuronal differentiation. In addition, CD44-null mice display a significant reduction in c-fos expression throughout the dentate gyrus granule cell layer after spatial learning [22], consistent with the notion that these excess immature neurons that accumulate in the granule cell layer may lack functional connectivity. All together, these data indicate that HA in the SGZ signals through CD44 expressed by NSCs to regulate NSC proliferation and early steps in neuronal differentiation.

HA regulates the activities of mature neurons

The mechanisms by which HA-CD44 signaling influences hippocampal neurogenesis are unclear. HA binding to CD44 may be one of many signaling events in the SGZ that cooperate to regulate the timing, onset, and progression of neurogenesis [30]. Alternatively, CD44 activation by HA may directly stimulate intracellular signaling to regulate NSC proliferation and differentiation. Interestingly, CD44 and HA may also play direct roles in regulating the activities of mature neurons. For example, we found that the baseline calcium levels in CD44 null neurons are significantly higher than those in wild type neurons, and that CD44 null neurons are less sensitive to KCl stimulation than wild type cells (our own unpublished findings). This phenotype is reminiscent of the phenotype observed in aged neurons [31–32] and in neurons affected by neurodegenerative disorders [33]. These data suggest that HA-CD44 interactions can influence the activities of calcium channels in mature neurons and further change hippocampal functions [25, 34]. However, we find that although CD44 expression is high in NSCs, it becomes undetectable as hippocampal neurons differentiate [22]. Thus, an alternative possibility is that cells from CD44 null animals aberrantly differentiate, resulting in altered calcium levels within the cells and altered hippocampal activity.

Unlike HA in NSC niches, many of the functions of HA in mature neurons have been linked to the role of HA in perineuronal nets (PNNs), specialized structures that wrap around neurons and proximal dendrites, with openings where synaptic inputs contact their underlying cells [35]. PNNs were first described as reticular structures by Golgi in the late 1800s [36, 37]. They are found in the CNS in a wide range of species, ranging from frogs to birds to mammals, including humans [37–39]. They form during development, and their overarching function is to stabilize synapses during adulthood and generally inhibit plasticity (see below). Despite their original discovery over 100 years ago, only recently has there been a focus on the role of PNNs in physiological brain functions, such as learning and memory, as well as in many pathologies, including schizophrenia, Alzheimer's disease, stroke, epilepsy, autism, drug addiction, and spinal cord injury [40]. Overall, PNNs play key

roles in neural development, synaptogenesis, neuroprotection, and experience-dependent synaptic plasticity [37, 41–44]. The increased excitement about understanding the role of PNNs in plasticity stems from findings that the removal of PNNs restores juvenile-like states of plasticity, as discussed in greater detail below and outlined in several reviews [35, 45–47].

PNNs are formed by the contribution of four families of ECM molecules. **(1)** HA is a significant component of PNNs, but unlike NSC niches, HA in PNNs appears to mostly form a backbone onto which other PNN molecules bind [11, 48]; **(2)** Chondroitin sulfate proteoglycans (CSPGs; primarily of the lectican family, including aggrecan, brevican, and versican), which bind via their amino-terminus to HA [49–52]. The composition of CSPGs in PNNs has been distinguished from that present in the loose ECM by using extraction procedures employing relatively harsh treatment (6 M urea), which separates components in PNNs from those in the loose ECM [53]. Further, the composition of these CSPGs varies within different brain regions [45, 54]. **(3)** Tenascins, proteins that form multimers and cross-link the lecticans to help stabilize PNNs [55]; and **(4)** HA and proteoglycan link proteins (HAPLNs; HAPLN1, 3 and 4 are found in the CNS). These proteins bind to both the HA backbone and CSPGs to stabilize PNNs [49, 56–58]. PNNs are most commonly identified by staining them with the plant lectin, *Wisteria floribunda* agglutinin (WFA; Fig. 2), which is thought to bind to terminal N-acetylgalactosamines beta 1 residues of glycoproteins [59], most likely on aggrecan; other methods for staining PNNs have also been used but probably bind to the same residues [59–62].

The HA synthesizing enzymes, HAS1, HAS2, and HAS3, are found in neurons with PNNs [45]. The different isoforms of HAS synthesize HA chains of different lengths [63, 64], and these may in turn impart PNNs with different functions that vary among brain areas. Which particular HAS enzymes are expressed on PNN-surrounded neurons varies dependent on the brain area and time point during development [45, 47]. For example, the spinal cord expresses HAS1 and HAS3, with only HAS3 in adult spinal cord [65]. In the mouse cortex and hippocampus, although all three enzymes are expressed, there is localization of HAS2 and HAS3 predominantly on cell bodies [66].

The mechanisms by which PNNs are attached to their underlying neurons has been examined in detail in the cerebellum [49]. Three HA receptors located in the CNS, CD44, RHAMM, and LYVE-1 [67], were examined, but none of these receptors was found in the PNNs of the cerebellum. The authors concluded that, of the three isoforms of HAS in mammals [68], neurons surrounded by PNNs have HA attached to their synthesizing enzyme, in this case, HAS2 and HAS3, as HAS1 was not found in PNNs in the cerebellum. The attachment of PNNs to their underlying cells via membrane HAS enzymes is supported by the observation that HEK cells can be transfected to generate HA and aggrecan, and these HEK cells in turn produce PNNs [56].

The removal of PNNs is accomplished most commonly by treatment with the bacterial enzyme called chondroitinase ABC (Ch-ABC; EC 4.2.2.4) from *Proteus vulgaris* NCTC 4636. This enzyme acts on CSs and CSPGs, and it also removes HA, HA binding proteins, and N-acetylgalactosamine [69, 70] as well as the HA binding region that binds proteins in the PNN [69]. The effects of Ch-ABC on PNNs are known to be long lasting: the enzyme

remains active for several days [71]. At 4 weeks post-Ch-ABC treatment, some PNNs reappear but their normal appearance does not occur for 5 months [69]. It remains unknown whether PNNs reappear on the same neurons or with the same composition. Hyaluronidase treatment also reduces PNN staining by WFA, consistent with its removal of two key components, aggrecan and link proteins [72]. However, the effects are much shorter lasting, with reappearance after several days [73]. While hyaluronidase treatment reduces the intensity of PNN staining by WFA, it does not influence the ECM that wraps around the initial segment of axons [74].

The role of HA in PNNs may depend on the cell type and the contents of the ECM

In many brain regions, PNNs are found mostly around fast-spiking, parvalbumin (PV)-containing GABAergic interneurons [75–78]. However, an increasing number of studies demonstrate that PNNs also surround glycinergic neurons [79] as well as glutamatergic neurons [49, 80–84], which can be both parvalbumin positive or negative [80, 81]. PNNs are located throughout the brain and spinal cord, but are unevenly distributed [85]. They form during development at different rates across the brain and spinal cord [86, 87] and are completed by early adulthood in the cortex of rodents [88], with differences in developmental rates among cortical subregions. Neural activity promotes PNN development, which occurs through changes in potassium and calcium conductance and through activation of glutamate receptors (N-methyl-D-aspartate receptors and calcium-permeable AMPA receptors) [77, 86, 89].

Interestingly, the development of PNNs occurs precisely at the time when critical period plasticity ends and when brain plasticity is greatly reduced. The core components of PNNs (detailed below) increase during this critical window of development [90]. PNNs have been heavily studied for their contributions to critical period plasticity within the visual system, motor system, and somatosensory system [88, 91–93]. PNNs limit plasticity in adulthood, but they can be degraded to reinstate juvenile-like states of plasticity, allowing for axon sprouting and regeneration of function in damaged neurons.

Several knockout (KO) mouse models have been generated to determine the contribution of the various HA-containing lecticans to PNNs. Versican V2 (a splice variant), neurocan, or brevican KO mice have largely normal PNNs [94], whereas aggrecan KO mice do not stain for WFA, indicating that aggrecan is a key contributor to PNN formation [62]. Given that these proteoglycans are absent during development, it is difficult to know how compensatory changes might preserve PNN structure. Overall though, the diversity of lecticans (e.g., [95]) and other components of PNNs, such as link proteins that are critical for PNN formation [58] and the length of the HA chain, are expected to confer different functions of PNNs across different regions of the brain and spinal cord, although exactly what these functions are remain unknown. In addition, other features of PNNs allow for further complexity. For example, the lecticans contain chondroitin sulfate (CS) chains that are sulfated at different positions during development, and this is believed to regulate plasticity in terms of axonal growth and guidance [96–99]. In addition, the sulfation pattern of CS chains continues to

change in old age, conferring an even greater inhibitory function to PNNs than is found in younger adults [100].

To date, the best studies examining the function of HA in PNNs have relied on hyaluronidase digestion of the ECM. Each of these studies provide clues about potential roles for HA in PNNs and associated ECM. An early study found that chronic exposure to hyaluronidase caused severe neurological dysfunction, such as rigidity, unresponsiveness and grand mal seizures [101]. The underlying mechanism of these phenomena is unclear due to the complex nature of the neuronal networks involved. However, more recent studies with better controlled delivery of hyaluronidases have revealed more specific roles linked to changes in the PNN ECM. For example, hyaluronidase treatment in the auditory cortex promotes cognitive flexibility in an auditory learning task in gerbils [102]. Hyaluronidase also decreased calcium influx through L-type channels in hippocampal dendrites and abolished long-term potentiation (LTP) in hippocampal (CA1 and CA3) synapses [25]. Addition of HA to hyaluronidase-treated hippocampal neuron cultures restored LTP, indicating that HA acts through L-type calcium channels to modulate synaptic function in the CA1/CA3 regions of the hippocampus. Digestion of HA also increases hippocampal sharp wave ripples observed during slow wave sleep [103], and a combination of Ch-ABC with hyaluronidase impairs fear memory when infused into the medial prefrontal cortex or dorsal hippocampus [104].

One area where HA in PNNs may play a critical role is in limiting the mobility of AMPA receptors (AMPA receptors) in the postsynaptic membrane. The rapid movement of AMPARs between junctional and extrajunctional sites allows desensitized receptors to be replaced by “fresh” responsive receptors. Treatment of mature PNN with hyaluronidase restored mobility of the AMPAR and synaptic plasticity [105]. This same study reported that PNN degradation did not affect the migration of NMDA receptors. However, there is evidence that the functional properties of NMDA receptors are affected by disruption of PNNs. Schweitzer and co-workers [106] reported that the PNN regulated the composition of NMDAR subunits, designated as GluN2A and GluN2B. During the development of the visual cortex and hippocampus, NMDARs are composed of the more adaptable GluN2B but after the end of the critical period the less plastic GluN2A predominates. In dissociated hippocampal culture the expression of GluN2A and GluN2B follow a similar time course (GluN2B being replaced by GluN2A). The dominant expression of GluN2A in mature cultures can be reversed treating the cells with hyaluronidase.

Each of these studies suggests that HA and HA-associated proteoglycans within PNNs could impact different aspects of neuronal activity. However, with the exception of knockout mice deficient in CSPGs or link protein, all studies to date have used hyaluronidase or Ch-ABC to assess the function of PNNs. A drawback to this approach is that the loose ECM is also digested, bringing into question whether damage to the loose ECM contributes to the neural plasticity effects. In addition, in studies that rely entirely on hyaluronidases, it is unclear if the physiological effects of hyaluronidases are due to loss of high molecular weight HA within PNNs or associated loose ECM, physiological effects of specific sizes of HA digestion products, or more generalized disruption of HA-associated proteoglycans. Future studies will need to develop more advanced methods to manipulate PNNs and their

component, including HA, by targeting their removal in a cell- and brain region-specific manner.

How changes in the HA-based ECM can impact neurogenesis and neuronal functions

The manipulation of HA and HA receptors in the SGZ and in PNNs could have a number of therapeutic benefits following different insults to the CNS that lead to altered neurogenesis or neuronal function. For example, there is a dramatic increase in NSC proliferation and an accumulation of immature neurons in the SGZ following seizures [107–109]. These changes are remarkably similar to the phenotypes observed in CD44 null mice and in wild type mice in which HA was disrupted in the SGZ [22]. Animals with seizure-induced increases in NSC proliferation are prone to subsequent seizures and associated cognitive difficulties [19, 27, 107, 110–113]. Furthermore, HA and levels of hyaluronan synthase-3 are altered in the hippocampus following seizures in rats [108] while degradation of HA with a hyaluronidase or treatment with CD44 function-blocking antibodies altered seizure-induced mossy fiber sprouting [109]. Recent studies support the notion that the cells demonstrating aberrant mossy fiber sprouting are aberrant adult-born dentate granule cells [114]. It is possible, therefore, that altering HA digestion or CD44 expression on in the SGZ or on mossy fibers following seizures could help limit seizure-associated cognitive deficits.

Excess accumulation of HA and CD44 overexpression could also influence changes in adult neurogenesis with aging. Neurogenesis in the SGZ decreases with aging, possibly contributing to age-related cognitive decline [115–120]. Decreased neurogenesis with aging is at least partly linked to reduced NSC proliferation. A number of studies have suggested that age-related changes in the NSC microenvironment could limit adult neurogenesis in old age [120–123]. Several studies have demonstrated that HA accumulates in the CNS with aging and in individuals suffering from age-related cognitive dysfunction [22, 124–126]. Importantly, we find that HA accumulates in the SGZ and the rest of the dentate gyrus with aging in mice [22]. This accumulation may, therefore, contribute to reductions in NSC proliferation and affect new neuron maturation, leading to age-related changes in hippocampal function. Developing strategies to prevent HA accumulation or excessive activation of CD44 in the SGZ could therefore be interesting approaches to prevent age-related declines in neurogenesis and associated cognitive disturbances.

Interestingly, the controlled regulation of HA and other components within PNNs may have the potential to influence conditions whose underlying cause is aberrant neuronal function. Consistent with the ability of PNNs to restrict plasticity in adulthood, their removal with either Ch-ABC or hyaluronidase restores several types of plasticity. Ch-ABC treatment restores ocular dominance plasticity in the visual cortex of adult animals [88], enhances object recognition memory [127], enhances reversal learning in the auditory cortex [102], promotes recovery of motor learning after spinal cord injury [128] and cortical ischemia [129], and influences extinction of fear conditioning [130]. However, removal of PNNs has also been shown to prevent certain types of plasticity imposed by strong stimuli, including

shock used for fear conditioning and drugs of abuse. Removal of PNNs prevents fear conditioning in the medial prefrontal cortex or dorsal hippocampus [104].

The removal of PNNs also prevents the effects of drugs of abuse, including the consolidation/reconsolidation of drug-associated memories and the erasure of memories [131–133]. We hypothesize that removal of PNNs is effective at both promoting or preventing plasticity depending on the type of stimuli used to induce plasticity. When relatively weak, physiological stimuli such as light or training of motor pathways are used to activate neurons, PNN removal allows for new synapse formation onto the underlying cells that restores function (e.g, ocular dominance plasticity). On the other hand, PNN removal may block the effects of stronger stimuli, including shock used in fear conditioning or drugs of abuse. Drugs of abuse induce metaplasticity [134], and this metaplasticity alters the ability of natural stimuli to further alter plasticity. In this case, PNN removal may prevent the drug-induced changes to restore the excitatory:inhibitory balance, likely by altering cell firing [135] and/or other molecules that bind to PNNs to regulate homeostatic plasticity [74].

PNNs are highly dynamic even during adulthood. For example, environmental enrichment in animals, in which rodents are housed together and given novel toys and exercise wheels, has been shown to either reduce or increase the number and/or the intensity of PNN staining by WFA [136–137]. Several different drugs of abuse have demonstrated increases or decreases in the intensity of PNN staining, depending on treatment and brain area [138–40]. The Sorg lab, (unpublished) and others [141] have even observed diurnal/circadian changes in the intensity of PNNs, suggesting a highly dynamic regulation of these structures. In some cases, some of the PNN components have been measured for what underlies these dynamic changes after exposure to drugs of abuse [139, 142], but multiple mechanisms may regulate the staining intensity of PNNs, including how tightly condensed the PNNs are, the sulfation patterns of the CSPGs, and the ability to capture inhibitory molecules or those that promote plasticity (e.g., BDNF). Regulation of HAS1-3 on neurons bearing PNNs produce HA that serves as the critical backbone structure of PNNs, but these have not yet been explored in the context of these dynamic changes. Future studies will need to dissect the contribution of these enzymes and the mechanisms by which they may be regulated, and in turn, how the properties of PNN-enwrapped neurons are altered.

Conclusions and Future Research Directions

Studies to date have demonstrated that HA, HA-associated ECM (including proteoglycans) and HA receptors each play roles in neurogenesis and in regulating the activities of mature neurons (Fig. 3). These roles are distinct depending on the point in neuronal differentiation, the location of the cells, and on age. For NSCs, HA appears to play a crucial role in regulating NSC expansion and early stages of neuronal differentiation and maturation. In mature neurons, HA is a component of PNNs where it contributes to the regulation of neuronal activity predominantly as a component of proteoglycans within PNNs. The growing evidence that HA is altered or elevated following CNS insults and during normative aging implicate HA and HA receptors as important players in CNS disease pathogenesis and injury responses.

It is intriguing to speculate that HA synthesis and catabolism are dynamically regulated during the course of normal CNS activity. For example, given that HA restricts NSC proliferation in the SGZ and prevents neurogenesis [22], it is possible that hyaluronidases or other mechanisms transiently digest the HA in NSC niches in an activity-dependent manner to promote NSC expansion and subsequent differentiation during learning or in response to other stimuli. What remains unclear is how and which hyaluronidases may be involved in such dynamic regulation. One candidate is CEMIP, which is expressed in the brain and has been linked to hippocampal learning and memory functions [143]. It is also unclear if the effects of HA or HA digestion in either the SGZ or in PNNs is due entirely to high molecular weight forms of HA, the disruption of such HA, or the accumulation of specific sizes of HA digestion products. Indeed, HA breakdown products that can signal through Toll-like receptors have been implicated in regulating the maturation of oligodendrocyte progenitors in CNS white matter [144, 145]. Finally, it will be important to understand which receptors regulate the activities of HA in both NSC niches and in PNNs. While CD44 appears to be crucial for regulating NSC proliferation in the SGZ, roles for other receptors in subsequent stages of neuronal differentiation and in mature neurons have not been fully investigated. Future studies that address these issues will provide insights into the precise mechanisms by which the HA-based ECM influences neurogenesis and neuron activity. Understanding these mechanisms will also make HA and HA receptors excellent candidates for a variety of therapeutic interventions aimed at restoring neurogenesis and neuronal functions in the damaged CNS.

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
CEMIP	Cell Migration-Inducing hyaluronan binding Protein
CNS	central nervous system
ECM	extracellular matrix
HA	hyaluronan
HAPLN	HA and proteoglycan link proteins
HAS	hyaluronan synthase
TMEM2	transmembrane protein 2
HYAL	hyaluronidase
NSCs	neural stem cells

PNNs	perineuronal nets
SGZ	subgranular zone

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Highlights

- The glycosaminoglycan hyaluronan (HA) regulates neural stem cell proliferation and differentiation as well as neuronal activity in mature neurons
- The HA-based extracellular matrix is dynamically regulated and can be influenced by a variety of stimuli and insults to the central nervous system
- HA and HA receptors may be effective targets for restoring neurogenesis and neuronal function in the injured brain or spinal cord.

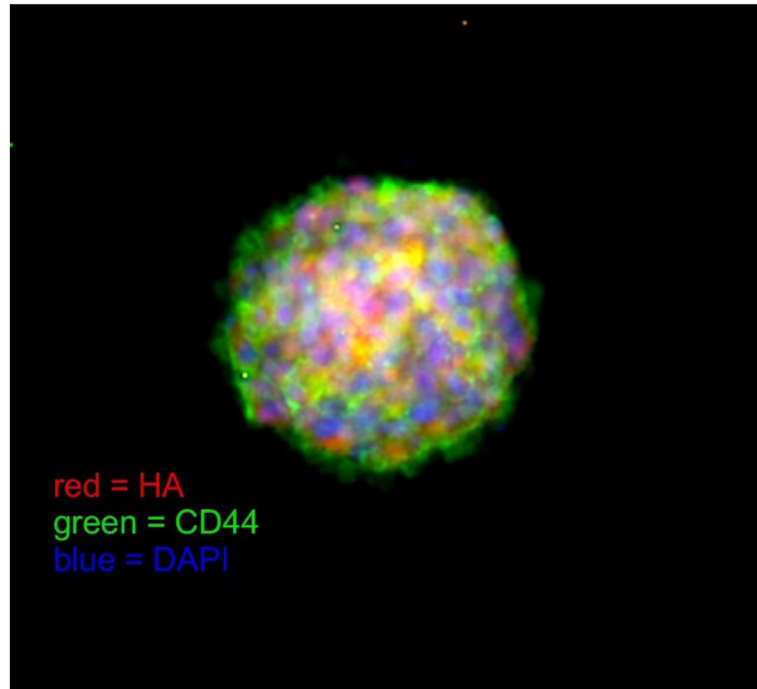


Fig. 1.

A whole-mount mouse SGZ-derived neurosphere labeled with HA (red, using a biotinylated HA-binding protein), CD44 (green) and DAPI (blue; to stain cell nuclei). As shown here, some of the outermost cells in neurospheres, which are undifferentiated NSCs, express both CD44 and HA (yellow staining). The degree to which this co-localization occurs varies from one neurosphere to another.

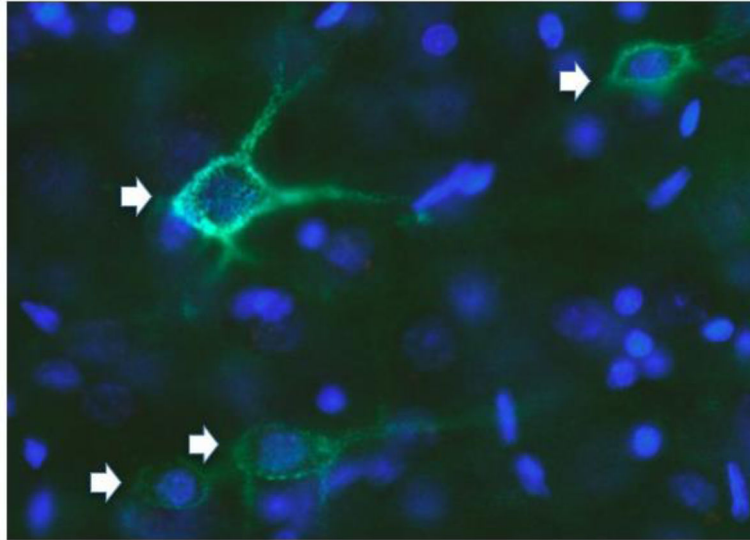


Fig. 2. Neurons in a section from mouse prefrontal cortex labeled with *Wisteria floribunda* agglutinin (WFA; green; arrows) to label PNNs and DAPI (blue; to stain cell nuclei). This image demonstrates the extent to which PNNs surround both neuron cell bodies (green staining surrounding DAPI-labeling) and processes.

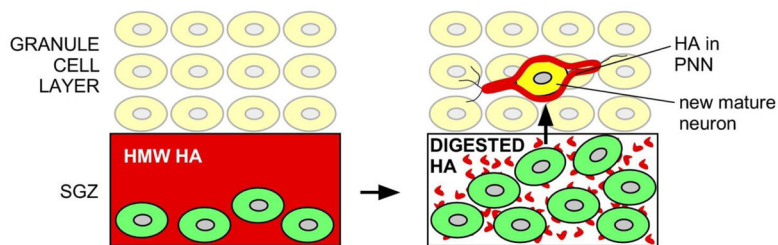


Fig. 3.

Distinct roles for HA in adult hippocampal neurogenesis and in mature neurons. HA (red) in the SGZ is believed to typically exist as a high molecular weight (HMW) molecule that keeps NSCs (green) in a quiescent state through signaling by CD44 expressed by NSCs. When NSCs need to expand in number, HA is digested, possibly by hyaluronidases like CEMIP, either relieving NSCs from the quiescence signals generated by HMW HA or by inducing proliferation in response to HA digestion products (small red dots). These NSCs migrate into the granule cell layer and differentiate into new neurons (yellow cell) that assemble PNNs with HA (red color surrounding the new neuron) around themselves. HA within these PNNs is also likely to be dynamically regulated to influence neuronal activity.