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The Role of Perineuronal Nets in Regulating Barrel Cortex Physiology

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The Role of Perineuronal Nets in Regulating Barrel Cortex Physiology

By

Philip Chu

Dissertation submitted to the Graduate Faculty in Psychology (Neuropsychology Subprogram) in partial fulfillment of the requirement for the degree of Doctor of Philosophy. The Graduate Center, City University of New York

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THE CITY UNIVERSITY OF NEW YORK
Abstract

The Role of Perineuronal Nets in Regulating Barrel Cortex Physiology

by

Philip Chu

Advisor: Joshua C. Brumberg, Ph.D.

The ability of the brain to adapt to changing environmental conditions is regulated by genetic and environmental factors. One component of the brain extracellular matrix, a scaffold of proteins and proteoglycans, tightly ensheaths the soma and proximal processes of neurons. These Perineuronal nets (PNNs) play protective and structural roles in the brain, but also regulate plasticity and behavior. Their developmental expression is highly attenuated following sensory deprivation, or pharmacological silencing of neuronal activity. Thus, PNNs contribute to the activity dependent regulation of plasticity in the brain. Although PNNs are relatively ubiquitous in the neocortex, little is known about the degree to which they impact the physiology of the diverse neuronal phenotypes that exist there. This research focused on determining the experience dependent maturation of PNNs in different cortical layers and whether the alterations to neuronal intrinsic properties following sensory deprivation could be explained by PNN reductions. Finally, we sought to determine what aspects of intrinsic and synaptic physiology are regulated by PNNs in the hope of providing future direction for understanding their fundamental role in the neocortex. This research produced a number of key findings: 1) PNN development in the granular and supragranular layers depend more on sensory input than the infragranular layers
; 2) sensory deprivation induces reductions in spike frequency, probability and mildly reduces input resistance, while significantly increasing action potential amplitude in fast spiking (FS) inhibitory interneurons; 3) enzymatic digestion of PNNs only mildly impac spike frequency and firing probability, but significantly reduces input resistance, and spike amplitude in FS inhibitory interneurons and 4) Perturbation of PNNs reduces the temporal range of spontaneous EPSPs onto FS cells and reduces the spontaneous EPSP frequency onto low threshold spiking putative interneurons. Other minor alterations to putative excitatory intrinsic properties were also detected. The similarity in features that are modulated by both sensory deprivation and perturbation of PNNs suggests that the presence of PNN may only serve as a partial mechanism for experience dependent regulation of physiology. Nevertheless, PNN perturbation impacts important features of intrinsic physiology that can affect inhibitory interneuron networks. These data are relevant to understanding the mechanisms of brain reorganization and plasticity and suggest that the gradual stabilization of brain extracellular matrix across development plays a role in stabilizing neuronal networks and their cognitive/behavioral outputs.

**Key Words:** Perineuronal nets, Barrel cortex, Physiology

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1. General introduction

Circuits serve to integrate inputs in order to instruct functional outputs. This reductionist perspective highlights one of the primary differences between classical computers and the biological brain. Rather than a fixed system that maximizes speed and efficiency, the biological brain is one that is capable of remarkable flexibility, which is manifested by the physical reorganization and adjustments of neuronal circuits. Ultimately, flexibility allows biological organisms endowed with complex nervous systems the ability to integrate new data into relatively established systems, and use them to adapt to changing environmental conditions.

Neuronal reorganization is modulated by various components of the brain parenchyma including myelin derived proteins, glia, and the extracellular matrix (ECM) (McGee et al., 2005; Pizzorusso et al., 2002; Tremblay et al., 2010). The ECM consists of a medley of proteins and polysaccharides that exist in the extracellular space, which comprises 40% of brain volume in the immature brain and 20% in the adult (Zimmermann & Dours-Zimmermann, 2008). The brain ECM is relatively ubiquitous, and likely exists to serve structural, homoeostatic and cell signaling functions similar to its role outside of the nervous system (Bonnans et al., 2014a).

A phenotype of ECM unique to the central nervous system are called Perineuronal nets (PNNs), aggregates of ECM that ensheath the cell bodies and proximal processes of neurons. First described by Camillo Golgi in 1898 as a "delicate covering", these structures were later dismissed by his most promising student, Ramon y Cajal, as a staining artifact due to the consuming debate between the proponents of the neuron doctrine and the reticular theorists.
(Celio et al., 1998). The first detailed description of PNNs by Camillo Golgi, the ensuing dismissal of their existence, and the ultimate posthumous vindication of his discovery, provides an ironic historical backdrop for our understanding of a ubiquitous brain structure that has been recently shown to resist neural reorganization.

In this report, I will first review the evidence for PNN regulation of plasticity in the brain. I will then describe the structure and pathway of the barrel cortex as a model system for studying experience dependent plasticity and the heterogeneous cell types that exist there. Next, I will discuss the evidence for experience dependent maturation of PNNs and elaborate on what is currently known about their composition, development and interactions in the central nervous system. I will focus on the evidence demonstrating that PNNs are involved in regulating plasticity, protecting specific neurons from oxidative stress and creating microenvironments on neuronal membranes. Finally, I will provide evidence for the role of PNNs in regulating the intrinsic and synaptic properties of different cortical neurons in the barrel cortex. In order to understand the malleable nature of brain function and behavior, it is important to understand how neuronal circuits can be regulated by structures that are so ubiquitous, that they are fundamental components of biology.

1.1. PNNs and Plasticity

A body of evidence has recently accumulated that demonstrates that PNNs in the brain are involved in regulating behavioral, structural and physiological plasticity. First, the maturation of PNNs correlates with the closing of early critical periods of plasticity (McRae et al., 2007; Pizzorusso et al., 2002), an early developmental period when the brain is particularly capable of reorganization. Although evidence exists that PNNs continue to mature into middle age in
rodents (Karetko-Sysa et al., 2014), they do not begin to appear until the developing neocortex starts to establish behaviorally relevant neural circuitry (Nowicka et al., 2009).

In the adult brain, experimentally destabilizing PNN levels reduces memory stability and promotes behavioral flexibility (Gogolla et al., 2009; Happel et al., 2014; Xue et al., 2014). Altering the expression and phenotype of PNNs in vivo also induces levels of cortical plasticity only seen during earlier stages of development (Carulli et al., 2010; Miyata et al., 2012; Pizzorusso et al., 2002). For example, Gogolla and colleagues (2009) demonstrated that enzymatically removing PNN components in the basolateral amygdala, a region involved in storing fear memories (Gale et al., 2004), leaves conditioned fear memories very susceptible to behavioral extinction which is typically only seen in juvenile animals. These experiments demonstrate that PNNs are involved in regulating critical periods of development, the times during early maturation when the brain and behavior, is particularly susceptible to experiential adaptations. However, some areas of the brain retain the ability to readily reorganize throughout life.

Other regions of the brain such as the hippocampus, are capable of reorganization into adulthood and maintain the ability to change synaptic strength that is correlated with learning and potentially leads to stable memory representations (Lynch, 2004). Importantly, altering the expression of PNNs has been shown to attenuate long term changes in synaptic strength in the hippocampus (Bukalo et al., 2001; Bukalo et al., 2007; Frischknecht et al., 2009; Kochlamazashvili et al., 2010) which may reflect a less saturated memory system (Lynch, 2004). On the other hand, in the perirhinal cortex, a structure important for objection recognition memory, both enzymatic and genetic attenuation of PNN expression extends objection recognition memory and enhances long term synaptic changes (Romberg et al., 2013). Consistent
with the findings in the perirhinal cortex, enzymatic reduction of PNN in the visual cortex both enhances long term synaptic changes, and decreases the stability of dendritic spines, small protrusions that serve as the post-synaptic sites for a large majority of excitatory synapses (de Vivo et al., 2013). Therefore, PNNs can impact synaptic strength and plasticity in opposite directions depending on the distinct neuronal circuits that exist in different functional areas.

Although, dendritic spines have been traditionally thought of as the physical manifestation of learning and memory due to their gradual stabilization in adulthood (Holtmaat et al., 2005) and sensitivity to sensory perturbations (Chen et al., 2015a; Chen et al., 2012) it has recently been proposed that the 'holes in the nets' of PNNs may also play a role in long term memory storage (Tsien, 2013). Like dendritic spines, PNNs also depend on normal sensory input during development to reach their mature expression patterns (McRae et al., 2007; Nakamura et al., 2009; Nowicka et al., 2009). Together, these findings suggest that PNNs are sensory dependent regulators of neuronal plasticity.

The aforementioned evidence linking PNNs with plasticity from areas throughout the sensory and limbic cortices have demonstrated the role of PNNs in regulating synaptic physiology, with somewhat diverging outcomes, simultaneously enhancing sensory cortex plasticity and limbic system flexibility, while in other regions enhancing recognition memory stability. The differences in plasticity outcomes likely reflects the behavioral paradigms, idiosyncratic circuits that are being recorded from in the different experiments, and potentially the cell type specificity of PNN expression. In fact, few studies (Cabungcal et al., 2013; Dityatev et al., 2007; Morishita et al., 2015) have examined the role of PNNs on the cell types that they ensheath.

The majority of neurons in the neocortex can be generally classified into those that
depolarize or hyperpolarize their post-synaptic partners, and are described as excitatory or inhibitory cells respectively. Interestingly, inhibitory cells have been reported to be preferentially ensheathed by PNNs (Foster et al., 2014). Only a minority of neocortical neurons are inhibitory and their experience dependent maturation, as demonstrated by morphological and physiological alterations following sensory deprivation, is well documented (Benevento et al., 1995; Chen et al., 2012; Jiao et al., 2006; Li et al., 2009; Sun et al., 2006). This sensory dependent maturation is then a major factor in closing the critical period of plasticity in the neocortex (Fagiolini & Hensch, 2000; Iwai et al., 2003). One type of inhibitory interneuron expressing the calcium binding protein parvalbumin (PV+) also expresses the greatest proportion of PNNs, (Nowicka et al., 2009). The conspicuous positioning of two factors, both dependent on experience for normal, but relatively late development, and both involved in regulating plasticity suggest the existence of a synergistic relationship. Since removal of PNNs induces cortical plasticity that is normally prevented by the maturation of inhibitory interneurons, it is possible that PNNs directly modulate inhibitory tone through the regulation of inhibitory interneurons in the brain, which may be detected using physiological measures for neuronal excitability.

The intrinsic physiology of a neuron is a broad set of features relating to the factors that govern its resting state, excitability and propagation of voltage depolarizations. These include passive properties such as input resistance (cell leakiness mostly driven by $K^+$ leak channels), or resting membrane potential (driven by mostly by $K^+$ equilibrium through leak channels, but also active currents (Lupica et al., 2001)) as well as active factors such as different voltage gated channels that are permeable to positively charged cations, that regulate and drive the action potential (Bean, 2007).

The expression of plasticity through the changes to the intrinsic physiological properties
of neurons has been well documented (reviewed in Zhang & Linden, 2003). For example, using the Aplysia conditioned gill reflex withdrawal paradigm, a benign siphon tap is classically conditioned to a tail shock which eventually results in a conditioned gill withdrawal reflex in response to the siphon tap. The siphon sensory neuron directly drives the motor neuron controlling the gill reflex in this preparation and both can be simultaneously recorded from. Following conditioning, Antonov and colleagues (2001) found both an increase in post-synaptic motor neuron activity following siphon tap, but also greater pre-synaptic sensory neuron activity. In the rodent neocortex, neurons that are persistently stimulated using extracellular electrodes show not only increases in synaptic strength, but also increased action potential firing frequency in response to depolarizing pulses delivered through a recording electrode (Sourdet et al., 2003). These changes appear to be driven by changes in intrinsic currents that regulate action potential firing and membrane potential (Sourdet et al., 2003). These studies demonstrate that plasticity can be expressed through an intrinsic phenotype, rather than just a synaptic one. Importantly, there is already evidence that PNN components are capable of binding to voltage gated Na$^+$ channels (Srinivasan et al., 1998), the cation channel that drives the rising phase of the action potential.

In order to understand the role of PNNs in regulating neuronal physiology and plasticity, I first sought to determine the experience dependent maturation of PNNs in different cortical layers in the barrel cortex, and evaluate the physiological impact of deprivation on two neuronal subtypes (presumed inhibitory and excitatory cell types) in the barrel cortex. To do this, I conducted histochemistry for PNNs following whisker trimming/restoration and counted distinct PNNs that encapsulated presumed neuronal cell bodies. In parallel, I conducted in vitro patch clamp electrophysiology on the sensory deprived barrel cortex and compared them to non-
deprived animals. I then sought to determine whether the alterations in intrinsic physiology following sensory deprivation could be mimicked through attenuation of PNNs alone. I also set out to determine the basic physiological role of PNNs on different neuronal phenotypes in the barrel cortex to isolate a potential mechanism for plasticity regulation in the brain. To this end, I recorded the intrinsic and synaptic properties from five electrophysiologically distinct neuronal cell types in the rodent somatosensory cortex using whole cell patch clamp methodologies.

2.1. The functional architecture of the barrel cortex.

The rodent somatosensory system is a useful model for studying the maturation and critical period of cortical circuits due to its relatively discrete and well mapped anatomical pathways. In addition, rodents are generally nocturnal with relatively poor eyesight and rely on non-visual senses such as olfaction, audition and somatosensation for survival, making the somatosensory system the dominant and thus most ethologically relevant sensory system to study within the confines of this particular animal model. Importantly, the cortical representation of the whisker somatosensory system is also highly enriched with PNNs (Karetko-Sysa et al., 2014; McRae et al., 2007; Nakamura et al., 2009; Nowicka et al., 2009).

The most peripheral aspect of the rodent somatosensory system is the large lateral and anterior projecting mystacial vibrissa which are embedded in the whisker follicles on the snout. These whiskers can be dynamically controlled to navigate the environment, recognize objects, and engage in social interactions (Sofroniew & Svoboda, 2015). In order to do this, rats protract and retract their whiskers at a frequency of 8Hz (range: 1-20 Hz) and can reliably detect the difference between a smooth texture and ones with 30 µm deep grooves spaced 90 µm apart (Carvell & Simons, 1990), similar to the discrimination ability of human finger tips (40-80 µm
During intense bouts of active whisking, rats typically whisk at 5-12 Hz whereas mice whisk at 14-24 Hz using intrinsic and extrinsic muscles consisting of mostly type 2B muscle fibers (Jin et al., 2004). Active whisking initiates during the second post-natal week of life (Landers & Zeigler, 2006) and clipping the whiskers from birth disrupts the rodents ability to discriminate between objects of different textures later on in life following whisker regrowth (Carvell & Simons, 1996) demonstrating an important role for normal experience in sensory processing.

Three major tracts exist for the nervous system pathway for whisker touch information, the lemniscal, the paralemniscal, and the extralemniscal pathways. For the lemniscal pathway, tactile information is carried from the whisker follicles by the infraorbital branch of the trigeminal nerve to the principal trigeminal nucleus of the brain stem where glutamatergic synapses are made onto the somatotopic representation of the whisker pad called barrelettes (Petersen, 2007). These inputs are then relayed to the ventral posterior medial nucleus (VPM) of the thalamus where barreloids, represent the whisker pad once more. Thalamocortical neurons of the VPM project mostly to layers 4 and 6A (Wimmer et al., 2010), but also layers 3 and 5B (Oberlaender et al., 2012). Layer 4 is where the somatopic representation of the entire whisker pad is mirrored in what is called the barrel cortex (Summarized in figure 1).

The para- and extralemniscal pathways diverge in the brain stem, with a parallel set of trigeminal nerve inputs innervating the rostral and caudal spinal interpolaris nuclei, which then project to the thalamic posterior medial nuclei (POM) and ventrolateral strip of the VPM respectively. The POM projects mostly to layer 1 and 5A of the barrel cortex (Wimmer et al., 2010), the secondary somatosensory cortex, and the motor cortex (reviewed in Petersen, 2007). The extralemniscal pathway projects to the motor cortex as well as the secondary somatosensory
cortex (reviewed in Feldmeyer et al., 2013).

Using these major pathways, the rodent somatosensory cortex, similar to other sensory cortices, serves as a gateway through which external sensory information is transformed into internal representations and processed for relevant behavioral output.

2.2. Cytoarchitecture of the Barrel Cortex

The somatosensory barrel cortex contains the somatopic representation of the contralateral whisker pad. The distribution of barrel cortex is columnar and subdivided into six layers, similar to other sensory cortices (Mountcastle, 1997). These six layers can also be further subdivided based on cellular morphology and function. Although originally recognized by Lorente De No in 1922 (Feldmeyer, 2012), the discrete somatopy was first correctly identified as the representation of the whisker pad by Woolsey and Van Der Loos in 1970.

Within the barrel cortex, coincident synchronous, but relatively weak thalamocortical inputs drive granular layer 4 (Bruno & Sakmann, 2006), which is transmitted through feed forward excitation into the intracolumnar supragranular layers 2/3, followed by signaling to the infragranular layers 5 and 6 (reviewed in Lübke & Feldmeyer, 2007). Neurons in both the supragranular and infragranular layers send cortico-cortico projections to secondary somatosensory cortex and the primary motor cortex, suggesting a role for motor integration during behaviorally relevant tasks. Neurons in lower layer 5 (5B), project to subcortical regions such as the pontine nuclei, tectum, thalamus, and spinal cord (reviewed in Lübke & Feldmeyer, 2007). Layer 6 provides potent cortico-thalamic feedback (Yang et al., 2014) that is 10x greater than the thalamocortical input it receives and may serve as a feedback gating mechanism for sensory inputs. Neurons in the supragranular and infragranular layers also project to striatum that
may serve to update the basal ganglia, a region important in motor control, on incoming sensory stimuli (Lübke & Feldmeyer, 2007).

In contrast to the canonical flow of information in the barrel cortex, Schubert and colleagues (2003) used patch clamp electrophysiology and glutamate uncaging to show that layer 4 pyramidal neurons also receive inputs from other cortical layers while spiny stellate cells do not. Recent studies have also discovered a parallel pathway in the barrel cortex. This study demonstrated in vivo that VPM projections to the infragranular layers drive action potential firing in these neurons, suggesting that a parallel pathway exists that can drive action based processing through feedforward projections to subcortical regions (Constantinople, & Bruno, 2013).

It has been known since the pioneering work of Ramon Y Cajal that a morphologically diverse population of neurons exist in the neocortex (Cajal 1899). Contemporarily, those morphologically diverse cell types have been divided into two superordinate functional classes of cells expressing either the major excitatory or major inhibitory neurotransmitters of the neocortex. Inhibitory interneurons in the neocortex express the principal inhibitory neurotransmitter in the nervous system, gamma-amino-butyric acid (GABA), are generally have less dendritic spines than excitatory neurons, and can be categorized based on their short range projecting axonal morphologies (Ascoli et al., 2008). Excitatory neurons express the principal excitatory neurotransmitter in the nervous system, the amino acid glutamate (Meldrum, 2000), carry dendritic spines on their dendrites, and can be classified by both dendritic and varying axonal morphologies.
2.3. Cortical neuron development

Inhibitory cell types are born and migrate from different locations compared to excitatory cell types. Whereas excitatory neurons proliferate from the subventricular zone, settling into the developing cerebral cortex in an "inside-out" fashion (Angevine & Sidman, 1961) by way of radial glia (Rakic, 1972), inhibitory cells originate from the subcortical ganglionic eminence and migrate in a long tangential pathway to their target locations in the neocortex (Anderson et al., 1997; Corbin et al., 2001). Excitatory neurons are directed along radial glia by the extracellular glycoprotein Reelin (D’Arcangelo et al., 1995), which is secreted by layer 1 Cajal Retzius neurons. However, it is uncertain the degree to which inhibitory interneurons require Reelin signaling for correct targeting in the neocortex (Caronia-Brown and Grove, 2011; Pla et al., 2006). Glutamatergic excitatory neurons make up the majority of neurons in the barrel cortex (~90% of ~19,000 neurons in a barrel cortex cortical column), whereas Gamma-Amino-Butyric Acid (GABA) inhibitory interneurons make up the remaining 11-13%, or about 2,200 inhibitory interneurons in a single cortical column (Meyer et al., 2011). These estimates are based on total counts of inhibitory interneurons marked with the GABA synthesizing enzyme, glutamic acid decarboxylase 67 (GAD 67) and may vary slightly between animals (Meyer et al., 2011).

The diverse and dynamic complexity of brain processing and behavioral output emerge from the activity of the nearly 90 billion neurons in the brain with only about 16 billion of them residing in the cerebral cortex (Azevedo et al., 2009). This relatively small population of neurons in the cerebral cortex are responsible for the major elements of representing, computing, and integrating sensory codes to make decisions for behavioral output and consciousness. The two major classes of cortical neurons, therefore, represent fundamental units in the biological
2.4. Characteristics of excitatory neurons

Glutamatergic neurons exist in two main morphological phenotypes, pyramidal neurons and spiny stellate cells which mostly populate layer 4. ~80% of neurons in the neocortex are pyramidal neurons, which are characterized by a pyramid shaped soma, a thick dendrite protruding from the apex of the pyramid (Apical dendrite) typically oriented towards the pial surface, and multiple basilar dendrites protruding from the base of the pyramid. (Molyneaux et al., 2007). These neurons typically fire in a regular spiking fashion, i.e. adapting trains of action potentials following suprathreshold depolarization (McCormick et al., 1985)

Dendrites have passive and active properties that influence the integration of synaptic signals through temporal and spatial summation, the non-simultaneous events that add in time and the adding of simultaneous events that occur in different spatial locations, respectively (Magee, 2000). Excitatory neuron dendrites also have small (~1µm) actin dense membrane protrusions called dendritic spines, which are the post-synaptic sites for mostly excitatory synaptic inputs. Dendritic spine stability is correlated with memories (Yang et al., 2009) and their morphology may provide electrical filtering properties for afferent synaptic depolarizations (Yuste, 2013). While most pyramidal neurons have their apical dendrites facing towards the pia mater, a small (<8.5%; Mendizabal-Zubiaga et al., 2007), but distinct population of pyramidal neurons are inverted, with their apical dendrites facing towards the white matter. These inverted pyramidal neurons are intracortical and callosal projecting with functions that are yet unknown (Mendizabal-Zubiaga et al., 2007). However, it has been recently shown that they have distinct physiological features compared to the more typical upright pyramidal neurons (Steger et al.,
Pyramidal cell axons which are generally thinner and more uniform in width compared to dendrites, protrude from the base of the soma. Pyramidal neuron axons can project long distances (tens of mm in the rodent). For example, pyramidal neurons in layers 5 and 6 provide thalamocortical feedback to the VPM and POM nuclei of the thalamus (Bourassa, Pinault, & Deschenes, 1995). Pyramidal neurons also project long range intracortically. For example, some neurons in layers 2/3 and 5A of the barrel cortex can project to the primary motor cortex (Mao et al., 2011) as well as to the secondary somatosensory cortex with behaviorally relevant connections for object localization and texture discrimination, respectively (Chen et al., 2013). Other long range pyramidal projections of the barrel cortex include targets in the perirhinal cortex, striatum, thalamic reticular nucleus, zona incerta, anterior pretectal nucleus, superior colliculus, pons, red nucleus, spinal trigeminal brain stem nuclei and callosal projections to the contralateral hemisphere (reviewed in Aronoff et al., 2010).

Spiny stellate cells reside mostly in layer 4, have multipolar dendrites that radiate out from their soma for short distances (~200-300µm), and typically project locally for short distances (Staiger et al., 2004) making them a class of excitatory interneuron. Electrophysiologically, they are similar to pyramidal and star pyramidal neurons and fire action potentials in adapting trains in response to depolarizing current pulses (Regular spiking; RS) (McCormick et al., 1985; Staiger et al., 2004). Although the lemniscal pathway provides strong innervation to layer 4 of the barrel cortex, spiny stellate neurons in the rodent only receive approximately 10-20% of their dendritic spine inputs from the thalamus (Benshalom & White, 1986; Staiger et al., 2004). In the cat visual cortex, only ~6% of synapses onto spiny stellate neurons arrive from the thalamus, with ~73% coming from intracortical sources (Ahmed et al., 2013).
However, the relatively sparse anatomical inputs onto spiny stellate cells from the somatosensory thalamus belies their importance in sensory processing. VPM inputs onto spiny stellate cells drive the barrel cortex through convergent and synchronous inputs (Bruno & Sakmann, 2006), have high feedforward connectivity with other spiny stellate cells (Feldmeyer et al., 1999) in the same cortical column (Lübke et al., 2003) to provide feedforward excitation to layer 2/3 in the lemniscal pathway (Feldmeyer et al., 2002).

Excitation without a "balance" of inhibition is insufficient to carry out the normal computations of the functioning brain (Isaacson & Scanziani, 2011). In fact, experimentally increasing excitation or decreasing inhibition can result in epileptiform or comatose states (Dudek & Sutula, 2007). Thus, a relatively small, but important population of inhibitory neurons in the brain play a critical role in sculpting the signals of the majority excitatory population.

2.5. Characteristics of inhibitory interneurons

Inhibition in the nervous system was first reported in 1863 by Ivan Setschenov who found that stimulation of the midbrain could inhibit spinal reflexes (reviewed in Fishell & Rudy, 2011). GABAergic projecting neurons are the principal inhibitory projection cells in systems such as the striatum and cerebellum. However, fewer inhibitory cells in the cerebral cortex project long distances (Tamamaki & Tomioka, 2010). I will therefore refer to inhibitory cells of the neocortex as inhibitory interneurons (locally projecting inhibitory cells). Additionally, although, inhibition can be induced by other neurotransmitter systems binding to G-proteins coupled receptors (Lee & Sherman, 2009) or G-protein coupled inward rectifying potassium channels (Padgett & Slesinger, 2010), ionotropic inhibition through GABA-A receptors (Sigel & Steinmann, 2012) is the primary fast mechanism for synaptic neuronal inhibition within the
Inhibitory interneurons play a number of important roles in the brain. For example, they are capable of generating gamma oscillations (Cardin et al., 2009) which are correlated with human attentive states (Herrmann et al., 2010). The relatively late maturation of inhibitory interneuron's also regulate developmental periods of enhanced plasticity, called critical periods (Hensch & Fagiolini, 2004; Iwai et al., 2003) and play a role in spatial memory (Murray et al., 2011). Inhibitory interneurons also regulate feed forward inhibition in local circuits to create dynamic windows for synaptic integration (Chittajallu et al., 2013; Sun et al., 2006). GABA antagonists iontopheretically introduced into the barrel cortex results in disinhibits surrounding barrels to principal whisker stimulation, indicating that inhibition normally serves to sharpen receptive fields (Foeller et al., 2005). Specific inhibitory networks are coupled through gap junctions (Beierlein et al., 2000; Gibson et al., 1999) and regulate excitatory neuronal populations (Beierlein et al., 2000; Royer et al., 2012). Interestingly, barrel cortex layer 4 inhibitory interneuron's receive stronger thalamocortical input than neighboring excitatory spiny stellate cells, providing a reliable system for sharpening the temporal interval for synaptic integration of somatosensory information (Cruikshank et al., 2007). However, not all inhibitory interneurons are the same.

Inhibitory interneurons are comprised of an incredibly diverse class of neurons in the cortex that have only recently begun to be systematically classified based on morphology, connectivity, and molecular markers (Ascoli et al., 2008; Kepecs & Fishell, 2014). Generally, the morphologies of inhibitory interneurons are aspiny or sparsely spiny with variable dendritic arborization patterns. The main morphological characteristic for inhibitory interneurons are their axonal arborization targets. Axo-somatic inhibitory interneurons are called basket cells, while
axo-axonic targeting interneurons are called chandelier cells. Although certain clear prototypes do exist, it can even be difficult for experts to classify inhibitory cells based purely on morphology (DeFelipe et al., 2013). The molecular expression of specific proteins has aided in the classification of interneurons. For example, basket cells and chandelier cells express the calcium binding protein parvalbumin (PV+) and collectively make up 40% of all inhibitory interneurons in the neocortex. PV+ basket neurons in layer 4 of the barrel cortex receive the greatest amount of thalamocortical drive (Cruikshank et al., 2007) and provide the most potent and direct source of feed forward surround inhibition to facilitate sensory integration in the barrel cortex (Brumberg et al., 1996; Bruno and Simons, 2002; Gabernet et al., 2005; Sun et al., 2006). PV+ chandelier cells on the other hand have been shown to make GABAergic excitatory axo-axonic synapses onto pyramidal neuron axon initial segments (AIS) (Szabádics et al., 2006).

In addition to morphology and molecular expression, different inhibitory interneurons generally have distinct electrophysiological properties. For example, PV+ interneurons respond with high frequency, non-adapting action potential trains during suprathreshold depolarizing current steps (Fast spiking; FS; McCormick et al., 1985). FS interneurons were later shown to express parvalbumin in the hippocampus (Kawaguchi et al., 1987) and their unique fast spiking properties were shown to be mediated by a fast repolarization due to the expression of the Kv3.1 voltage gated potassium channels (Erisir et al., 1999). In the somatosensory cortex, PV+ interneurons mostly reside in lower layer 4 (Meyer et al., 2011) and receive powerful thalamocortical input (Cruikshank et al., 2007). Interestingly, these PV+ feedforward inhibitory circuits are latent neonatally, but are recruited following the first post-natal week of life, coincident with the closing of the critical period for gross reorganization of the barrel field (Daw et al., 2007). Intracortical excitatory neurons in layer 4 of the barrel cortex make reliable
glutamatergic synapses onto FS interneurons that demonstrate facilitating excitatory post-synaptic potentials during high frequency synaptic transmission (Michael Beierlein, Gibson, & Connors, 2003) as well as electrical gap junction mediated synapses between other FS inhibitory interneurons (Gibson et al., 1999). Outside of the somatosensory cortex, the dysfunction of these inhibitory interneurons has been correlated with symptomology associated with schizophrenia and epilepsy (Curley & Lewis, 2012; Schwaller et al., 2004).

Axo-dendritic inhibitory interneuron phenotypes generally synapse onto different portions of dendrites or dendritic spines (Markram et al., 2004). Martinotti cells are a type of bitufted inhibitory interneuron that express the neuropeptide somatostatin (SST+) and target the apical dendrites of pyramidal neurons. SST+ interneurons mostly reside in layer 3, upper layer 5 (5A) and upper layer 6 (Meyer et al., 2011). They can target the distal dendrites of layer 5 pyramidal neurons in layers 2/3 to mediate disynaptic inhibition between neighboring pyramidal neurons whose cell bodies are hundreds of µm away in layer 5 (Silberberg & Markram, 2007). SST+ Martinotti cells have adapting responses to depolarizing current injections and unique “rebound spikes” following the offset of hyperpolarizing current pulses. Rebound spikes are mediated by T-type calcium currents which are deinactivated at membrane potentials hyperpolarized to the typical resting membrane potential (Suzuki & Rogawski, 1989). SST+ cells that express rebound spikes are called low threshold spiking (LTS) and are thought to mediate the neuronal network transition from tonic to phasic firing (Suzuki & Rogawski, 1989). Interestingly, these interneurons are hyperpolarized during whisker deflection, and depolarize during whisker contact, a sensory circuit that likely disinhibits the apical dendrites of layer 5 pyramidal neurons when feed forward processing is necessary (Gentet et al., 2012b). In layer 4 of the barrel cortex, LTS cells receive intracortical inputs from excitatory neurons that are
Generally unreliable (characterized by high failure rates during paired synaptic recordings), but make gap junction electrical synapses with other LTS interneurons as well as inhibitory synapses with mostly RS and FS interneurons (Beierlein et al., 2000).

Neurogliaform cells are multipolar interneurons that express have a characteristically late spiking response to depolarizing current pulses and reside in layers 1-3 in the somatosensory cortex (Fishell & Rudy, 2011). These cells can fall under a class of interneurons expressing the ionotropic serotonin receptor 5HT3aR (Lee et al., 2010). However, subtypes of neurons in the superficial layers also express a combination of the neuropeptide vasointestinal protein (VIP), reelin and the calcium binding protein calretinin (Fishell & Rudy, 2011). Interestingly, 5HT3aR, PV+ and SOM are three interneurons that cover nearly 100% of all inhibitory interneurons in the barrel cortex (Lee et al., 2010; Rudy et al., 2011). Electron microscopy and multiple patch clamp recordings from the somatosensory cortex have recently demonstrated that neurogliaform cells release GABA to inhibit their own axons, as well as the axons of other neurons expressing metabotropic GABA-B receptors (Olah et al., 2009). Recent work in the barrel cortex has also demonstrated a circuit specific role for neurogliaform cells in attenuating feed forward inhibition, typically mediated by PV+ interneurons (Chittajallu et al., 2013). Although the role of inhibitory interneurons in the functional circuitry of the barrel cortex is still under intense investigation, the large diversity of interneuron form and function likely adds to the computational complexity of the cerebral cortex and promotes its adaptations to environmental changes.

2.6. Experience dependent plasticity of the neocortex

The brain is a dynamic organ that is capable of adapting its circuitry to environmental
conditions. Primary sensory regions crystallize their excitatory and inhibitory circuits at discrete
time points during early development called critical periods of plasticity, in a sensory dependent
manner. Seminal work conducted by Hubel and Wiesel (1970) demonstrated that the normal
electrophysiological segregation of the two eye representations in the binocular zone of the
primary visual cortex became disturbed following monocular deprivation through eyelid suturing
for several days during the fourth post-natal week, an effect which could not be induced in the
mature brain. This delimited period of sensory dependent maturation called the critical period of
ocular dominance plasticity (ODP), was later confirmed in the rodent primary visual cortex and
extended for normal receptive field properties and visual acuity (Fagiolini et al., 1994).
Shrinkage of the ocular dominance columns of the deprived eye were visualized by transynaptic
labeling the binocular zone of the primary visual cortex using tritiated proline during the
previously defined critical period (Hubel, Wiesel and Levay, 1977). Early sensory deprivation
has also been shown to impact the morphology of neurons in the visual pathway (Wiesel &

At the level of the single cell, inhibitory interneurons appear to show particularly
dramatic, although not exclusive, alterations to their physiology, morphology and connectivity
following sensory deprivation generally resulting in altered morphologies and reduced
excitability (Chittajallu & Isaac, 2010; De Marco García et al., 2015; Jiao et al., 2006; Li et al.,
2009; Sun, 2009a). Interestingly, the critical period for ODP has recently been shown to be under
the control of the maturation of inhibitory signaling in the visual cortex (Fagiolini et al., 2004),
which can be induced earlier (Iwai et al., 2003) or later (Harauzov et al., 2010) by increasing or
decreasing inhibitory activity respectively.

Early ablation of a whisker follicle leads to large scale neuronal shrinkage of that
whiskers representation in the barrel cortex (Van der Loos & Woolsey, 1973b) that has a critical period between P0-P4 (Rebsam et al., 2005). As such, gross reorganization of post-synaptic cell bodies making up individual barrels have not been shown to be induced by follicle ablation at older ages (Although subtle alterations to neuronal morphology do occur). This anatomical result is confirmed using physiological recordings in layer 4 using more benign deprivation experiments. Plucking all whiskers but one, does not anatomically reorganizes the whisker representation, but results in an increased spread of whisker evoked responses in adjacent barrels. This effect decreases substantially by P4 in layer 4 of the barrel field (Fox et al., 1992).

Early sensory deprivation (P0-P30) through whisker clipping, an even less invasive procedure that does not typically result in barrel structure reorganization, affects pyramidal neurons in layer 6 by increasing their cell body size, basilar dendritic length, decreasing their apical dendritic length and decreasing their dendritic spine density (Chen et al., 2015; Chen et al., 2012). The effect on layer 6 neuron dendritic spines is restricted to the first post-natal month and is not impacted by adult deprivation (P100-P130), indicating a critical period for layer 6 neurons. Early sensory deprivation also prevents the gradual pruning of spines in layers 2/3 and 4 (Chen et al., 2015; Zuo et al., 2005), indicating that the normal down regulation of dendritic spine densities across development requires sensory input maturity (Orner et al., 2013). However, the effect on layer 4 dendritic spines, as well as layer 4 spiny stellate cell dendritic orientations is not restricted to the first post-natal month (Chen et al., 2015b; Tailby et al., 2005) which suggests that morphological rearrangement at the level of the synapse continues to occur into adulthood.

Following maturation, the brain becomes less susceptible to reorganization, but does not become fixed. Dramatic manipulations to sensory organs can induce large scale reorganization even into adulthood. For example, digit amputation results in reorganization of the deprived
fingers representations in the adult owl monkey (Merzenich et al., 1984). However, more benign manipulations also lead to reorganization. Following the aforementioned single whisker spared experiment, layer 2/3 of barrel cortex shows increased receptive fields beyond the critical period for layer 4 (Fox et al., 1992). Physiologically, long term adult whisker trimming induced sensory deprivation reduces the average whisker evoked responses of barrel cortex neurons (Popescu & Ebner, 2010) as well as alters their receptive fields properties, resulting in a less preferential response of neurons in a barrel to their normal principal/columnar whisker (Shoykhet et al., 2005). Interestingly, increased excitability due to reduced lateral inhibition between adjacent barrels can be immediately detected after plucking adjacent whiskers (Kelly et al., 1999). The alterations in receptive field sizes are likely due to rapid alterations in barrel cortex neuron response properties (Kelly et al., 1999) due to rapid changes in inhibition (Jiao et al., 2006; Land & Akhtar, 1987; Li et al., 2009; Sun, 2009b) which is followed by reductions in the molecular markers for inhibitory interneurons (Jiao et al., 2006; Land & Akhtar, 1987) and structural reorganization in excitatory cells that reduces their dendritic orientation specificity (Tailby et al., 2005).

Once cortical circuits have organized, sensory deprivation also reduces the density of myelin (Trim from P30-P60; Barrera et al., 2013) (which may also play a role in regulating plasticity through myelin derived proteins such as Nogo; McGee et al., 2005), and thalamocortical axons within their target barrels (Trim for 2-3 weeks from P90; Oberlaender et al., 2012) as well as upregulates dendritic spine densities in layer 4 (Trim from P100-P130; Chen et al., 2015), perhaps reflecting a homeostatic upregulation of post-synaptic cells in a presynaptically scarce environment. However, certain types of PNNs are not altered following adult (P30-P60) whisker trimming (McRae et al., 2007). Thus, although the timing and magnitude of
the required sensory alteration is greater, certain components of the adult brain retain the ability to adapt and reorganize.

Overall, while dramatic sensory deprivation can reorganize the entire representation of sensory organs in the cortex during the critical period, alterations to dendrites, axons and neuronal physiology still occur in the adult brain. From these experiments, we can predict that the cortical plasticity induced in most circumstances occurs in a stepwise fashion, with alterations to neuronal physiology occurring first, followed by morphological plasticity at the level of single neurons then circuits, and finally resulting in large scale representational reorganizations depending on the scale and timing of the change in normal sensory input. Together, these studies have both laid the groundwork for our understanding of how experience through sensory input can shape the brain while raising questions about the mechanisms by which that may occur. Recent evidence has demonstrated a role for components in the neuronal environment, such as PNNs, in regulating their maturation, function and plasticity.

3. Features of Perineuronal nets

3.1. PNN Composition

The dense circuitry and diverse identity of neurons in the barrel cortex, like many other cortical systems reflects the sophistication of the computational abilities that emerge from the brain. The external environment that these cellular ensembles are embedded in are composed of a complex architecture of ECM that may protect, compartmentalize, and regulate the physiology of specific cell types. PNNs are a soma and proximal neurite localized phenotype of ECM that have a diverse and heterogeneous molecular make-up. Therefore, in order to appreciate their role in regulating the diverse cell types that exist in the cortex, it is necessary to first understand what
PNNs are made of.

The major constituents of the PNN microstructure are hyaluronic acid (HA), chondroitin sulfate proteoglycans (CSPGs), phosphocan and tenascin-r (TN-R) that are linked together with several key link proteins (Carulli et al., 2010; Oohashi et al., 2002; Wang & Fawcett, 2012). HA is a large (10^3-10^4 kDa) and linear non-sulfated polymer of n-acetylglucosamine and glucoronic acid that is not linked to a protein through covalent bonds and is synthesized within the inner leaflet of the plasma membrane (Toole, 2000). Following application of the HA digesting enzyme Hyaluronidase (H-ase) the entire PNN structure is removed while other enzymes such as chondroitinase ABC (chABC) that do not principally target HA, leave many CSPG components preserved (Deepa et al., 2006; Köppe et al., 1997).

Brain CSPGs such as aggrecan bind to HA (Fryer et al., 1992; Figure 2). Recent work showed that heterologous expression of the HA synthesizing enzyme Hyaluronan synthase (HAS) in human embryonic kidney cells that do not normally express PNNs, can result in a diffuse form of ECM that is consolidated into PNNs with the presence of the CSPG Aggrecan and the link protein Crtl-1 (Kwok et al., 2010). Therefore, it seems likely that HA is linked to the membrane via HAS and serves as a backbone for the entire PNN structure while other components serve to aggregate the structure into its mature form.

CSPGs consist of a collection of different sulfated disaccharide repeats called glycosaminoglycans (GAG) that are highly electronegative and are polymerized into long chains and can be covalently attached to the central domain on a family of core proteins called lecticans. A commonly used marker is the lectin Wisteria Floribunda Agglutinin (WFA). Derived from the flowering wisteria plant, WFA labels n-acetylgalactosamine carbohydrates that are present in GAG chains on PNNs and has been used extensively to identify their presence in the central
The lectican family is characterized by their conserved globular N and C terminal domains and includes brevican, neurocan, versican, and aggrecan (Galtrey & Fawcett, 2007; Yamaguchi, 2000) which differ in core protein size and number of GAGs (Zimmermann & Dours-Zimmermann, 2008). Aggrecan, versican and neurocan, bind to HA through their G1 N-terminal domain, a proteoglycan interaction which is stabilized by link proteins (Nieduszynski et al., 1980).

Aggrecan was the first lectican to be identified using antibody labeling. Derived from immunizing mice with the gray matter of cat spinal cord, the Cat-301 antibody was shown to be present on the soma, proximal dendrites, and around axosomatic synapses under electron micrographs (Hockfield & McKay, 1983). Cat-301 as well as Cat-304, Cat-315, and Cat-316 were later shown to bind to aggrecan across a broad array of tissues (e.g. central nervous system, lung, aorta, trachea, tendon, and articular cartilage) (Fryer et al., 1992) and brain regions (Matthews et al., 2002) in a generally non-overlapping cell type specific manner that covers all the cortical layers in the sensory neocortex. The difference between the Cat series of antibody reflect the epitope locations that are being recognized, either on the core protein (Cat-301) or the carbohydrates themselves (Cat-315, Cat 316). In general specific epitopes from the Cat series label a minority of PNNs compared to other markers (Karetko-Sysa et al., 2014).

It is important to note that the highly electronegative lectican GAG chains have been shown to bind Ca$^{2+}$ (Vigetti et al., 2008) and have been proposed to regulate neuronal physiology through this cation buffering capability (Härtig et al., 1999). Suggestive evidence for modulation of physiology by the lecticans comes from anatomical studies showing colocalization of aggrecan, brevican, and neurocan along the axon initial segment and soma of neurons (Brückner
et al., 2006), the colocalization of versican and brevican at the nodes of ranvier (Bekku et al., 2009; Dours-Zimmermann et al., 2009; Horii-Hayashi et al., 2008) as well as physiological studies demonstrating alterations to intrinsic physiology following enzymatic digestion of PNNS (Dityatev et al., 2007).

Another component group of ubiquitous ECM molecules are the Tenascins (Tn) come in several alternatively spliced isoforms (R,C,X,W/N). Only Tn-R and Tn-C are present in the brain (Zimmermann & Dours-Zimmermann, 2008). Tn-R binds to lecticans in a calcium dependent manner and has been shown to interact with cell surface receptors to also modulate synaptic physiology and plasticity of neuronal circuits (Bukalo et al., 2001; Bukalo et al., 2007; Saghatelyan et al., 2000; Srinivasan et al., 1998; Figure 2).

Another mechanism by which PNN components may regulate the function of neurons is through access with the intracellular machinery that regulates cell function. PNN components are theoretically capable of interacting with intracellular cascades, for example, phosphocan interacts with Tn-R, neuronal cell adhesion molecules and lecticans (Maurel et al., 1994; Milev et al., 1998). Cotransfection studies and voltage clamp recordings have demonstrated that RPTPβ functionally interacts with the inactivation state of the voltage gated sodium channel α subunit (Ratcliffe et al., 2000).

PNN components also interact with neurons through their interactions with other extracellular components. For example, the astrocyte expressing RPTPβ has been shown to promote neurite outgrowth through its interactions with neuronal cell adhesion molecules (Sakurai et al., 1997), while the secreted isoform of RPTPβ (Phosphocan) inhibits neurite outgrowth, (Milev et al., 1994). Interestingly, phosphocan is also present in brain PNNs (Deepa et al., 2006; Haunso et al., 1999), suggesting a role of developed PNNs in regulating neuronal...
reorganization and plasticity.

Overall, PNN components have been shown to interact with intracellular mechanisms as well as extracellular proteins to regulate, early neuronal growth and physiology. However, the role of PNNs in regulating different neuronal phenotypes has yet to be explored.

3.2. Cellular source of PNN components

The diverse and heterogeneous presence of different molecular components likely reflects many roles in structural integrity and the regulation of normal cellular function (Bonnans et al., 2014b). However to date, a full census of PNN component expression has not been conducted in any one mature brain region, which complicates direct analysis of their impact on established neuronal circuits. However, specific molecular markers such as WFA, as well as antibodies for various core proteins and receptors, have been important in determining the general overlap of PNNs with different neuronal subtypes.

The cell type synthesis and secretion of PNN components are both neuronal (Lander, Zhang, & Hockfield, 1998) and glial in origin (Giamanco & Matthews, 2012; John et al., 2006; Probstmeier et al., 2000). Recent work has demonstrated that WFA detected PNNs, phosphocan, and HABP are still present in dissociated brain cell cultures following inhibition of glial cell maturation using cytosine β-D-arabinofuranoside (AraC) (Miyata et al., 2005). Using a similar methodology, Giamanco and Matthews (2012) showed that Aggrecan can still express without glial presence, whereas normal Brevican, Tn-R and hyaluronan expression required the presence of glial cells. Other studies have also demonstrated that using >95% pure astrocyte cultures, TnR is still expressed (Meiners & Powell, 1995). Brevican has also been shown to specifically colocalize with astrocytes (John et al., 2006). Alternatively, Tn-R is also produced in
oligodendrocyte isolated cell cultures, where it plays a role in facilitating their early developmental migration (Probstmeier et al., 2000).

The mature PNN is comprised of a complex and heterogeneous scaffolding that is uniquely positioned to modulate cellular function. Interestingly, the expression of their different components generally become upregulated peri- and post-natally (Milev, Maurel, et al., 1998; Zimmermann & Dours-Zimmermann, 2008). Together, these results show that different components of PNNs derive from different cellular phenotypes. However, PNNs are still capable of forming without all of their components. Studies have shown that incomplete PNNs, due to genetic deletion of specific link components, leaves the adult brain susceptible to juvenile levels of reorganization (Carulli et al., 2010). These findings suggest that either the impact of PNNs on neuronal circuit’s scales with their density, or that specific components may play different roles.

3.3. Regulation of early developmental patterning

PNN components appear to play distinct roles at different stages of early neuronal maturation. For example rat stem cells transfected with the lectican versican differentiate into cells with neuronal phenotypes, while transfection of co-cultured astrocytes with versican promotes neurite outgrowth (Wu et al., 2004).

Neuron and glial secreted components of PNNs play a role in patterning the developing nervous system. In cell culture systems, Tn-R and Phosphocan, the secreted splice variant of RPTPβ have been shown to inhibit neurite outgrowth (Meiners & Powell, 1995; Milev, Chiba, et al., 1998). However, once neurites have begun to develop, CSGPs affect their guidance and targeting. In the early visual system, CSGPs regulate the axonal guidance of ganglion cells in the retina (Brittis et al., 1992) and retinal ganglion axons at the optic chiasm which are perturbed
following chABC digestion (Chung et al., 2000). RNA interference of versican in the optic tectum also results in perturbed retino-tectal presynaptic terminals (Yamagata & Sanes, 2005).

Evidence also exists for the ECM regulating of the developing neocortex. Using a unique model of cortical development, embryonic thalamic neurons were plated on a developing forebrain slice which possesses areas that both promote (intermediate zone, subplate) and repel (cortical plate) extending thalamocortical axons. Interestingly, chABC digestion of the forebrain slice enhanced extension of thalamocortical axons into the cortical plate (Emerling & Lander, 1996). Together, these results demonstrate that PNNs produced by both neurons and glia, regulate early development by promoting neuronal stem cell differentiation and regulating neurite outgrowth.

3.4. Sensory dependent maturation

PNNs mature relatively late in post-natal development (Carulli et al., 2010; Nowicka et al., 2009; Pizzorusso et al., 2002). Several lines of evidence have recently demonstrated that normal levels of neuronal activity are required for PNN maturation. First, normal PNN maturation in dissociated cell cultures is abolished by tetrodotoxin blockade of voltage gated sodium channels (Dityatev et al., 2007). PNNs are also reduced following early and prolonged dark rearing in the visual cortex and following early whisker trimming in the barrel cortex (Lander, Kind, Maleski, & Hockfield, 1997; McRae et al., 2007; Nakamura et al., 2009; Nowicka et al., 2009) which has been shown to reduce neuronal population activity (Popescu & Ebner, 2011) and inhibitory interneuron intrinsic properties specifically (Jiao et al., 2006; Sun, 2009a). Monocular deprivation was also shown to induce a reduction of both Cat-315 and WFA in the deprived visual cortex, but only when the GAD inhibitor 3-mercaptopropionic acid
(MPA) was used to also reduce inhibitory neurotransmission (Harauzov et al., 2010).

Interestingly, monocular deprivation and subsequent environmental enrichment also reduced WFA detected PNN expression, coincident with reductions in GABA signaling (Sale et al., 2007). These results clearly demonstrate a reliance of PNN maturation on both activity and a potential reliance on GABAergic signaling.

### 4. Cell type specific modulation of function by PNNs

#### 4.1. PNNS expression by excitatory neurons

Evidence exists that PNNs may also regulate excitatory axon regeneration in the spinal cord where a body of evidence has demonstrated that enzymatic digestion of PNN components promote axon regeneration (Bradbury et al., 2002) and spinal cord reorganization and functional recovery following stroke (Soleman et al., 2012). Furthermore, mice with a genetic knockout for n-acetylgalactosaminyltransferase-1 (CSPG biosynthesis enzyme) recover from spinal cord injury better than wild type mice (Takeuchi et al., 2013). These results suggest a general role of PNN components in regulating reorganization of neuronal circuits.

Although, PNNs are relatively ubiquitous in the adult brain (Matthews et al., 2002; Zimmermann & Dours-Zimmermann, 2008), only diffuse type WFA detected PNNS have been shown to ensheath pyramidal shaped neurons (Wegner et al., 2003). Retrograde labeling of projection neurons using biotinylated dextran amine (BDA) in multiple regions of the brain including the primary somatosensory cortex, have also identified a very small percentage of pyramidal shaped neurons expressing PNNs (Alpár et al., 2006). However this study relied on morphological distinctions rather than molecular markers for identifying excitatory neurons such as CAMKII or Neurogranin (Singec et al., 2004; Tighilet et al., 1998) that may have facilitated a
more precise excitatory neuron identity. Furthermore, a small population of GABAergic projection neurons are present in the neocortex (Tamamaki & Tomioka, 2010), complicating the colocalization of PNNs with cell identity. Nevertheless, studies examining the synaptic impact of PNN alteration in excitatory circuits provide a clue to their general role in the brain.

4.2. PNN expression by inhibitory interneurons

Current evidence shows that PNNs predominantly ensheath PV+ inhibitory interneurons in the barrel cortex (McRae et al., 2007; Nowicka et al., 2009) although this association appears to decrease during late adulthood (Karetko-Sysa et al., 2014). The colocalization proportion in all layers of the barrel cortex approach ~70% (Nowicka et al., 2009). Others have reported strong colocalization patterns between PNNs and PV+ interneurons in the visual cortex (Cabungcal et al., 2013; Miyata et al., 2012; Ye & Miao, 2013) and basal forebrain (Morris & Henderson, 2000). Using the 5HT3aR-BAC GFP mouse, we have found that PNNs are not expressed by any 5HT3aR expressing inhibitory interneurons, thus precluding neurogliaform cells from expression of these molecules (Figure 3). Others have reported colocalization of WFA detected PNNs with calretinin, calbindin two markers that are also expressed by SOM+, which has also been shown to express a proportion of PNNs in the hippocampus (Karetko-Sysa et al., 2014; McRae et al., 2010). These SOM+ interneurons could potentially make up the 30-40% of non PV+ WFA expressing PNNs in the neocortex (Nowicka et al., 2009). In the brain stem, ~70% of PNNS ensheath GAD+ inhibitory interneurons (Foster et al., 2014).

4.3. Impact of PNNs on excitatory networks

Although the extent of PNN expression by excitatory neurons is not completely known,
recent studies have elucidated the role of PNNs in regulating excitatory circuits in the hippocampus. These studies quantify the impact of PNNs on electrophysiologically detected plasticity, such as long term potentiation (LTP) or long term depression (LTD) which is due to persistent strengthening or weakening in synaptic strength that is widely considered to be the synaptic basis for learning and memory. Long term alterations are due to both pre-synaptic and post-synaptic mechanisms (Yang & Calakos, 2013). Short term changes which can reflect dynamical information processing are measured using a pair of high frequency stimulations of presynaptic axons to measure paired pulse ratio, which can lead to paired pulse facilitation (PPF) or paired pulse depression (PPD) of the second evoked synaptic response. This form of plasticity which does not persistently alter synaptic strength is generally attributed to vesicle availability, calcium buffering, and release probability in the presynaptic terminal (Fioravante & Regehr, 2011b).

Using a homozygous TnR knockout mouse (TnR -/-), Bukalo and colleagues (2001) demonstrated that LTP was reduced while basal synaptic rise slopes were increased. chABC digestion of PNNs perturbed both LTP and LTD in CA1 of the hippocampus following stimulation of the theta burst stimulation of the schaffer collaterals, but did not alter basal synaptic transmission (Bukalo et al., 2001). Using a brain specific link protein knock out (Crtl1) transgenic mouse, neurons of the perirhinal cortex showed increased LTD and significantly reduced PPF, an affect which was mimicked exactly by chABC hydrolysis of PNNs (Romberg et al., 2013). Furthermore, enzymatic digestion of PNNs significantly reduces the isolated IPSC amplitudes on excitatory neurons in the primary visual cortex (Liu et al., 2013). Enzymatic digestion of PNNs has also been shown to induce increased spine motility and LTP in the visual cortex (de Vivo et al., 2013). Most recently, it has been demonstrated that Brevican is involved in
maintaining fast synaptic transmission at the calyx of Held synapse in the medial nucleus of the trapezoid body (Blosa et al. 2015), a brain stem structure involved in sound localization. These results suggest that while PNNs are typically not ensheathing excitatory neurons, excitatory synapses and synaptic plasticity can still be modulated by PNNs on inhibitory cells. Alternatively, direct modulation of inhibitory interneurons may still impact excitatory networks.

4.4. The role of PNNs on inhibitory interneurons

Interestingly, the relatively late maturation of PNN development corresponds with the maturation of inhibitory interneuron markers like parvalbumin (Nowicka et al., 2009). The maturation of PV+ interneurons depends on brain derived neurotrophic factor (BDNF) (Huang et al., 1999) and transcription factors such as the homeoprotein Otx2 (Sugiyama et al., 2008). Sensory deprivation through dark rearing significantly reduces the expression of both PV+, PNNs and Otx2, while cortical infusion of exogenous Otx2 rescues expression of all three markers, and initiates early onset of the critical period for ODP (Sugiyama et al., 2008). Enzymatic digestion of PNNs with chABC prevents the normal expression of Otx2 while a synthetic peptide targeting the chondroitin sulfate 6 (CS6 of the n-acetylgalactosamine portion of the GAG chain) binding site for Otx2 prevents its accumulation which prevents the aggregation of WFA detected PNNs, and inhibits PV+ cell maturation (Beurdeley et al., 2012).

The finding that Otx2 regulated PV+ maturation was confirmed through the cell autonomous upregulation of immature (CS6) sulfation patterns in PV+ cells using in utero electroporation, which served to reduce the expression of WFA detected PNNs as well as Otx2 expression (Miyata et al., 2012). In contrast to previous work, the reductions in PNNs and Otx2 did not reduce the population count of PV+ interneurons, suggesting compensatory mechanisms.
for PV+ cell maturation without Otx2 (Miyata et al., 2012). However, these authors did find that PV+ interneurons had immature intrinsic properties and prolonged excitatory discharge in their visually evoked responses (Miyata et al., 2012). Together these results highlight the importance of PNNs in promoting the maturation of PV+ interneurons. Consistent with these findings Balmer and colleagues (2009) used a zebra finch model to show that depriving juvenile birds from their normal song tutor experience during the critical period, resulted in decreased PNNs and inhibitory interneurons within the HVC sensorimotor nucleus. The remaining PNNs correlated with the complexity of the bird song (Balmer et al., 2009). These results suggest that PNNs are involved in stabilizing adult circuits following the critical period through their interactions with PV+ interneurons.

Recent evidence has shown that PNNs play a two-fold role in the fate of PV+ interneurons. Iron induced cell death (FeCl₃ intracortical injection) results in greater cell death for non-PNN associated cells as indicated by Fluoro-Jade B staining (Suttkus et al., 2012). Using a glucose and oxygen deprivation model for brain damage, a recent study found that exogenous chondroitin sulfate reduced the degree of various cell death markers associated with oxidative stress (Martín-de-Saavedra et al., 2011). Glutamate cysteine ligase is the rate limiting enzyme for the synthesis of glutathione, a major antioxidant and redox regulator. Using a transgenic model that is missing the modulatory subunit of the glutamate cysteine ligase (Gclm), Cabungcal and colleagues (2013) found increased oxidative stress which preferentially damaged PV+ interneurons without PNNs, but not those with PNNs. Importantly, enzymatic digestion of PNNs with chABC, along with experimentally induced redox dysregulation, resulted in a significant reduction in PV+ cell counts, whereas chABC alone did not impact PV+ cell counts (Cabungcal et al., 2013). Interestingly, a recent study has demonstrated that cell autonomous redox
dysregulation in PV+ interneurons specifically reduces both PV+ cells and PNNs, which corresponded with a cortical phenotype that lacks a closing point for critical period ODP in the primary visual cortex (Morishita et al., 2015). Using four PNN knock out lines (aggrecan +/−, brevican +/−, Tn-R +/− and Hapln +/−), it was also recently shown that aggrecan, Tn-R and Hapln were specifically important for protection of PV+ interneurons from iron induced oxidative stress (Suttkus et al., 2014). In the spinal cord, TnR enriched PNNs have also been shown to play an anti-adhesive role for activated microglia, perhaps serving a protective role for motorneurons during periods of trauma and inflammation (Angelov et al., 1998). Overall, the current literature demonstrates that PNNs play an important role in regulating the development and protection of inhibitory interneurons in the brain which is important for the regulation of cortical circuit plasticity.

PNNs may also directly regulate the fundamental intrinsic physiology of the neurons they ensheath. A recent study has demonstrated that chABC enzymatic digestion of hippocampal dissociated cell cultures results in decreased action potential after-hyperpolarizations and decreased action potential initiation thresholds of morphologically identified interneurons, suggesting that PNNs play a role in regulating the excitability of mature cells that they ensheath (Dityatev et al., 2007). There is also growing evidence that the mechanisms by which PNNs modulate inhibitory interneuron function is through physical interactions with the cellular components that govern their physiology.

4.5. Molecular interaction of PNNs with neurons and their effect on physiology

In addition facilitating growth factor uptake and protecting from oxiditative stress, PNNs also interact directly with neurons. Although the functional outcome of this interaction have yet
to be shown in neurons, the mature sulfate phenotype of CSPGs (CS4) has been shown to
directly bind Ca\(^{2+}\) (Vigetti et al., 2008) demonstrating the buffering capability of PNNs, which
was proposed as a potential mechanism for regulation (Härtig et al., 1999). Interestingly, PNN
lecticans also appear to require divalent cations to bind to glycolipids on the cell surface of the
membrane (Miura et al., 1999), potentially providing a cation dependent compartmentalization
barrier to mobile proteins in the cell membrane.

The restriction of mobile receptors in the cell membrane was demonstrated by
Frischknecht and colleagues (2009) and provides a potential mechanism for regulating synaptic
physiology. In that study, dissociated hippocampal cell cultures with quantum dot single particle
tracking of the GluR1 subunit of the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
(AMPA) receptor was used along with fluorescence recovery after photobleaching. Using these
techniques, they showed that enzymatic digestion of PNN components using the enzyme
hyaluronidase (which cleaves hyaluronic acid backbones on PNNs), resulted in increased AMPA
receptor trafficking from extra-synaptic to synaptic locations and resulted in decreased PPD
(Frischknecht et al., 2009). This study demonstrated that ECM molecules, presumably isolated
around neurons as PNNs, can create compartmentalized microenvironments along the neuronal
membrane to regulate their basic synaptic physiology.

It has also been demonstrated that specific epitopes of CSPG components can regulate
the activity of neurotransmitter receptors and ion channels. The PNN Component, Tn-R interacts
with neurotransmitter receptors through the human natural killer site (HNK-1) epitope which
impacts the strength of GABA-A mediated inhibitory post synaptic conductances (IPSCs) as well
as GABA-B mediated G-protein coupled inward rectifying potassium channels (GIRK)
(Saghatelyan et al., 2000; Saghatelyan, 2003). Together these results demonstrate that PNNs
both link to the cell membrane to regulate neurotransmitter receptor mobility, and to the receptors themselves in a subtype specific manner, putting them in important positions to regulate synaptic communication. These authors later demonstrated that GABA-A agonists such as muscimol and a GABA-B antagonist rescued LTP (Bukalo et al., 2007).

The consequence of regulating synaptic communication is the modulation of plasticity at the synapse. For example, enzymatic digestion of PNNs as well as a trangenic knockout of a PNN link protein (Crtl1), results in decreased PPD and increased LTD in the perirhinal cortex, a functional outcome correlated with increased object recognition (Romberg et al., 2013). In contrast, LTP was decreased by chABC digestion of PNNs in the CA1 region of the hippocampus (Bukalo et al., 2001) which is likely due to a PNN deficiency resulting in threshold increase for LTP (Bukalo et al., 2007). These reductions in LTP in Tn-R−/− were also rescued by phosphotase blockers (Bukalo et al., 2007), suggesting that intracellular mechanisms through phosphatase interactions (e.g. RPTPβ) may be involved in the PNN regulation of LTP.

A direct interaction between PNNs and L-type Ca2+ channels were reported by Kochlamazashvili and colleagues (2010), who demonstrated that the disruption of LTP following H-ase was rescued by exogenous hyaluronic acid and an L-type calcium channel potentiator. Interestingly, these authors had previously proposed a mechanism in Tn-R−/− mice by which GABA-A signaling is reduced, GABA-B is upregulated, which results in intracellular cascades within excitatory neurons that elevate the threshold for LTP (Bukalo et al., 2007). Overall, these results point to a cascade of events which span the extracellular space to the intracellular domains that can change the balance between excitation and inhibition.

Although much work has been done on the role of PNN components and synaptic physiology, less is known about how their impact on intrinsic physiology. Although there is some
evidence that PNNs do interact with neurons to regulate intrinsic physiology. In cell culture, it has been demonstrated that the PNN component Tn-R can bind with the β subunit of voltage gated Na⁺ channels, potentiating their activity (Srinivasan et al., 1998; Xiao et al., 1999). In addition, the aforementioned work of Kochlamazashvili and colleagues demonstrated a potential interaction with L-Type Ca²⁺ channels (Kochlamazashvili et al., 2010). PNNs have also been shown directly bind to Ca²⁺ (Vigetti et al., 2008) which functionally regulates Ca²⁺ diffusion (Hrabetová et al., 2009). These results suggest that PNNS may interact with ion carrying channels and Ca²⁺ ions themselves to affect the membrane potential and availability of free Ca²⁺. These interactions, which appear to depend on the fundamental the biochemistry of the structures, may provide a conserved role for PNNs to regulate the excitability of neurons in the brain.
Chapter Two: Aims

PNNs are specialized ECM aggregates around specific types of neurons in the brain. They may play roles in regulating plasticity (Pizzorusso et al., 2002), as well as promoting the development (Beurdeley et al., 2012) and protecting PV+ FS inhibitory interneurons from oxidative stress (Cabungcal et al., 2013). It has been previously demonstrated that the maturation of PNNs is activity (Dityatev et al., 2010) and sensory dependent (Lander et al., 1997; McRae et al., 2007), similar to the normal maturation of inhibitory interneurons (Jiao et al., 2006; Morales, Choi, & Kirkwood, 2002; Sun, 2009a).

However, it is unknown whether the maturation of PNNs in all the cortical layers require sensory input, and whether the experience dependent regulation of plasticity by PNNs occurs through their impact on the intrinsic or synaptic physiology of cortical neurons. It is also unknown whether those effects would be restricted to inhibitory interneurons in the barrel cortex, where there is a relatively high packing density of neurons with and without PNNs. Therefore, in order to reveal the mechanisms for plasticity regulation in the brain, it is important to characterize the role of PNNs in regulating the functional characteristics of neocortical neurons.

Aim 1: Determine the laminar dependence of PNNs on sensory input

In order to understand the mechanisms of cortical plasticity, I will first characterize the experience dependent maturation of PNNs in the different cortical layers of the barrel cortex. Mice will be sensory deprived through bilateral whisker trimming from P0-P30. A subset of animals will have their whisker regrown for 22 days until P52 to evaluate the impact of sensory restoration on PNN levels. Coronal sections including the barrel cortex
cut at 300µm will be used to evaluate the effect of sensory on PNN expression in the barrel cortex. Sections will be imaged using light microscopy, captured using a camera attachment, and quantified offline using Neurolucida explorer (MBF Bioscience Inc.).

**Aim 2: Determine the role of PNN components in regulating intrinsic neuronal physiology.**

Enzymatic digestion of the PNN followed by *in vitro* whole cell recordings of will allow for the elucidation of the function of different PNN components. The enzyme chABC digests the PNN GAGs which exist on the side chains. These components are highly electronegative. The effect of chABC on neuronal intrinsic properties (e.g. membrane potential, action potential amplitude) will be compared to that of control slices using whole cell patch clamp techniques.

**Aim 2: Determine the role of PNNs in regulating the synaptic properties of neurons and interneurons in the neocortex.**

Previous reports have demonstrated that PNNs can modulate synaptic transmission in the hippocampus, it is not clear if this is a generalizable finding. To expand on this literature, *in vitro* patch clamp recordings from different neuronal phenotypes will be conducted from layers 2/3, 4 and 5 of the barrel cortex. Spontaneous synaptic inputs onto each cell type will first be evaluated, followed by an evaluation of thalamocortical synaptic inputs onto layer 4 neurons in the barrel cortex. Current clamp will be used to record and then analyze excitatory post-synaptic potential amplitude, rise slope, decay slope and frequency of spontaneous inputs. For thalamocortical inputs, amplitude and paired pulse depression will be evaluated. All data will be analyzed offline using Clampfit (Axon Instruments).
Chapter 3: Introduction (1):

Determining the laminar dependence of PNNs on sensory input in the barrel cortex

The barrel cortex has distinct critical periods for PNN maturation (McRae et al., 2007) which likely serve to crystallize neuronal circuits (Carulli et al., 2010; Gogolla et al., 2009; Miyata et al., 2012; Pizzorusso et al., 2002) and protect FS cells from oxidative stress (Cabungcal et al., 2013; Morishita et al., 2015a).

Although much work has been done in the visual cortex, it is less understand how the experience dependent maturation of PNNs in the barrel cortex may be reflected in its laminar distribution. PNNs are distributed across the layers of the barrel cortex heterogeneously, with the greatest densities in layer 4, followed by layer 5, and finally layers 2/3 and 6. Their distribution roughly follows the proportions of FS PV+ interneurons that are distributed throughout the cortical layers (Nowicka et al., 2009). Previous research has shown that major reorganization of the post-synaptic organization of barrel field somas are limited to alterations in the four postnatal days (Rebsam et al., 2005; Van der Loos & Woolsey, 1973a) while plasticity in the layers 4 to layer 2/3 synapse, induced by trimming all whiskers but one, reaches a critical period during the second post-natal week of life (Lendvai et al., 2000). Although morphological plasticity at the subcellular level (dendrites, dendritic spines, axons) appears to be generally preserved in adulthood (Chen et al., 2015, 2012; Tailby et al., 2005). These results suggest that a sensory dependent and layer specific effect of PNN maturation may differentially impact early development. Because sensory deprivation in the juvenile animal, but not adult reduces PNN expression (McRae et al., 2007) as well as results in altered neuronal physiology (Jiao et al., 2011; Kelly et al., 1999;
Popescu & Ebner, 2011; Shoykhet et al., 2005; Sun, 2009b), we sought to determine that relationship by first characterizing the layer specific sensory dependency of PNNs in the barrel cortex.
Chapter 4: Materials and Methods (1)

Determining the laminar dependence of PNNs on sensory input in the barrel cortex

4.1. Animals

Anatomical and physiological experiments were conducted with white laboratory adolescent Swiss mice of either sex (CD-1, postnatal day 28-52; (see table 1 for the number of animals in each experimental group; Charles Rivers Laboratory, Wilmington, MA) in accordance with the Institutional Animal Care and Use Committee of Queens College, CUNY (Protocol #100) and NIH guidelines for responsible use of animals in research.

4.2. Sensory Deprivation

Two groups of mice (see table 1 for sample sizes) had their whiskers bilaterally trimmed every other day for 30 days starting from postnatal day (P)0, the day of birth. Whiskers were trimmed with microspring scissors to a length of < 1 mm. Animals older than P15 were sedated using isofluorane (Hospira Inc.) to prevent struggling during the trimming procedure. The trimmed group (Trimmed) was sacrificed at P32 and acute coronal slices were prepared as described below. The sensory deprivation regrow (Trim-Regrow) group had their whiskers trimmed for their first 30 days of life and then their whiskers’ were allowed to regrow for an additional 22 days, when they were then sacrificed and processed in the same way as the trimmed group. A control group (non-trimmed control) of mice did not have their whiskers trimmed, but were handled regularly from P15 until P32.
4.3. Acute slice preparation

Juvenile CD-1 mice were placed under deep anesthesia via an intraperitoneal injection of Euthasol (Virbac ANADA, Catalog# 200-071) at 0.5-1 mL/gram until unresponsive to a toe pinch. Animals between P35 and P40 were used. An incision was then made to reveal the calvaria, followed by quick decapitation. The brain was subsequently removed and placed in chilled (~2º C) and oxygenated (95% O₂, 5% CO₂) ACSF for ~1 min with the following composition in mM: NaCl 125, 2.5 KCl, 0.5 MgCl₂, 2 CaCl₂, 25 NaHCO₃, 1.25 NaH₂PO₄, 25 D-Glucose. Following incubation in sACSF the brain was blocked along the coronal plane to include the primary somatosensory cortex (S1) using the anatomical markers of the anterior commissure and anterior regions of the hippocampus to signal the start and end of the barrel cortex. The tissue was then superglued (cyanoacrylate) to a cold stage, submerged in chilled and aerated sACSF, and sectioned using a vibratome (Leica VT 1000S) in the coronal plane. Slices were cut at 300-350 μm and included the posterior medial barrel subfield using anatomical references (George Paxinos, 2012). Slices were removed using a paint brush and maintained at room temperature (~25º C) in standard ACSF solution. Slices from trimmed and control animals were placed for 1 hour in either a custom slice incubation chamber (see below) or a standard incubation chamber (Edwards and Konnerth, 1992) after which they were placed in chilled 4% paraformaldehyde in 0.01 M phosphate buffered saline (PBS). Following a 3-5 day fixation at 4°C, slices were histochemically labeled for PNNs (see below).

4.4. Histochemistry

All sections were stained for PNN using Wisteria Floribunda Agglutinin (WFA), a standard lectin that labels the N-acetylglycosamine side-chain of chondroitin sulfate
proteoglycans. A subset of the slices that were recorded from were labeled with FITC-WFA (Vector Laboratories) to confirm the presence and absence of PNNs in control tissue and following chABC digestion. For histological analyses free floating sections were rinsed in 0.1M PBS (3 x 10 min). Rinses were done in between all the subsequent steps unless indicated. Sections were quenched for endogenous peroxidase activity (2% H₂O₂, 1% MEOH in 0.1M PBS; 1 hour), blocked for 30 min using 1% BSA (Fisher Biotech, Catalog # 9048-46-8), and incubated in biotinylated Wisteria Floribunda Agglutinin (WFA; 1:500; Sigma Aldrich, Catalog #B-1355) for 2 hours. For visualization purposes a standard ABC kit reaction was conducted (Vector Laboratories, Catalog# PK-400) for 1 hour preceded and followed by 0.1M PBS with 0.05% Tween (Sigma Aldrich, Catalog #P1379) rinses. Finally, 3,3’-diaminobenzidine (Sigma Aldrich, Catalog #D4293) reaction was used as the chromogen (~10 min, 1 3.5 mg tablet / 5 ml to produce 0.7 mg/ml) with H₂O₂ to catalyze the reaction. Sections were washed and then immediately mounted on gelatin coated slides, dried overnight and either counterstained with Nissl stain (Thionin), or immediately dehydrated in graded alchohol baths, followed by defatting in xylene substitute followed by coverslipping using Permount mounting medium (Fisher Scientific, Catalog #SP15-100).

4.5. PNN analysis

Slices were viewed using an Olympus (BX-51) microscope with a high resolution digital camera (Optronics Microfire) connected to a dedicated PC. Images were taken in 4x (Numerical aperture (NA=0.10) and 10x (NA=0.25) magnifications and analyzed on a standard Windows desktop computer using Neurolucida and Neuroexplorer (MBF Biosciences Inc.). Nissl stains conducted on WFA-labeled sections were used to define the pial surface and layer VI white
matter border and distinguish between individual cortical laminae (Figure 4).

PNN were identified based on their circular hollow centers, or lattice-like mesh around cell bodies, with distinctive short radial processes. Only PNNs that included staining around processes were counted. PNN density was calculated as PNNs counted/area (mm$^2$) within a defined lamina contour. Relative densities were calculated by taking the ratio of chABC hemisphere density and control hemisphere density. A 2-factor mixed ANOVA was used to analyze the layer vs. experimental condition, followed by pair-wise posthoc Tukey analyses. All comparisons used an $\alpha$ level of 0.05 to indicate statistical significance using Statistica (Statsoft Inc). All data are reported using standard errors of the mean (SEM).
Chapter 5: Results (1):

Determining the laminar dependence of PNNs on sensory input in the barrel cortex

5.1. Laminar distribution of PNNs in the barrel cortex

Under control conditions, labeling of PNN chondroitin sulfated proteoglycan side chains demonstrated that all the cortical lamina have significantly different PNN densities from each other (Figure 4 B,C,E). Layer 4, the primary thalamic input layer in the barrel cortex, consistently showed the highest density of PNN aggregates (181.1±9.3/mm²) followed by layer 5 (143.5±12.4/mm²), then layers 2/3 (120.6±10.0/mm²), with layer 6 showing the lowest density (93.9±9.5/mm²).

5.2. Effect of bilateral whisker trimming on PNN distribution in the barrel cortex

Following 30 days of bilateral sensory deprivation, layer 4 continued to have the greatest expression of PNNs (131.87±4.31/mm²), layer 6 remained at the lowest density (70.65±4.02/mm²). However, layers 2/3 and 5 were no longer significantly different from one another (102.11±2.3/mm²; 95.35±3.55 respectively; See Figure 5B,D). This reflected a deprivation-induced change in layers 4 (27.2% reduction, p=0.0008) and 5 (33.5% reduction, p=0.001), and no change in layers 2/3 (15.3% reduction, p=0.71) or 6 (24.8% reduction; p=0.37). Across all layers PNNs were significantly reduced following sensory deprivation (average 28.4% reduction; p=0.008; See Figure 5B,D). Regrow animals showed the same pattern of PNN density as the Trimmed group across the cortical layers. However, layer 2/3 (80.4±4.2/mm²), layer 4 (125.2±6.5/mm²), layer 5 (91.8±5.2 /mm²), and layer 6 (74.5±5.1/mm²; See Figure 5C,D) showed significantly lower levels of PNN expression following whisker regrowth compared to
non-trimmed controls, that were not different from the Trimmed condition. Thus, there was no recovery of PNN levels in the deprived barrel cortex even after a period of sensory restoration roughly equivalent to the period of sensory deprivation. This is similar to our previous results (McRae et al., 2007) and supporting the hypothesis that the critical period for PNN development in the barrel cortex lies within the first postnatal month.
Chapter 6: Discussion (1):

Determining the laminar dependence of PNNs on sensory input in the barrel cortex

6.1. PNNs are reduced following sensory deprivation and do not recover following sensory restoration

Prolonged bilateral sensory deprivation reduced the density of PNNs in the barrel cortex. Our current findings (27% PNN reduction in layer 4) correspond closely with our previous work, which showed that labeling of the aggrecan phenotype of PNNs is reduced by 22% in layer 4 of the barrel cortex following 30 days of unilateral sensory deprivation from birth (McRae et al., 2007). Interestingly, unilateral deprivation in our previous work did not show reductions in WFA detected PNNs. However, others have reported that checkerboard deprivation and whisker plucking deprivation do impact the expression of WFA detected PNNs (Nakamura et al., 2009; Nowicka et al., 2009). Our current bilateral deprivation paradigm likely resulted in an overall greater sensory impact than unilateral trimming, a result we have also seen when evaluating dendritic spine density and morphology (Chen et al., 2015). Bilateral deprivation results in overall reductions in the in vivo responsiveness of layer 2/3 and 4 RS and FS type cells, whereas unilateral deprivation can lead to upregulation of sensory evoked spiking in layers 2/3 and in the layer 4 septum in response to controlled whisker deflections (Popescu and Ebner, 2010). Since it has previously been shown that the expression of PNNs are activity dependent (Dityatev et al., 2007). The greater impact of bilateral deprivation on activity levels, may explain the reduction of WFA detected PNNs, which were not reduced with unilateral deprivation under the same trimming schedule (McRae et al., 2007). Our current findings add to a body of evidence that shows that the quantity and pattern of sensory deprivation is important for the molecular
expression of PNNs in the barrel cortex. Together, our results suggest that a gradient of activity levels define the expression of WFA detected PNNs in the barrel cortex.

Our results also point to a mechanism by which the different functional layers of the barrel cortex regulate plasticity. Due to their inhibitory features on early development, we can predict that reductions in PNNs enhance dendritic and spine motility. Previous work from our lab has shown that interneurons in layers 6 show increased dendritic arborization following sensory deprivation, whereas pyramidal neurons show increased basilar dendritic spines in layer 4 and a decrease in layer 6 (Chen et al., 2015, 2012). If PNNs do in fact inhibit neurite outgrowth and reorganization, then we would expect less PNNs in regions that are more dynamic following sensory deprivation. Consistent with this hypothesis, we find that layer 4 shows a significant reduction in PNNs following deprivation.

Dendritic spines are usually dynamic during development, and become stable later in adulthood (Yang et al., 2009). The significant reduction of PNNs that we detect in layer 2/3 may reflect an environment that is no longer supportive of receptive field tuning and sensory dependent pruning of dendritic spines (Lendvai et al., 2000; Zuo et al., 2005) due to a degradation of the underlying inhibitory interneuron signaling capabilities. In the current study, layer 6 does not show significant reductions of PNNs following prolonged whisker trimming. However, it is possible that their relatively low basal level of expression (~50% of layer 4 in control) requires minimal sensory evoked activity to develop and may reflect a different role for PNNs in that lamina, one that is not necessarily involved in regulating experience dependent plasticity. However, little is known about the specific function of the PV+ and PNN sparse layer 6.
Functional implications of loss of PNN recovery following sensory restoration

In the adult mouse brain aggrecan mRNA levels fall across development which is mirrored by an increase in protein expression (Carulli et al., 2010). This suggests that a relatively large amount of aggrecan mRNA transcription occurs in early postnatal life, followed by a delayed and prolonged period of increased mRNA translation of aggrecan protein. Sensory deprivation from birth during the critical period reduces aggrecan mRNA even further by one postnatal month and prevents protein levels from reaching adult levels. Interestingly, sensory restoration did not recover adult levels of aggrecan protein (McRae et al., 2007) nor adult levels of WFA expressed PNNs (current work). Therefore, neonatal whisker trimming may lead to long term or permanent changes to PNN levels in the barrel cortex, enabling an extended post-critical period enhancement of plasticity such as seen in the visual cortex following chABC digestion of PNNs and following sensory deprivation (Iwai et al., 2003; Pizzorusso et al., 2002).
Chapter 7: Introduction (2):

The role of PNNs in regulating the intrinsic properties of neurons in the barrel cortex.

Although the work of Dityatev and colleagues (2007) first demonstrated in the hippocampus that PNN digestion impacts the intrinsic physiology of interneurons, but not pyramidal neurons, nothing is known about how PNNs regulate the intrinsic physiology of the diverse cell types that exist in the somatosensory barrel cortex. To this end, I first sought to characterize the experience dependent maturation of PNNs in a laminar dependent manner. In parallel, I used sensory deprivation to determine whether the mechanism for the experience dependent maturation of intrinsic properties were related to PNNs. I then sought to experimentally perturb the expression of PNNs using the chABC enzyme, then recorded from multiple putative excitatory and inhibitory cell types in the somatosensory barrel cortex.

Due to the demonstrated Ca\(^{2+}\) buffering nature of PNNs and their proposed interactions with calcium channels (Hrabetová et al., 2009; Kochlamazashvili et al., 2010; Vigetti et al., 2008), we expected that SST+ interneurons with L-type Ca\(^{2+}\) channels could show alterations in their rebound spikes. We further expected that FS interneurons, the cell-type expressing a large majority of PNNs, would show discrete alterations to their action potential dynamics due to the demonstrated interactions of PNNs with β subunit of the voltage gated Na\(^{2+}\) channel (Srinivasan et al., 1998; Xiao et al., 1999). We hypothesized that these two interneuron subclasses would also show changes to their synaptic inputs, due to previous findings in the hippocampus that demonstrates alterations to synaptic physiology. Shedding light on the experience dependent maturation of PNNs and their regulation of neuron in the barrel cortex is important for
understanding how the brain is able to normally maintain stability and yet undergo plastic changes under changing environmental conditions.
Chapter 8: Materials and Methods (2):

The role of PNNs in regulating the intrinsic properties of neurons in the barrel cortex.

8.1. Animals

Anatomical and physiological experiments were conducted with white laboratory adolescent Swiss mice of either sex (CD-1, postnatal day 28-45; see Table 1 for the numbers of animals and cells in each experimental group, Charles Rivers Laboratory, Wilmington, MA) in accordance with the Institutional Animal Care and Use Committee of Queens College, CUNY and NIH guidelines for responsible use of animals in research.

8.2. Sensory Deprivation

Two groups of mice (see Table 1 for sample sizes) had their whiskers bilaterally trimmed every other day for 30 days starting from post natal day (P)0, the day of birth. Whiskers were trimmed with microspring scissors to a length of < 1 mm. Animals older than P15 were sedated using isofluorane (Hospira Inc.) to prevent struggling during the trimming procedure. The trimmed group (Trimmed) had their whisker clipped until P30. Trimmed animals used for electrophysiological experiments were sacrificed between P32-P36 (n=3). A third control group (non-trimmed control) of mice did not have their whiskers trimmed, but were handled regularly and were also anesthetized from P15 until P30.

8.3. Single slice incubation chamber fabrication

We developed a single slice incubation chamber to maintain low volumes of solvent (artificial cerebral spinal fluid, ACSF) to accommodate proportionally smaller volumes of
reagent (chABC) to reduce the cost per experiment. The single slice incubation chamber was produced using the bulbous tips (Figure 6A) of polyethylene disposable transfer pipettes (Biologix Research Corp., Catalog # 30-0138), transected 3 ml polypropylene pipetter tips (United Laboratory Plastics, Catalog # UP 4082-JS), and nylon mesh nets (10 μm grid size; Figure 6A). The bulbous ends of three transfer pipettes were transected using a razor blade to create three chambers (Figure 6B). The slice chamber (top) was 1 mm in length, the middle (liquid chamber) and bottom (air chamber) were both ~3.5 mm in length. A pipetter tip was transected (2.5 mm from the cylindrical end; 12 mm diameter) so that it fit snugly into the top chamber, with room for a mesh net (~1.9 mm²) to be wedged in place between the two chambers. The top chamber was then perforated with a 26.5 gauge syringe needle to create a 3x3 grid on the curved end (Figure 6B, piece 3). For the middle chamber, the rounded end of the cap was sliced to produce a ~400 μm diameter circular hole. An additional hole was cut for the bottom chamber (500-600 μm diameter). The hole in the bottom chamber was fit with a female luer (for 1/16” ID tubing; World Precision Instruments, Catalog# 13156) and reinforced using parafilm to make it relatively air tight (Figure 6C,D). The female luer was then fitted with silicon tubing (Tygon tubing, Catalog # 3350, 1/32” inner diameter x 3/32” inner diameter) which was then adapted to a multi air valve controller (World Precision Instruments, Catalog# 14059). Each disposable transfer pipette chamber fit snugly into the other to form three sequential chambers stacked on top of each other (Figure 6E-H).

8.4. Acute slice preparation

Juvenile CD-1 mice were placed under deep anesthesia via an intraperitoneal injection of Euthasol (Virbac ANADA, Catalog# 200-071) at 0.5-1 mL/gram until unresponsive to a toe.
pinch. Animals between P35 and P40 were transcardially perfused with ice-cold sucrose artificial cerebral spinal fluid (sACSF – see below) using a 10 ml syringe to improve slice viability for our recording experiments. An incision was then made to reveal the calvaria, followed by quick decapitation. The brain was subsequently removed and placed in chilled (~2\(^\circ\) C) and oxygenated (95% O\(_2\), 5% CO\(_2\)) sACSF for ~1 min with the following composition in mM: 250 Sucrose, 2.5 KCl, 3.0 MgCl\(_2\), 25 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 25 D-Glucose.

Following incubation in sACSF the brain was blocked along the coronal plane to include the primary somatosensory cortex (S1) using the anatomical markers of the anterior commissure and anterior regions of the hippocampus to signal the start and end of the barrel cortex. The tissue was then superglued (cyanoacrylate) to a cold stage, submerged in chilled and aerated sACSF, and sectioned using a vibratome (Leica VT 1000S) in the coronal plane. Slices were cut at 350-400 \(\mu\)m and included the posterior medial barrel subfield (PMBSF) using anatomical references (George Paxinos, 2012). Slices were removed using a paint brush and maintained at room temperature (~25\(^\circ\) C) within the single slice incubation chamber, or under standard conditions in ACSF solution of the following composition; in mM: NaCl 125, 2.5 KCl, 0.5 MgCl\(_2\), 2 CaCl\(_2\), 25 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 25 D-Glucose. We used a low Mg\(^{2+}\) solution in order to enhance spontaneous synaptic activity in our recordings. In experimental conditions, 10 \(\mu\)l of chABC (Seikagaku, Sigma) was dissolved in 990 \(\mu\)l (1:100 vol./vol.) of ACSF to result in a final concentration of 0.2918 units/ml and added to the slice chamber for preincubation. To match our recording and enzymatic digestion experimental controls, slices from trimmed and control animals were placed for 1 hour in either the slice incubation chamber or a standard incubation chamber (Edwards and Konnerth, 1992) after which they were placed in chilled 4% paraformaldehyde in 0.01 M phosphate buffered saline (PBS). Following a 3-5 day fixation at
4°C, slices were histochemically labeled for PNNs. Electrophysiological recordings from trimmed animals were conducted using ACSF of the following composition in mM: NaCl 124, KCl 2.5, NaH$_2$PO$_4$ 1.2, NaHCO$_3$ 24, HEPES 5, Dextrose 12.5, MgSO$_4$ 2, CaCl$_2$ 2 and compared to controls using the same solution.

The gas flow rate in the middle chamber was adjusted so that there was a steady ~50-60 bubbles/minute. The ~30-40 bubbles/minute liquid chamber flow rate resulted in no bubbles forming in the slice chamber, where the sections were maintained. The gas flow rate was adjusted to maximize air flow in the air and liquid chamber and to provide sufficient air pressure to prevent any water from the liquid chamber from leaking into the air chamber. This flow rate was also needed to allow air to flow through the air and liquid chambers while preventing any bubble formation in the slice chamber that would agitate the tissue. Slices were placed into the slice chamber and incubated in preparation for histology or whole cell patch clamp recordings described below. Slice viability in the incubation chamber was equivalent to those under standard incubating procedures as described below.

### 8.5. Graded enzymatic digestion of PNN

Histochemistry was performed in a subset of sections to determine a baseline for PNN expression in posterior medial barrel subfield regions in the coronal plane as well as expression after a specified time in chABC. The sections were separated down the midline, with one of two hemispheres randomly assigned to either “control” incubation (ACSF only; n=4), or digestion using chABC (1:100 vol./vol. in aerated ACSF; n=6), an enzyme that cleaves glycosaminoglycan side chains in chondroitin sulfate proteoglycans (Yamagata et al., 1968) as well as the hyaluronan backbone (Galtrey et al., 2007). Sliced hemispheres were incubated in the enzyme in one of four
temporal intervals (30, 60, 90, or 120 min) then removed and immediately fixed in cold 4% paraformaldehyde for 3-5 days, followed by histochemical staining. Hemisphere controls were maintained in normal aerated ACSF for the same length of time as their digestion counterparts and subsequently fixed and stained using the same procedures for comparison purposes.

8.6. Histochemistry

PNN labeling was conducted as previously described (see materials and methods chapter 4.4).

8.7. PNN analysis

PNN analysis was conducted as previously described (see materials and methods chapter 4.5).

8.8. Electrophysiology

Sections were produced as described above and incubated for the one hour in either normal aerated ACSF or aerated chABC ACSF (1:100) solutions in the single slice incubation chamber. Following either condition, slices were transferred into a submersion chamber for whole cell patch clamp. Borosilicate pipettes were pulled (Sutter P-87) to produce electrodes of 4-8 MΩ tip resistance. Glass electrodes were filled with intracellular solution of the following composition in mM: K-Gluconate 120, KCl 20, NaCl 10, Hepes 10, EGTA 0.5, Mg-ATP 2, Na-GTP 0.3 (290-310 mOsm). Slices were visualized using a Bx51WI microscope with infrared differential interference contrast optics (IR-DIC) with camera attachment (Retiga Ex). Although cells were recorded “blind” with regard to their expression of PNNs, the relative proportions of
PNN expression on different cell types has been well documented. GABAergic interneurons (Foster et al., 2014), particularly GABAergic PV+ interneurons express a large percentage of WFA detected PNNs, (Nowicka et al., 2009), with a substantially smaller proportion being expressed by excitatory pyramidal neurons (Wegner et al., 2003). Although the relative proportions of PNN expression on excitatory and inhibitory interneurons may change across development (Karetko-Sysa et al., 2014), the age range we chose likely contained PNNs predominantly expressed by inhibitory interneurons, which display fast spiking and low threshold spiking properties (Markram et al., 2004).

Cells were recorded using PClamp (Version 10, Molecular Devices) under current clamp methods as previously described at room temperature (Ramos et al., 2008). Signals were amplified with a Multiclamp 700A (Molecular Devices), digitized with a Digidata 1440A, low pass filtered at 10 kHz and sampled at 20 kHz (Molecular Devices). After achieving a stable membrane potential (Vm < -55 mV, ability to generate action potentials (APs) in response to a depolarizing pulse), the cells’ intrinsic properties were examined. The resting membrane potential was assessed by recording without depolarizing pulses. The cells’ intrinsic properties were assessed by injecting current from -250pA to +250pA in incrementing steps of 50pA each lasting 1 second with an interstimulus interval of 1000 ms. Following data collection, all traces were analyzed offline using Clampfit (Molecular Devices). The maximum input resistance (R_{in}) was calculated by taking the slope of the voltage current relationship in current steps that elicited no APs. Resting membrane potentials (RMP) were collected during 10-20 ms prior to onset of each current step. Latencies before spike onset and amplitude change before spike onsets following a current step were measured manually for all steps eliciting spikes. Spike threshold, amplitudes and afterhyperpolarization (AHP) were measured from baseline by manually setting
markers at threshold and lowest AHP for both first and last spike for each current intensity. Only stimulation currents between depolarizing steps of 150-250 pA were included in the final analysis for these parameters due to the fact that a subset of cells did not fire to +50 pA and +100 pA of stimulation. Rebound spike latency, amplitude and slopes were measured by analyzing the change between the moment of a hyperpolarizing current step offset and the threshold for the initiation of the rebound spike. All other parameters of rebound APs were measured in the same way as described above. Spiking probability was defined as the proportion of cells firing their first AP at each stimulation current between each group.

AP half widths, rise slopes, decay slopes, rise times, and decay times were analyzed using the threshold detect feature in Clampfit by setting the baseline marker at threshold for each first and last spike in response to depolarizing current pulses and setting a detection marker at 0.1 mV above the baseline marker. Analysis of interspike intervals (ISI) and AP firing rates were done using threshold detection by setting the baseline marker at RMP and setting a detection marker at the approximate half width of the spike train amplitude. For spontaneous synaptic potentials, gap free traces (5-15 min) were filtered at 1000 Hz (Gaussian), manually baseline adjusted, and excitatory post synaptic potentials (EPSPs) were isolated using event detection. Event detected EPSPs were then analyzed as isolated units in Clampfit. Both unitary and compound EPSPs were included in the analysis. Events with amplitudes below 0.75 mV were excluded from the analysis due to the observations of noise in some of the recordings. For measurements of the EPSP kinetic properties, isolated EPSPs were manually sorted so that five unitary EPSPs for each amplitude bin (0.75-1 mV, 1-2 mV, 2-3 mV, 3-4 mV, >4 mV) were analyzed using threshold detection. Unitary EPSPs were selected based on temporal order from the onset of the gap free recording so that the events were added until reaching a sample of five. Thus, the analysis of half width, rise
slope and decay slope contained 25 EPSPs for each cell. Independent t-tests were used to evaluate effects between groups (control and chABC digested). An α level of 0.05 was used to determine statistical significance. All data are reported using SEM.

8.9. Cell type identification

We recorded from two electrophysiologically identified cell types following sensory deprivation. Fast spiking cells (FS) were classified by their short AP half widths at half amplitude (<1 ms) and minimal spike frequency adaptation. Regular spiking- single spiking cells (RS-S) exhibited marked spike frequency adaptation and relatively long AP half widths at half amplitude (>1ms; McCormick et al., 1985). For our PNN digestion experiments, we expanded our analysis to three additional putative excitatory and inhibitory cells, classified based on electrophysiological properties in response to families of depolarizing and hyperpolarizing current pulses. Regular spiking-doublet cells (RS-D) were those we classified as having similar spike frequency adaptation and spike half widths as regular spiking-single cells, but consistently discharged a pair of closely spaced APs at the onset of their spike train (Staiger et al. 2014). Burst firing neurons fired a burst of three or more spikes at the onset of a spike train (Bursting). RS-S, RS-D and bursting neurons are putatively excitatory and release glutamate in the cortical circuit. Low threshold spiking neurons (LTS) fired rebound bursts of one or more APs following the offset of an injected hyperpolarizing pulse. FS neurons are well known to be PV+ GABAergic interneurons of basket or chandelier morphology while LTS neurons have been previously identified as GABAergic SOM+ inhibitory interneurons of Martinotti type morphology in the neocortex (Rudy et al., 2011).
Chapter 9: Results (2):

The role of PNNs in regulating the intrinsic properties of neurons in the barrel cortex.

9.1. Alterations in intrinsic physiology following sensory deprivation

Sensory reduces the whisker evoked responsiveness of the barrel cortex (Popescu and Ebner, 2010), while PNN maturation depends on activity (Dityatev et al., 2007; McRae et al., 2007). Alterations in neuronal activity could potentially result in a reduction in PNNs or alterations in PNN expression may impact intrinsic physiology. To approach this second possibility, we sought to determine whether alterations in intrinsic physiology resembles that which occurs following perturbation of PNNs alone. Enzymatic digestion of PNNs in vitro also allowed us to target multiple cell types from the barrel cortex, and characterize the alterations to intrinsic physiology among these different neuronal phenotypes.

Following one month of sensory deprivation, we recorded from FS interneurons (Control n=9, Trim n=7) and RS neurons (Control n=10, Trim n=9) in acute slices from mouse barrel cortex. Relative to control, FS-trim interneurons showed an average reduction in spike frequency, which was significant when spike frequencies were collapsed across all depolarizing stimulation currents (FS control: 56 Hz, FS trim: 32 Hz; p=0.045; Figure 7D). We did not detect this effect in RS neurons (RS control: 6.7 Hz, RS trim: 8.6 Hz; p=0.5; Figure 7C). The reduction in spike frequency was caused by alterations in spiking probability. The average stimulation needed for FS firing increased from +125 pA to +178.6 pA (p=0.02; Figure 7E) following sensory deprivation. RS neurons showed a trend in the opposite direction, with increased firing probabilities following sensory deprivation. For RS neurons, the average stimulation current decreased from +190 pA in controls to +155.5 pA in the Trim group (p=0.26; Figure 7F)
suggested slightly increased excitability due to decreased inhibition.

Interestingly, we also detected increases in spike amplitude in FS interneurons following sensory deprivation (first spike at +250 pA; FS control: 54.5 mV; FS trim: 63.9 mV; p=0.005; Figure 8G) which did not occur for RS neurons (first spike at +250 pA; RS control: 83.8 mV; FS trim: 80.5 mV; p=0.57; Figure 8H). RS neurons showed slight but non-significant increases in $R_{in}$ (RS control: 120.3 MΩ; RS trim: 148.2 MΩ; p=0.06; Figure 8G) while FS interneurons, similar to previous reports using unilateral sensory deprivation (Sun et al. 2009), showed average reductions in $R_{in}$, which however, did not reach significance in our bilateral deprivation assay ($R_{in}$; FS control: 176.8 MΩ; FS trim: 148.3 MΩ; p=0.13; Figure 8G), which did not correlate with firing probability (Pearsons r; Control: p=0.11, Trim: p=0.34). Overall, we saw that on the average, FS interneurons became less excitable following sensory deprivation, but had greater spike amplitudes whereas RS neurons were slightly more excitable and showed no changes to their spike amplitudes.

9.2. Graded digestion of PNN in the mouse somatosensory cortex

Since sensory deprivation alters many physiological processes in neurons (Barrera et al., 2013; Chen et al., 2012; Fox et al., 1992), we sought to isolate the independent effect of PNN reduction on the intrinsic physiology of cortical neurons by conducting enzymatic digestion on acute slices derived from a separate group of animals. Consistent with other reports, two hours of chABC incubation eliminated all perineuronal nets from acute sections (Figure 9H,I; Bukalo et al., 2001). It appeared that following 90 min of digestion, some PNNs existed in a cell surface condensed or internalized form (Dityatev et al., 2007), suggesting a possible compensatory
upregulation of PNN component synthesis following our acute slice preparation. Under control conditions PNN expression at 30 min was lower than that at 60 minutes, 90 minutes, and 120 min (30 min vs. 60 min, p=0.01; 30 min vs. 90 min, p=0.03; 30 min vs. 120 min, p=0.01). PNN density values of control slices between 60 min, 90 min and 120 min were not different, which is consistent with previously published reports that conducted transcardial perfusion with fixative prior to sectioning and staining of slices for PNNs (Nowicka et al., 2009). This suggests that the acute sectioning procedure of live slices disrupted the normal PNN levels at the surface of the slice, but is compensated for during the slice recovery phase prior to patch clamping. Unfortunately, we were unable to unequivocally determine whether internalization or upregulation of PNNs occurred in our chABC digested or control sections due to the high expression of PNN labeling that often covered the surface of the cell bodies.

Following 30 min of chABC digestion, the average PNN density decreased slowly (21% reduction; p=0.059; Figure 9B,I), then rapidly between 30 and 60 min of digestion (69% reduction; p=0.01; Figure 9D,I). PNN reduction plateaued by 90 min (65% reduction; p=0.009; Figure 9F,I) and PNNs were undetectable following 120 min of digestion (>99% reduction; Figure 9H,I).

9.3. Slice viability

Acute slices from adolescent mice (P28-P40) were sectioned and incubated in the slice incubation chamber for 1 hour before visual inspection under IR-DIC microscopy. Adolescent rather than juvenile mice were specifically chosen for recordings to match ages of our sensory deprived and enzymatic digestion conditions. Further, PNN regulation of neuronal physiology likely increases across development as their expression does not approach maturity until P30.
The incubation chamber was able to maintain slice integrity and viability during prerecording incubation with volumes of 1 mL for at least 2 hours (the longest duration tested). The slices contained many cells with smooth shiny surfaces, a hallmark of healthy cells. These cells had stable and hyperpolarized membrane potentials (-55 to -80 mV) and consistently fired AP’s in response to depolarizing current steps. Importantly, the single slice incubation chamber produced slices of comparable health to slices held under standard conditions (Edwards and Konnerth, 1992) and showed no electrophysiological differences to their control counterparts in standard incubation (small volume control: n=7 RS cells, data not shown). Additionally, all cellular phenotypes were found in relatively normal proportions based on our physiological recordings. Due to the lack of differences between the two chambers, all control cells were grouped together.

9.4. **Intrinsic properties of cortical neurons after chABC digestion**

We recorded from slices following 60 min of chABC digestion (69% reduction) to evaluate the effect of PNN perturbation on the intrinsic properties of neurons in the mouse neocortex. We chose 60 min due to our findings that the effect on overall PNN density at this time period more closely approximated the effects of bilateral sensory deprivation than later time points and did not result in the appearance of intracellular PNNs which occurred at 90 min of chABC digestion. Further, we wanted to maximize the viability of the slice, which was optimal at 1 hour post sectioning. After patching onto cells, we gave families of depolarizing and hyperpolarizing current pulses to identify five different cellular phenotypes (FS control: n=7, FS chABC n=8; RS-S control n=18, RS-S chABC n= 33; RS-D control n= 6, RS-D chABC n= 13;
LTS control n=8, LTS chABC n=7; Bursting control n=4, Bursting chABC n=6)

Following chABC digestion, slices were still viable and in some cases cells were relatively easier to patch compared to control slices.

**9.5. Regular spiking-single, Regular spiking-doublet cells and bursting cells**

At our lowest depolarizing current step +50 pA, 11/25 (44%) of RS-S control cells fired whereas only 4/33 (12%) of RS-S chABC cells fired (Figure 10E). At the same stimulation current, 4/6 (67%) RS-D control cells fired, whereas only 5/13 (38%) of RS-D chABC cells fired (Figure 10F). There was also no difference in bursting cell firing at 50 pA of stimulation (Burst control firing: 4/5, 80%; Burst chABC firing: 5/6; 83%, Figure 10G). Although RS-S cells (Control: n=18; chABC n=33) did not show changes to overall spike amplitude (Table 2), there were significant increases to the spike decay slope for both first and last spikes at 150pA compared to RS-S control (+150 pA First spike: p= 0.2, Last spike: p=0.014, Figure 11A) with similar results for larger magnitude stimulation pulses. This change in decay slope did not significantly impact RS half widths (+150pA Control vs. chABC digested; First spike: p=0.09; Last spike:p=0.06). A minimal decrease in decay slope was present for RS-D cells (Control n=6; chABC n=12) and only significant for the first spikes at +200 pA of stimulation (Figure 11B, p=0.02) while not present for bursting cells, nor FS and LTS cells (Table 2). Other than a minor, non-significant change in AP decay slope at +200 pA, no significant changes were detected for bursting cells in any of the measured parameters for bursting cells (Control n= 4; chABC n=6; Table 2) following enzymatic digestion of PNNs with chABc.

Thus, RS-S and RS-D, putative excitatory neurons showed slightly decreased spiking firing probability and AP decay slopes, but were otherwise not greatly impacted by PNN
digestion.

9.6. Fast spiking neurons

If the loss of alterations to intrinsic physiology following sensory deprivation were due to a paucity of PNNs, then chABC digestion could potentially mimic those effects. In contrast to the significant reduction in spike probability seen after whisker trimming, chABC FS cells only showed a trend towards reduced firing probability compared to FS controls (FS Control firing: 7/7, 100% FS chABC firing: 4/8, 50%; Figure 12D). chABC FS cells also showed significantly lower input resistances than FS controls (n=8; FS control: 242±26 MΩ; FS chABC: 146±16 MΩ; p=0.009; Figure 13AB). The higher current thresholds needed for AP generation seen after chABC digestion are likely a result of the changes to input resistances as the average input resistances for chABC are correlated with the minimum current needed to elicit firing (Pearsons r=0.78, p=0.024; Figure 13B), which was not the case for FS control (r=0.48, p=0.34).

We measured RMP 10-20 ms prior to the onset of the depolarizing current pulses, thus we were able to detect whether positive stimulation altered the RMP following ~1 sec of baseline after each pulse. We found that FS chABC interneurons had a more depolarized RMP compared to FS control (Figure 13D). This effect was magnified with each sequential depolarizing step which resulted in gradual hyperpolarizing RMP for FS control interneurons (within group main effect: p=0.03), but did not occur for FS chABC interneurons. Overall, the average RMP across +150, +200 and +250 pA for FS chABC interneurons was significantly more depolarized than the average RMP for FS chABC (FS chABC: -59.2±1.4mV; FS control: -64.6±1.6 mV; independent t-test p=0.03; Figure 13E). The more depolarized average RMP of the FS chABC cells also negatively predicted the stimulation current necessary to elicit spiking activity
(Pearsons r= -0.86, p=0.029, Figure 13E). The low variability of the stimulation current threshold for FS controls precluded a correlation analysis on those cells. The effects on RMP in particular appear to be an independent effect of chABC, as this was not seen following sensory deprivation.

Unexpectedly, ChABC digestion of the PNN resulted in decreased AP amplitude compared to FS controls when measured from threshold (+150 pA First spike: p=0.002, Last spike: p= 0.015; Figure 14A,B,C). The spike amplitude reduction occurred consistently for the first spike in the train and to a lesser degree for the final spike (Figure 14A,B). However, this effect was significant for all but the stimulation current of +250 pA for the last spike. On average, AP amplitude reduction ranged from 12-19% (Figure 14C). Interestingly, following PNN digestion neither the APs of LTS cells, the other putative inhibitory cell type (Figure 14D) nor any of the putative excitatory cell types were impacted (see Table 2 for quantitative breakdown in response to a +150 pA, 1 second duration step pulse). Additionally, Pearson’s correlations did not find significant relationships between input resistances, or RMP and AP amplitude for control or chABC treated FS interneurons (data not shown). Overall, the effect of PNN digestion on FS interneurons significant reduced spike amplitude, input resistance, and increased RMP were generally distinct from those that occurred following sensory deprivation.

9.7. Low threshold spiking cells

LTS cells showed reduced spiking probability following chABC during +50 pA of stimulation (LTS Control firing: 8/8, 100%; LTS chABC firing: 7/8, 87.5%; Figure 12E), but no other prominent alterations in their depolarization induced properties. The main feature for our classification of LTS cells was the rebound spiking following the offset of a hyperpolarizing current step. These rebound spikes are driven primarily by T-type Ca\(^{2+}\) currents which are
transient and activate at low thresholds following their deinactivation at hyperpolarized membrane potentials (Zhan et al., 2014). Previous reports have implicated chondroitin sulfate components of PNNs in the extracellular buffering of Ca$^{2+}$ ions (Vigetti et al., 2008), thus we analyzed the rebound spike properties of LTS interneurons. The offset of hyperpolarizing current steps (-250 to -150 pA) typically elicited rebound APs (Figure 12B) and so were measured in our sample of control (n=8) and chABC treated (n=7) neurons. The latency and voltage change between the offset of the hyperpolarizing step and onset of a rebound spike can be used to estimate the Ca$^{2+}$ contribution to the rebound spike.

In our sample, all LTS cells fired rebound spikes at the offset of -250 and -200 pA in both control and chABC. However, at -150 pA only a subset of chABC LTS cells (3/6= 50%) fired rebound spikes whereas all of the LTS control cells fired rebound spikes (8/8=100%). At -100 pA, 88% of LTS controls fired rebound spikes, whereas only 17% of LTS chABC cells fired rebound spikes (data not shown). Additionally, the slopes of the T-type Ca$^{2+}$ currents were significantly decreased following chABC (p’s<0.05; Figure 15A,B) without changes to the time course nor maximum amplitude of I_T (Figure 15C,D). LTS chABC cells also showed reduced rebound spike rise slopes (-250 pA: p=0.04, -200 pA: 0.04, -150 pA: p=0.2) which may be a consequence in alterations to I_T slope. Although the effects on putative I_T that we observe are subtle, these results suggest that PNN component regulation of Ca$^{2+}$ conductance's seen previously in xenopus laevis photoreceptors may also present in the mammalian neocortex (Vigetti et al., 2008). In sum, the impacts of PNN digestion were most pronounced in GABAergic cells which correlates with the known expression pattern of PNNs in the barrel cortex.
Chapter 10: Discussion (2):

The role of PNNs in regulating the intrinsic properties of neurons in the barrel cortex.

10.1. Sensory deprivation and intrinsic physiology

Previous reports have demonstrated that sensory deprivation shifts critical period plasticity (Morales et al., 2002) while affecting the physiology of FS interneurons by reducing their input resistance, decreasing excitability, and increased frequency adaptation (Sun, 2009b) resulting in reduced inhibitory drive onto excitatory cells (Jiao et al., 2006). Similar to what has previously been reported, we found significant alterations in the intrinsic physiology of FS interneurons in the barrel cortex following prolonged sensory deprivation. Reduced spike frequency and reduced spiking probability both contribute to reduced FS excitability, similar to that which occurs following chABC digestion of PNNs.

Unexpectedly, and in contrast to the reduced spike amplitudes that we recorded in FS interneurons following chABC digestion, prolonged sensory deprivation slightly increased the spike amplitude of FS interneurons. Similar to our findings, auditory deprivation increases the action potential amplitude of neurons in the bird nucleus mangocellularis by increasing sodium currents (Kuba et al., 2010). However, that study also found increased excitability, which did not occur in our experiments (Kuba et al., 2010). Overall, our findings demonstrating reductions in spike frequency resemble those of Sun et al (2009). This reduction in FS inhibitory activity likely results in decreased FS feedforward excitation in the sensory circuit and increased receptive fields (Fox et al., 2003) which is found following sensory deprivation (Shoykhet et al., 2005).
10.2. PNNs and intrinsic physiology

Following chABC digestion of PNNs, we observed additional changes in intrinsic physiology that have not been previously reported and were not observed in our sensory deprivation assay. Subtle alterations in FS, LTS, RS-S, and RS-D neuron active properties, as well as reductions in firing probability were selectively altered following PNN digestion, but not after sensory deprivation. It is currently unclear what mediates the relatively non-specific change in spiking probability and relatively specific changes in active properties across the four aforementioned cell types that we observed following chABC digestion. Although chABC digestion alone has not been shown to increase markers of oxidatitive stress nor decrease PV+ interneuron expression (Cabungcal et al., 2013) we cannot rule out that increased oxidation-reduction reactions following PNN perturbation may alter the intrinsic physiology of neurons without significantly affecting cell viability. Further, recent work has shown that redox dysregulation in PV+ interneurons, but not excitatory neurons can impact cortical plasticity (Morishita et al., 2015a).

However, since the complete taxonomy of PNN subtypes and diffuse components are not well defined our results may be influenced by a separate population of PNN that has yet to be explored. Some PNNs apparently ensheathe glutamate expressing neurons (Wegner et al., 2003), and long range projection neurons (Alpár et al., 2006) and recent reports have shown colocalization of PNNs with Somatostatin, a putative marker for another class of inhibitory interneurons (McRae et al., 2010). Since we detect subtle alterations to putative excitatory RS-S neurons but not RS-D nor bursting, these neurons may be a distinct class of PNN expressing excitatory neurons.

The alterations in LTS rebound spiking are suggestive of a modulatory role of PNNs in
regulating Ca\(^{2+}\) flux. The 4 and 6 sulfated forms' of chondroitin sulfate proteoglycans are present in PNNs and are the developmentally mature and juvenile dominant forms respectively (Miyata et al., 2012). The 4 sulfated version has a high affinity for Ca\(^{2+}\) binding (Vigetti et al., 2008). Regulation of synaptic physiology by PNNs has already been shown to occur through L-type Ca\(^{2+}\) channels (Kochlamazashvili et al., 2010) and our results suggest that there may also be an interaction with the T-type Ca\(^{2+}\) currents. In the context of barrel cortex function, a reduction in T-type Ca2+ slope could reduce the repolarization and subsequent tonic firing of SOM+ inhibitory interneurons during the state switch from whisker deflections to quiet wakefulness (Gentet et al., 2012b). I would therefore hypothesize that PNNs in the barrel cortex that impact LTS interneurons are involved in regulating synaptic integration of incoming inputs from other cortical regions, such as the motor cortex (Kinnischtzke et al., 2014).

It is unclear how PNNs may regulate the AP amplitudes of FS cells. However, it has been reported that components of PNNs link to the β-subunit of voltage gated Na\(^{+}\) channels (Srinivasan et al., 1998). Both membrane bound receptor tyrosine phosphatases (RPTPβ) and its catalytically inactive secreted form (phosphacan) which forms PNNs, interact with voltage gated Na\(^{+}\) channels. It has been previously demonstrated that phosphorylation of Na\(^{+}\) channels decreases peak Na\(^{+}\) currents (Hilborn et al., 1998) whereas dephosphorylation through RPTP-β increases peak Na\(^{+}\) currents (Ratcliffe et al., 2000). Thus, chABC digestion of phosphocan (Deepa et al., 2006) may result in decreased intracellular phosphatase activity which would in turn reduce peak Na\(^{+}\) influx during the AP, resulting in smaller amplitude APs as was observed rather than the increased AP amplitudes found after sensory deprivation. Additionally, the disruption of PNNs associated with the β subunit of Na+ channels may also result in reductions of peak voltage gated Na\(^{+}\) channels, resulting in reduced action potential amplitude (Xiao et al.,
The overall consequence of reduced action potential amplitudes would be a reduced, or less reliable synaptic release of GABA from FS interneurons. This effect would be consistent with a reduced inhibitory tone which characterizes early developmental periods before the onset of the critical period of plasticity (Fagiolini & Hensch, 2000; Fagiolini et al., 2004; Iwai et al., 2003) and could reflect a more permissive environment for synaptic rearrangement.

We also find selective reductions in FS interneuron input resistance in response to chABC digestion of PNNs. Some components of PNNs, such as extracellular matrix glycoprotein tenascin-R (TN-R), interact with GABA-B receptors to affect K\textsuperscript{+} conductances. Antibody competition for the TN-R binding site increases K\textsuperscript{+} conductance in hippocampal neurons (Saghatelyan et al., 2003). Tenascin receptor knock out mice also show similar deficits in hippocampal long term potentiation (LTP) to slices treated with chABC (Bukalo et al., 2001), which suggest that chABC may also impact FS input resistance through chABC mediated dysregulation of TN-R and K\textsuperscript{+} conductances. These alterations could potentially also mediate the changes in RMP of FS interneurons following chABC and requires further experimental investigation. Ultimately, changes in input resistance and RMP seem to predict decreases in firing probability, which would also functionally reduce the sensitivity of FS interneuron networks to feed forward excitation in the barrel cortex. Overall, our results demonstrate that the effect of sensory deprivation on intrinsic physiology is distinct from the alterations that occur following chABC digestion.

We conclude that PNNs play an important role in normalizing the intrinsic physiology of FS interneurons to maintain a balanced level of excitability, but that maturational deficiencies following sensory deprivation likely have mechanisms separate from that of PNN regulation.
Chapter 11: Introduction (3):

The role of PNNs in regulating the synaptic properties of neurons in the barrel cortex.

In the postnatal brain, components of PNNs begin to mature to adult levels (>P30) in approximate parallel to the progressive crystallization of brain circuits (Carulli et al., 2010; Karetko-Sysa et al., 2014; Nowicka et al., 2009). Drawing inspiration from the early models of critical period plasticity of the visual system, seminal work from Pizzorusso et al., (2002) demonstrated that enzymatic digestion of PNNs in the visual cortex reactivated juvenile levels of ODP in the adult brain. These results were later corroborated using a brain specific knock out of the link protein Crtl1 which perturbed the normal accumulation of PNNs and lead to prolonged ODP beyond the critical period (Carulli et al., 2010). Non-cell autonomous upregulation of an immature CSPG sulfation phenotype (CS6) also lead to a dramatic persistence of ODP in the adult mouse primary visual cortex (Miyata et al., 2012), demonstrating that inducing even immature PNN component sulfation states is permissive for plasticity in cortical circuits. Interestingly, transgenic induction of cell autonomous redox dysregulation in FS cells, impacting those cells without PNNs more (Cabungcal et al., 2013), also caused prolonged ODP in the mouse visual cortex (Morishita et al., 2015a) suggesting that PNN regulation of plasticity occurs through the underlying FS inhibitory interneurons that they ensheath.

Excitatory and inhibitory synapses from VIP+ interneurons are readily found apposed to PV+ interneurons, the cell type that also expresses a great deal of PNNs (Hioki et al., 2013; Kameda et al., 2012; Nowicka et al., 2009). This suggests that synaptic inputs onto PV+ interneurons may be regulated by PNNs. Therefore, I measured the spontaneous
synaptic inputs onto different neuronal cell types to determine whether chABC digestion of PNNs would alter them. I also measured the thalamocortical responses evoked by thalamic fiber stimulation in layer 4 of the barrel cortex. We predicted that if PNNs regulate the input resistance and RMP of FS interneurons, and the L-type Ca2+ slopes of LTS interneurons, then depolarizing synaptic inputs onto these cell types should also be altered following chABC digestion.
Chapter 12: Materials and Methods (3):

The role of PNNs in regulating the synaptic properties of neurons in the barrel cortex.

12.1. Animals

Anatomical and physiological experiments were conducted with white laboratory adolescent Swiss mice of either sex (CD-1, postnatal day 28-45; see Table 1 for the number of animals in each experimental group, Charles Rivers Laboratory, Wilmington, MA) in accordance with the Institutional Animal Care and Use Committee of Queens College, CUNY and NIH guidelines for responsible use of animals in research.

12.2. Acute slice preparation

Animals were sacrificed and brains were prepared for sectioning as described earlier (See materials and methods Chapter 8.4.) for slices used for spontaneous synaptic recordings. For thalamocortical stimulation, the brain was glued (cyanoacrylate) onto an agar block (4% agar in ACSF) and sectioned 55° from midline in the thalamocortical plane (Agmon & Connors, 1991).

Following sectioning, slices were removed using a paint brush and maintained at room temperature (~25°C) within the single slice incubation chamber, or under standard conditions in ACSF solution of the following composition; in mM: NaCl 124, KCl 2.5, NaH₂PO₄, 1.2, NaHCO₃ 24, HEPES 5, Dextrose 12.5, MgSO₄ 2, CaCl₂. In experimental conditions, 10 μl of chABC (Seikagaku, Sigma) was dissolved in 990 μl (1:100 vol./vol.) of ACSF to result in a final concentration of 0.2918 units/ml and added to the slice chamber for preincubation. To match our recording and enzymatic digestion experimental controls, slices from trimmed and control animals were placed for 1 hour in either the slice incubation chamber or a standard incubation
chamber (Edwards and Konnerth, 1992) followed by placement into the submerged patch clamp recording chamber.

12.3. Graded enzymatic digestion of PNN

Sections were prepared for chABC digestion as described earlier (See materials and methods chapter 8.5.). Briefly, thalamocortical slices were incubated in the enzyme for 60 min, then placed into the patch clamp recording chamber for experiments. Control slices were maintained in normal aerated ACSF for the same length of time as their digestion counterparts and subsequently placed into the recording chamber for the recording session.

12.4. Electrophysiology

Borosilicate electrodes were pulled and patch clamp procedures were conducted as described previously (See materials and methods chapter 8.8.). For spontaneous synaptic potentials, neurons were patched and recorded for 10 minutes in current clamp gap free mode. Offline gap free traces (5-15 min) were filtered at 1000 Hz (Gaussian), manually baseline adjusted, and excitatory post synaptic potentials (EPSPs) were isolated using event detection. Event detected EPSPs were then analyzed as isolated units in Clampfit. Both unitary and compound EPSPs were included in the analysis. Events with amplitudes below 0.75 mV were excluded from the analysis due to the observations of noise in some of the recordings. For measurements of the EPSP kinetic properties, isolated EPSPs were manually sorted so that five unitary EPSPs for each amplitude bin (0.75-1 mV, 1-2 mV, 2-3 mV, 3-4 mV, >4 mV) were analyzed using threshold detection. Unitary EPSPs were selected based on temporal order from the onset of the gap free recording so that the events were added until reaching a sample of five.
Thus, the analysis of half width, rise slope and decay slope contained 25 EPSPs for each cell. Independent t-tests were used to evaluate effects between groups (control and chABC digested). An α level of 0.05 was used to determined statistical significance. All data are reported using SEM.

### 12.5. Stimulation

A beveled concentric bipolar stimulation electrode (FHC Inc.) was visually guided towards the thalamocortical fibers at the lateral edge of the thalamus. Stimulation current was adjusted so that a series of pulses (1Hz) resulted in 50% failure to elicit EPSPs. The minimal current stimulation was then increased by 5%, which generally resulted in reliable EPSP generation for each pulse. Paired stimulation pulses of 20 pulses at 1Hz, 5Hz, 10Hz, 20Hz and 50Hz were then used to determine short term plasticity (Fioravante & Regehr, 2011; Schulz et al., 1994). Stimulated EPSPs were analyzed offline using Clampfit 10. Traces were Gaussian filtered at 1000 Hz, baseline adjusted using mean subtraction of the prior onset of stimulation, averaged across traces and analyzed using the statistics functions of Clampfit 10. Traces resulting in failures were not included in the averaging or analysis.

### 12.6. Cell type identification

Cells were characterized and sorted as described earlier (See materials and methods 8.9.). Briefly, intrinsic physiological responses to families of depolarizing and hyperpolarizing current pulses were used to sort cells as putative excitatory (e.g., regular spiking) and putative inhibitory neurons (e.g., fast spiking).
Chapter 13: Results (3):

The role of PNNs in regulating the synaptic properties of neurons in the barrel cortex.

13.1. Spontaneous synaptic potentials

Previous reports have demonstrated that evoked IPSPs onto excitatory cells are altered following enzymatic digestion of PNNs in the visual cortex (Liu et al., 2013), however it is unknown how synaptic events are regulated by PNNs in the barrel cortex. Therefore, we used spontaneous synaptic events as an indicator of changes in synaptic connectivity following enzymatic PNN digestion.

Putative excitatory neurons showed no alterations in their EPSP amplitudes or the distribution of their frequencies. RS control neurons had a total average EPSP frequency of 1.46±0.24Hz which was not significantly different then RS chABC frequency of 1.2 ±0.18Hz (p=0.39; Figure 16A,B). Doublet control cells had an average EPSP frequency of 2.03±0.50 relative to the average spontaneous EPSP input onto doublet chABC cells, 1.8±0.45 (p=0.73; Figure 16C,D). Bursting cells showed no difference in their spontaneous EPSP inputs either (Burst control: 2.16±0.41; burst chABC: 2.14±0.43; p=0.97; Figure 16E,F).

On the average, LTS interneurons showed decreased spontaneous EPSP frequencies following chABC digestion (Figure 17A,B). The reduction in LTS EPSP frequency was largely driven by decreases EPSP frequencies from 1 mV and higher (LTS control: 2.45±0.34 Hz; LTS chABC: 1.29 ±0.25Hz; p=0.02), which reached significance for the 1-2 mV and 2-3 mV amplitude bins (0.75-1 mV: p=0.8, 1-2 mV: p=0.02, 2-3 mV: 0.015, 3-4 mV: p=0.08, >4mV: p=0.1; Figure 17B). These decreases in higher amplitude EPSPs drove a shift in the overall distribution of their spontaneous EPSPs, favoring smaller EPSPs (0.75-1mV bins) relative to
higher amplitude EPSPs (Figure 17A).

In FS cells, PNN digestion using chABC resulted in a slightly greater number of EPSPs compared to control which did not reach significance (FS control: 2.83±0.15; FS chABC: 4.05±0.78; Figure 17D). However, we observed altered temporal characteristics in the EPSPs of FS cells. Measuring the half widths and temporal characteristics of FS cells yielded a significant decrease in EPSP half width for FS cells (Figure 18A) driven mostly by increases in decay slope for the 1-2 mV, 2-3 mV, and >4 mV bins (1-2 mV: p=0.004, 2-3 mV: 0.09, 3-4 mV: p=0.04, >4mV: p=0.006; Figure 18B). The decreases in half width at the 3-4 mV level appeared to be driven more by increases in rise slopes (FS control: 0.86mV/ms; FS chABC: 2.17 mV/ms; p=0.036, Figure 18C). Although LTS cells showed decreased EPSP frequencies, those remaining did not show changes to their half widths (LTS control: 19.15±2.28ms; LTS chABC: 19.8±2.75ms; p=0.85; Figure 18,E,F). The minimal intrinsic and synaptic alterations found in excitatory cells following chABC suggests that the effect is due to PNN digestion rather than a non-specific effect of the enzyme. In summary, EPSPs onto inhibitory interneurons are specifically modulated by PNNs, whereas inputs onto excitatory neurons are unaffected which is correlated with the observed expression patterns of PNNs.

13.2. Thalamocortical stimulation

For our preliminary data, the thalamocortical evoked EPSPs on chABC digested FS cells (n=2) were compared with non-digested FS cells (n=2). My preliminary evidence using chABC enzymatic digestion of PNNs in the thalamocortical slice shows that evoked EPSP amplitudes onto FS interneurons are similar relative to control FS cells at 10 Hz paired stimulation (FS control: 1.28 ±0.22 mV; FS chABC: 0.82±0.02mV; p=0.13) and 50Hz (FS control: 1.4±0.23mV;
FS chABC: 1.18±0.13; p=0.07; Figure 19). Half widths of the EPSPs are also similar at 10 Hz (FS control: 10.2±0.25ms; FS chABC: 9.44±0.58ms; p=0.29) and 50 Hz (FS control: 11.3±0.41; FS chABC: 10.8±0.72; p=0.92). However, paired pulse ratios following chABC show trends towards reductions at 10 Hz (2nd/1st; FS control: 0.75±0.12; FS chABC: 0.91±0.03) at 50 Hz (2nd/1st; FS control: 0.88±0.11; FS chABC: 1.18±0.08; Figure 19).
Chapter 14: Discussion (3):

The role of PNNs in regulating the synaptic properties of neurons in the barrel cortex.

14.1. PNNs and synaptic physiology

Previous research has examined the role of the extracellular molecule TN-R and its carbohydrate HNK-1 in modulating synaptic transmission in area CA1 of the hippocampus and found increased mini IPSCs and increased EPSP slopes following antibody and genetic manipulations (Saghatelyan et al., 2000; Bukalo et al., 2001). PNN digestion alone also alters the IPSCs onto excitatory neurons in the visual cortex (Liu et al., 2013), increases their post synaptic AMPA receptor mobility in the hippocampus (Frischknecht et al., 2009) and enhances dendritic spine motility to promote long term potentiation (de Vivo et al., 2013). Following PNN digestion, we show that FS interneurons exhibit significantly decreased half widths. I suspected a potential interaction between the FS reduction in input resistance, depolarized RMP and the alteration in spontaneous synaptic input. However, I found no correlations between RMP and total frequency of EPSPs nor any individual amplitude bin of EPSPs (data not shown). Large reductions in EPSP drive onto FS PV+ interneurons could be a result of reduced synchronous thalamocortical (Bruno & Sakmann, 2006), or potent intracortical inputs (Kameda et al., 2012). Interestingly, excitatory neurons (RS-S, RS-D, bursting) do not show changes to the frequencies of their spontaneous EPSPs and little changes to their intrinsic properties. However, we did not examine the role of IPSPs on the neurons we recorded from. More detailed experiments are needed to elucidate whether thalamocortical or intracortical inhibitory and excitatory synapses are modulated at the site of the axonal bouton by the surrounding PNNs.

At the same time, EPSP frequencies observed in LTS cells were significantly decreased
and showed a shift towards lower amplitude spontaneous EPSPs. These differential impacts have been observed previously whereby excitatory neurons can have facilitating synapses onto somatostatin+ interneurons while simultaneously having depressing synapses onto PV+ interneurons (Reyes et al., 1998). Although it is tempting to speculate that changes in spontaneous EPSPs may be driven by alterations in spine dynamics or AMPA receptor mobility, more work is needed to specify the mechanism for the results that we observed. Nevertheless, our results show that PNNs do play a role in regulating the spontaneous EPSPs of FS and LTS inhibitory interneurons in the barrel cortex.

These results indicate that PNNs play a large role in regulating neuronal physiology through the temporal and frequency characteristics of spontaneous synaptic inputs. While the effect of PNNs could be post-synaptic or pre-synaptic, my preliminary thalamocortical stimulation results show that first EPSP following stimulation is reduced in amplitude, and paired pulse ratio on FS cells is subsequently reduced following chABC, similar to previous findings in the hippocampus, that found reduced PPD following hyaluronidase digestion of PNNs (Frischknecht et al., 2009). A post-synaptic effect is also consistent with our preliminary observations of reduced first EPSP amplitude. One theory as to how PPD occurs is that there is a delay to new vesicle binding due to the time it takes to clear bound neurotransmitter vesicle components from the presynaptic active zone (reviewed in Fioravante & Regehr, 2011a). This would be possible if disruption of PNN components also impacted cell adhesion molecules associated with both the presynaptic and post-synaptic membrane. Interestingly, PNN components do interact with neuronal cell adhesion molecules (Maurel et al., 1994; Milev, Chiba, et al., 1998).

Reductions in PPD may also indicate reduced Ca\(^{2+}\) influx resulting in reduced vesicular
release (Reviewed in Fioravante & Regehr, 2011), which could occur following digestion of Ca\textsuperscript{2+} buffering PNN components (Vigetti et al., 2008). Because our preliminary work shows reductions in first EPSP rather than second EPSP amplitude, it seems unlikely that altered Ca\textsuperscript{2+} influx is at play. In addition, the increased temporal dynamics of spontaneous EPSPs in FS cells suggests that glutamate may be diffusing out of the synaptic cleft at greater rates than normal. These effects should be explored further in future work.

In the context of sensory processing, PNNs may ultimately regulate the excitability of these critical inhibitory network to provide stabilization to a maturing brain (Gentet et al., 2012a; Swadlow, 2003). Future work should examine whether prolonged stimulation intervals result in eventual depression and the interaction between evoked EPSPs and IPSPs.
Chapter 15: General Discussion and Concluding Remarks

Overall, our results demonstrate that PNNs specifically regulate the active and passive properties of FS interneurons, modulate FS and LTS interneurons spontaneous synaptic properties, and more diffusely impact the firing probability of FS, LTS, RS-S, and RS-D neurons in the barrel cortex. Our preliminary data also suggest that PNNs regulate glutamatergic thalamocortical short term plasticity. Although the mechanisms are unclear, our findings suggest that both sensory deprivation and PNNs may play a greater role in regulating the intrinsic physiology of inhibitory interneurons, but that prolonged sensory deprivation produces some qualitatively different effects on neuronal physiology than acute PNN perturbation. However, the overall effect of the alterations on intrinsic physiology appear to point towards an altered excitatory inhibitory balance in the barrel cortex. The excitatory-inhibitory balance has been proposed to be one mechanism by which the brain regulates developmental plasticity (Hensch & Fagiolini, 2004) while PNNs have been proposed to be another (Gogolla et al., 2009; Pizzorusso et al., 2002). Our results bridge the gap between the two proposed mechanisms, whereby PNNs have a direct impact on the intrinsic excitability of neurons in the barrel cortex via their passive, active and spontaneous synaptic properties.
Table 1. Sample sizes for control and experimental conditions. TC: thalamocortical.

<table>
<thead>
<tr>
<th>Number of animals in each condition</th>
<th>Control</th>
<th>Sensory deprived</th>
<th>Sensory deprived regrew</th>
<th>Control recorded for trim</th>
<th>Trim recorded</th>
<th>ChABC intrinsic and spontaneous synaptic</th>
<th>ChABC intrinsic and spontaneous synaptic ChABC TC stim</th>
<th>Control for TC stim</th>
<th>ChABC TC stim</th>
</tr>
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<tbody>
<tr>
<td>n=</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>15</td>
<td>18</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2. Summary for intrinsic properties in five neuronal cell types. Bold numbers indicate p-value <0.05.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Condition</th>
<th>IR</th>
<th>Resting Membrane Potential (mV)</th>
<th>First spike threshold (mV)</th>
<th>Last spike threshold (mV)</th>
<th>First spike AHP (mV)</th>
<th>First spike time to AHP time (ms)</th>
<th>First spike height (mV)</th>
<th>First spike half width (ms)</th>
<th>Last spike half width (ms)</th>
<th>Last spike amplitude from threshold (mV)</th>
<th>Last spike amplitude from threshold (mV)</th>
<th>Last spike rise slope (mV/ms)</th>
<th>Last spike decay slope (mV/ms)</th>
<th>Last spike rise time 10-90% (ms)</th>
<th>Last spike decay time 10-90% (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS-S</td>
<td>Control</td>
<td>18-69,4±1.2-39,5±1.1-34,3±1.3-46,3±1.6-47,1±1.7</td>
<td>28,5±4,9-33,0±3.5</td>
<td>1,0±0.1</td>
<td>2,3±0.1</td>
<td>91,1±2.3</td>
<td>79,9±2.5</td>
<td>147,4±8.4</td>
<td>115,4±8.4</td>
<td>*31,4±1.6</td>
<td>*23,9±2.6</td>
<td>60,5±0.0</td>
<td>0,6±0.0</td>
<td>2,4±0.1</td>
<td>*2,9±0.2</td>
<td></td>
</tr>
<tr>
<td>RS-S</td>
<td>chABC</td>
<td>39-68,8±0.9-39,1±1.1-35,8±1.0-47,2±1.2-48,0±1.2</td>
<td>227,5±2.3</td>
<td>31±1.9</td>
<td>1,3±0.1</td>
<td>2,0±0.1</td>
<td>78,2±1.5</td>
<td>57,6±0.5</td>
<td>155,7±6.5</td>
<td>229,0±6.9</td>
<td>*37,9±2.2</td>
<td>*32,3±2.2</td>
<td>20,5±0.0</td>
<td>0,6±0.0</td>
<td>2,1±0.1</td>
<td>*2,3±0.1</td>
</tr>
<tr>
<td>RS-D</td>
<td>Control</td>
<td>6-63,6±2.4-39,9±1.5-37,6±1.9-48,3±1.2-48,0±1.2</td>
<td>12,7±9.6</td>
<td>17±3.8</td>
<td>3,0±0.1</td>
<td>1,6±0.1</td>
<td>84,7±3.3</td>
<td>71,6±3.5</td>
<td>152,2±8.6</td>
<td>211,4±3.2</td>
<td>*33,7±4.0</td>
<td>*33,7±4.0</td>
<td>50,5±0.0</td>
<td>0,5±0.0</td>
<td>1,2±0.1</td>
<td>1,9±0.2</td>
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<tr>
<td>RS-D</td>
<td>chABC</td>
<td>17-67,5±1.9-41,7±1.8</td>
<td>38,6±2.1-44,0±2.0</td>
<td>49,0±2.0</td>
<td>16,1±5,7</td>
<td>92,9±4,5</td>
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<td>1,8±0.1</td>
<td>84,7±3.3</td>
<td>74,7±3,6</td>
<td>163,6±16,6</td>
<td>31,0±15,2</td>
<td>*43,2±4.5</td>
<td>*33,7±4.0</td>
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<td>Burst</td>
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<td>39,8±4.8</td>
<td>44,7±5.6</td>
<td>46,5±5,4</td>
<td>4,1±0.5</td>
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<td>3,3±0.1</td>
<td>1,9±0.2</td>
<td>81,7±4,7</td>
<td>74,7±2,6</td>
<td>36,2±15,9</td>
<td>101,5±8,1</td>
<td>*47,5±6,0</td>
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<td>Burst</td>
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<td>8-59,0±0.9-35,4±2.1</td>
<td>31,0±2.3</td>
<td>37,0±3,8</td>
<td>39,9±2.0</td>
<td>5,9±2.0</td>
<td>13,3±3,5</td>
<td>3,3±0.1</td>
<td>1,5±0.1</td>
<td>69,0±7,3</td>
<td>62,7±5,0</td>
<td>71,8±18,2</td>
<td>98,7±11,1</td>
<td>*48,5±8,9</td>
<td>*37,7±5.0</td>
<td>50,5±0.0</td>
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<tr>
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<td>41,5±1.6</td>
<td>65,0±1.1</td>
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<td>7,2±0.2</td>
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<td>655,8±2,3</td>
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<tr>
<td>FS</td>
<td>chABC</td>
<td>6-58,4±2.2-40,4±4,7</td>
<td>37,5±4,7</td>
<td>56,9±4,4</td>
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<td>58,7±3,5</td>
<td>555,2±4,0</td>
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<td>-25,7±11,9</td>
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<td>Control</td>
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<td>48,4±1.8</td>
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<td>93,1±9,4</td>
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<td>LTS</td>
<td>chABC</td>
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<td>51,7±3,5</td>
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<td>66,9±3,0</td>
<td>31,9±10,7</td>
<td>98,4±3,9</td>
<td>*59,4±13,2</td>
<td>*42,2±10,0</td>
<td>50,4±0,0</td>
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</table>
Figure 1. Whisker to barrel pathway. Texture information is detected by mechanoreceptors in the whisker follicle and transmitted to the brain stem nuclei and mapped onto whisker representations called barrelettes (not shown). This information is then relayed to two contralateral thalamic nuclei, where the whisker pad is once again represented by discrete clusters of neurons. This thalamic representation is called the barreloids (not shown). Thalamocortical neurons from the barreloids are then innervate the cortical layers of the primary somatosensory cortex barrel field (barrel cortex).
Figure 2. Perineuronal nets ensheath the soma and proximal processes of neurons in the central nervous system. Their molecular make up is heterogenous, but generally consist of hyaluronic acid, chondroitin sulfate proteoglycans, Tenascin-R and a variety of link proteins (e.g. Crtl1, Bral1). They link to the cell through either direct interaction with the phospholipid membrane or through surface receptors such as the transmembrane hyaluronan synthesizing enzyme Hyaluronan synthase (HAS). PNN components such as tenascins have been shown to interact with the β subunit of the voltage gated Na\(^{2+}\) channel. In addition, it has been hypothesized that PNNs serve as restrictive barriers for synaptic reorganization and contact. Presumably, PNNs are capable of regulating both intrinsic excitability and synaptic plasticity (see text).
Figure 3. Lack of colocalization of Perineuronal nets with 5HT3aR expressing inhibitory interneurons. have been shown to primarily ensheath fast spiking inhibitory interneurons expressing parvalbumin (PV+; Nowicka et al., 2009). Another major class of inhibitory interneuron is one that expressed somatostatin (SOM+). Recent work has indicated that PNNs may colocalize with those types of interneurons in the hippocampus (McRae et al., 2010) and with neurons that also express SOM in the neocortex (Karetko-Sysa et al., 2014). A recent report has demonstrated that nearly 100% of all inhibitory interneurons express three markers; PV, SOM and 5HT3aR expressing interneurons (Lee et al., 2010; Rudy et al., 2011). Although a full census has of PNN colocalization has yet to be taken in the neocortex, we show above that (unpublished work) that PNNs do not colocalize at all with 5HT3aR expressing interneurons.
Figure 4. Laminar specific distribution of PNN. Nissl staining reveals dense barrel structures in coronal sections of mouse somatosensory cortex (WM=white matter), A. Different laminar densities are also evident after WFA labeling of PNN in coronal sections. Barrel structures can be occasionally be visualized as well, B. Staining and labeling using Nissl and WFA reveals a high degree of overlap in cortical expression, C. PNN expression is highly distinct, ensheathing somata, proximal dendritic processes, and axon initial segments and largely absent from the cytosol, D. The highest density of PNN in mouse barrel cortex is layer 4, data represent population means and 1 SEM, E.
Figure 5. Bilateral sensory deprivation reduces expression of PNNs in the mouse barrel cortex. Coronal sections of mouse barrel cortex region in control (A), bilaterally trimmed conditions (B), and regrow (C). Bilateral sensory deprivation significantly reduces PNN density in layers 4 and 5. Average PNN density across cortical layers is also reduced. Following whisker regrow, PNN densities remained significantly lower than control and not different from trim, (D).

** p<0.01. Error bars represent 1 SEM.
**Figure 6. Small volume incubation chamber.** The slice incubation chamber consists of four sequential chambers transected from three disposable transfer pipettes and one disposable pipette tip. Blue lines indicate approximate locations to cut (see text for measurements), A. Using a syringe needle, puncture a grid of nine holes into the bottom of piece three to allow airflow into the slice chamber. Attach the female luer into the silicon tubing, B. The other end of the female luer is fit into the hole of piece one and secures using parafilm to make relatively airtight, creating the air chamber, C. Then piece two is fit into piece one to form the liquid and air chambers. After turning on air flow, the liquid chamber is filled with 1-2 ml of water and the airflow is adjusted to ~ 1 bubble per second, D. Piece four is put into piece three to wedge in the mesh netting and form the slice chamber, E and F. After achieving proper air flow, the slice chamber is fit into the liquid chamber and filled with appropriate reagent (e.g. ACSF). If bubbles formed in the slice chamber, it was removed and out the remaining bubbles were dislodged, making sure the side of the slice chamber are dry and then the chamber was put back in place. Completed slice incubation chamber is shown and diagrammed, H and I.
Figure 7. Bilateral sensory deprivation reduces FS excitability. Representative traces from FS (A) and RS-S (B) neurons in the mouse barrel cortex under control and sensory deprived conditions. 30 days of bilateral sensory deprivation reduces FS spike frequency and firing probability (D, E). RS-S interneurons spike frequency and probabilities are not significantly impacted by the same deprivation (C,F). No significant alterations were found for input resistances (G). * p<0.05; ** p>0.01. Error bars represent 1 SEM.
Figure 8. FS spike amplitudes, but not RS-S spike amplitudes are altered following prolonged sensory deprivation. FS spike amplitudes were significantly increased following 30 days of whisker trimming, an effect which did not occur for RS-S neurons. * p<0.05; ** p>0.01. Error bars represent 1 SEM.
**Figure 9. Enzymatic digestion using chABC rapidly reduces PNN levels in mouse barrel cortex.** Control coronal sections (A,C,E,G) and hemisphere matched sections exposed to 1:100 chABC (B,D,F,H), incubation times are indicted on micrographs. Insets show higher magnification examples of PNNs at each time point, scale bars represent 40 µm. Relative density of chABC digested to hemisphere matched controls reveal rapid fall in PNN expression from 30 min (21% reduction), 60 min (64% reduction), 90 min (65% reduction) and 120 min (>99% reduction). These effects varied across layers, but on average show a relatively linear decrease in expression between 30 min and 2 hours. I. Asterisks indicate significant differences relative to controls. Data represent laminar and cross laminar means. *p<0.0
Figure 10. Alterations in putative excitatory neuron intrinsic active properties following enzymatic PNN digestion. Representative recordings from RS-S (A), RS-D (B) and bursting (C) neurons from the barrel cortex. Spike frequency is not significantly impacted by 60 min of chABC digestion of PNNs for putative excitatory neurons (D). RS-S neurons show a slight decrease in firing probability, which is not as apparent for RS-D (F) or bursting neurons (G).
Figure 11. Regulation of RS-S spike properties by PNNs. RS-S decay slopes are significantly decreased following PNN digestion (A). RS-D neurons show decreases in decay slope only in response to the maximal +200 pA stimulation intensity condition, for the first evoked AP, but are otherwise unaffected, (B). Neither bursting cells, FS, or LTS cells show changes to decay slopes following chABC (see Table 1), (F). *p<0.05, **p<0.01. Error bars represent 1 SEM.
Figure 12. chABC digestion of PNNs alters FS excitability. Representative traces of FS and LTS interneurons, two putative GABAergic phenotypes in the barrel cortex under control and PNN digested conditions (A). 60 min of PNN enzymatic digestion using chABC subtly alters the spike frequency (C) and firing probability of FS interneurons (D), but not LTS interneurons (C,E). Error bars represent 1 SEM.
Figure 13. Alterations in FS input resistance and RMP are correlated with changes in firing probability. Input resistance was measured as the membrane voltage change following a family of hyperpolarizing current pulses (A). 60 minutes of enzymatic digestion of PNNs using chABC reduces input resistance for FS interneurons and not other neuron types (A). Alterations in FS input resistance negatively correlates with firing probability after chABC digestion (B), but not in control (data not shown). Average FS RMP is more depolarized following chABC digestion (C), which also negatively correlates with firing probability. * p<0.05; ** p>0.01. Error bars represent 1 SEM.
Figure 14. Fast spiking interneurons show reduced spike amplitude following enzymatic digestion of PNNs. Spike amplitude measured from threshold is reduced in FS cells at +150 pA and +200 pA for first and last spikes in trains of APs, (A). Representative traces evoked by 1 second depolarizing pulses of +150 pA show superimposed FS control (black trace) and FS chABC (gray trace), (B). Average reduction in spike amplitude following chABC digestion averages between 10-20%, (C). Putative inhibitory LTS cells spike amplitudes are not affected by PNN digestion, (D). *p<0.05, **p<0.01. Error bars represent 1 SEM.
Figure 15. Regulation of LTS $I_T$ by PNNs. LTS $I_T$ current measured between the offset of a hyperpolarizing current pulse and rebound spike threshold. $I_T$ current slopes are reduced following chABC digestion of PNNs (A,B). However, neither the max amplitude of the $I_T$ current nor the latency from hyperpolarization offset to rebound spike threshold is significantly altered by chABC (C,D).*p<0.05. Error bars represent 1 SEM.
Figure 16. Putative excitatory neuron spontaneous synaptic physiology is unchanged following chABC digestion of PNNs. Representative traces of spontaneous EPSPs from RS-S (A,B top), RS-D (C-D top) and bursting neurons (E,F top). No significant alterations in EPSP amplitude proportion (A,C,E) or frequency (B,D,F) were detected following chABC digestion of PNNs. Average frequencies were also unchanged (B,D,F insets).
Figure 17. LTS spontaneous post synaptic potentials show changes in spike amplitude distribution and frequency following PNN enzymatic digestion. Spontaneous PSPs were binned based on amplitude and calculated as a percentage of total PSPs measured (A,C) and relative to total recording time (B,D). Representative spontaneous PSP traces (A, B,C,D, top) and quantification (A-D, bottom). LTS cells show increases in the 0.75-1 mV range of PSPs relative to all PSPs, and a reduction of the 2-3 mV amplitude PSPs, (A, bottom). LTS interneurons showed reductions in the frequency of 1-2 mV and 2-3 mV PSPs (B, bottom) which reduced total average PSP frequency (B, inset). FS cells showed no significant changes in the proportion of PSPs, nor PSP frequency. Note differences in scale for B and D. *p<0.05, **p<0.01. Error bars represent 1 SEM.
Figure 18. FS PSP temporal properties are altered following enzymatic PNN digestion. FS PSP half widths are reduced following PNN enzymatic digestion at all spike amplitude bins, (A). Corresponding increases in PSP decay slopes (B) and rise slopes (C). LTS cells do not show changes to spontaneous PSP temporal properties (D,E,F). *p<0.05, **p<0.01. Error bars represent 1 SEM.
Figure 19. Reductions in amplitude and paired pulse depression (PPD) following chABC.

Thalamocortical slices were taken and the existing fibers of the VPM thalamus were stimulated using a concentric stimulating electrode. Preliminary results (FS control n=2, FS chABC n=2) show that the first evoked EPSP amplitude is reduced following chABC. PPD is also reduced following chABC digestion, as a result of the initial EPSP amplitude reduction. EPSP half width appears unaltered.


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