Published in final edited form as:
*Eur J Neurosci*. 2012 December ; 36(11): 3471–3482. doi:10.1111/j.1460-9568.2012.08268.x.

# Persistent decrease in multiple components of the perineuronal net following status epilepticus

**Paulette A. McRae**[1,2], **Esther Baranov**[1], **Stephanie L. Rogers**[1], and **Brenda E. Porter**[1,2]

[1]Department of Pediatrics, Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

[2]Department of Neurology, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

## Abstract

In the rodent model of temporal lobe epilepsy, there is extensive synaptic reorganization within the hippocampus following a single prolonged seizure event, after which animals eventually develop epilepsy. The perineuronal net (PN), a component of the neural extracellular matrix, primarily surrounds inhibitory interneurons and under normal conditions restricts synaptic reorganization. The objective of the current study was to explore the effects of status epilepticus (SE) on PNs in the adult hippocampus. The aggrecan component of the PN was studied, acutely (48 hours post-SE), sub-acutely (1 week post-SE), and during the chronic period (2 months post-SE). Aggrecan expressing PNs decreased by one week, likely contributing to a permissive environment for neuronal reorganization and remained attenuated at 2 months. The SE exposed hippocampus showed many PNs with poor structural integrity, a condition rarely seen in controls. Additionally, the decrease in the aggrecan component of the PN was preceded by a decrease in hyaluronan and proteoglycan link protein 1 (HAPLN1) and hyaluronan synthase 3 (HAS3), which are components of the PN known to stabilize the connection between aggrecan and hyaluronan, a major constituent of the extracellular matrix. These results were replicated *in vitro* with the addition of excess KCl to hippocampal cultures. Enhanced neuronal activity caused a decrease in aggrecan, HAPLN1, and HAS3 around hippocampal cells *in vivo* and *in vitro* leaving inhibitory interneurons susceptible to increased synaptic reorganization. These studies are the foundation for future experiments to explore how loss of the PN following SE contributes to the development of epilepsy.

### Keywords

Aggrecan; perineuronal net; seizure; HAPLN1; HAS3

## Introduction

Temporal Lobe Epilepsy (TLE) is a common form of focal epilepsy associated with major synaptic remodeling and rewiring of neuronal circuitry. In animal models of TLE, SE (a

Corresponding Author: Paulette A. McRae, The Children's Hospital of Philadelphia, Abramson Research Building, Rm 512, 3516 Civic Center Blvd, Philadelphia, PA 19104, Telephone: 215-590-4585, Fax: 215-590-3779, mcraep@email.chop.edu.

prolonged seizure) is followed by a latent period when synaptic reorganization and mossy fiber sprouting occur (Gombos et al., 1999; Shibley and Smith, 2002). This reorganization is thought to promote the progression of epilepsy. Alterations in inhibitory synaptic transmission combined with synapse reorganization may have an essential role in initiating and ultimately maintaining the seizure prone condition of the brain. The mechanisms for these changes are still being determined.

Importantly, the integrity and stability of synapses require complex interactions with the extracellular environment. The perineuronal net (PN) is a unique extracellular matrix (ECM) structure that forms a lattice-like configuration around the synapses on the somata and proximal dendrites of a subset of interneurons (Hockfield et al., 1990; Brückner et al., 1993), and is therefore uniquely positioned to influence synaptic integrity and stability (Frischknecht et al., 2009).

Chondroitin sulfate proteoglycans (CSPG), particularly members of the lectican family, which include versican, neurocan, brevican, and aggrecan are the major components of the PN (Yamaguchi, 2000). Neurocan, a brain specific lectican expressed primarily during development (Yamaguchi 1996) re-appears in adulthood following SE (Kurazono et al., 2001; Matsui et al., 2002). SE in adult rats leads to increased expression of brevican cleavage products (Yuan et al., 2002). Previously we found a transient increase in aggrecan expression following early-life SE (McRae et. al., 2010). Phosphacan, another CSPG, forms PNs, however, after SE there is a decrease in phosphacan-expressing PNs surrounding parvalbumin interneurons in the hippocampus (Okamoto et al., 2003). Suggesting that seizures can alter components of the PN, but the specific proteins involved, the direction and duration of changes may vary.

Aggrecan is found almost exclusively in the PN expressed in adulthood (Matthews et al., 2002; Dino et al., 2006). During development decreased activity attenuates aggrecan expression (Sur et al., 1988; Guimaraes et al., 1990; Kind et al., 1995, 2012; Lander et al., 1997; McRae et al., 2007), while increased activity augments expression (McRae et al., 2010). Importantly, once mature synapses are established and ensheathed by aggrecan containing PNs, they are stable and subject to little reorganization (Hockfield et al., 1990; McRae et al., 2007). Hyaluronan synthases (HASs) and hyaluronan and proteoglycan link proteins (HAPLNs) stabilize interactions between lecticans and hyaluronan. *In vivo* loss of HAPLN1 decreased PN production (Carulli et al., 2010). *In vitro* co-expression of aggrecan, HAPLN1, and HAS3 was necessary for the formation of compact PNs (Kwok et al., 2010).

The goal of the current study was to determine the effects of SE on various components of the PN. We found that increased neuronal activity leads to a temporal cascade of changes in PN expression, which may contribute to increased plasticity in the hippocampus making the environment conducive to aberrant synaptic reorganization.

## Materials and Methods

### Animals and experimental groups

The institutional animal care and use committee at the Children's Hospital of Philadelphia approved all procedures used in this study. Male Sprague-Dawley rats ≥250 grams, from Charles River (Wilmington, MA, USA), were randomly assigned into either the control group or the experimental group. Rats were singly housed in standard plastic cages with corncob bedding and *ad libitum* access to food and water.

### Seizure Induction

Status epilepticus was induced in the experimental group using the pilocarpine-HCl chemoconvulsant rodent model of epilepsy. All animals were given intraperitoneal (i.p.) injections of methyl-scopolamine (1 mg/kg) 30 minutes prior to injections of pilocarpine. The experimental seizure group received intraperitoneal pilocarpine HCL (385 mg/kg) with a 1/2 dose administered one hour later until the animals reached stage V SE as described by Treiman et al. (1990). Only rats that reached stage V SE were included in the experimental group. The control group received 1/10 of a dose (38 mg/kg) of pilocarpine. The experimental seizure group received an i.p. injection of diazepam (6 mg/kg) one hour after the onset of stage V SE, while the control group received the same dose one hour after the low dose pilocarpine injection. The seizure group received an additional half dose (3 mg/kg) one hour after the initial diazepam injection.

### Immunohistochemistry

Rats were deeply anesthetized with isofluorane and underwent transcardiac perfusion with 0.1 M PBS, followed by 4% phosphate-buffered paraformaldehyde, pH7.4. The tissue was post-fixed in paraforamldehyde overnight, then immersed in 30% sucrose in phosphate buffer. Forty-micron sections were cut on a cryostat, and free-floating sections, or coverslips from primary neuronal cultures were incubated at 4°C overnight in the primary antibodies Cat-315 which detects a glycoform of aggrecan (mouse IgM 1:10; a gift from Dr. Russell Mathews, SUNY Upstate, Syracuse, NY, USA), Wisteria Floribunda Agglutinin (1:750; Vector labs, Burlingame, CA, USA), parvalbumin (mouse IgG 1:50; Chemicon, Temecula, CA, USA), HAPLN1 (goat 1:50; R&D Systems, Minneapolis, MN, USA), or HAS3 (rabbit 1:50; Santa Cruz; Santa Cruz, CA, USA) with 0.5%Triton X-100 in DMEM. The next day the sections were rinsed with phosphate buffer then incubated with Alexa fluorescent-conjugated goat anti-mouse, goat anti-rabbit, or donkey anti-goat secondary antibodies (Invitrogen, Carlsbad, CA, USA) and 0.5%Triton X-100 in DMEM. 4′ 6-diamidino-2-phenylindole DAPI (1:1000; Molecular Probes, Carlsbad, CA, USA) with 0.5%Triton X-100 in DMEM was applied after the secondary antibody staining was complete. Sections/coverslips were rinsed with phosphate buffer and mounted onto glass slides using Prolong Antifade mounting medium (Invitrogen) or Vectashield mounting medium with DAPI (Vector Labs, Burlingame, CA, USA).

## Cell Counts

Stained sections were visualized using a Zeiss Axioplan microscope (Thornwood, NY, USA). Quantitative analyses were performed at 20x and colocalization was verified at 40x magnifications. Stereological methods were employed using the fractionator method (Gunderson et al., 1986), which provides an unbiased estimate of the total number of cells. Systematically-randomly sampled (SRS) 40 μm thick sections through the dorsal hippocampus, spanning from approximately −1.344 mm through −2.30 mm posterior to bregma, were evaluated. The SRS was every $10^{th}$ section through the hippocampal structure, which yields between 6–8 sections per animal. The estimated number of cells was based on the following formula:

$$N=\sum Q \times 1/ASF \times 1/SSF \times 1/TSF$$

ASF: area sampling fraction; SSF: number of sections sampled; TSF: thickness of sampling fraction

## RT-PCR

Fresh frozen whole hippocampal tissue samples were homogenized on ice with a sonicator. The *mir*Vana Isolation Kit (Ambion Inc., Austin, TX, USA) was used to extract RNA from the tissue. RNA concentrations were measured with a spectrophotometer (NanoDrop ND1000). Five micrograms of purified RNA per 8 μl were reverse transcribed with the SuperScript II reverse transcription kit and random hexamers (Invitrogen). The cDNA concentrations were quantified with a spectrophotometer and diluted so that either 500 ng per (2μl) sample underwent real-time PCR in a 384-well plate. Each master mix was prepared using the Taqman Universal Master Mix (Applied Biosystems, Branchburg, NJ, USA) and a probe for aggrecan (Acan Rn01477603_m1), HAPLN1 (Rn00569884_m1), or HAS3 (Rn00515092_m1) (Applied Biosystems, Carlsbad, CA, USA). Each sample was assayed in triplicate to minimize error and matched to a standard curve of rat cortex cDNA. The real-time PCR assay was executed by an SDS 7900HT thermocycler (Applied Biosystems), comprised of a 2 min cycle at 50 °C, followed by a 10 min cycle at 95 °C, and 40 cycles of 15s at 95 °C and 60 s at 60 °C. Data was expressed as a percent change relative to control values in the same run.

## Neuronal Cultures

Standard culture techniques were used as described in (Cummings et al., 1996; Wilcox et al., 1994). Pregnant Sprague-Dawley rats were anesthetized and underwent cervical dislocation. Hippocampi from E17-19 rat embryos were dissected and trypsinized in Dulbecco's minimum essential medium (DMEM) (Whittaker Bioproducts, Walkersville, MD, USA) containing 0.027% trypsin at 37°C for 15 minutes. They were triturated in media consisting of DMEM supplemented with 10% fetal calf serum (Hyclone Lab, Nampa, ID, USA). Dissociated cells were plated in 35-mm Petri dishes with glass coverslips (Bellco, Vineland, NJ, USA) coated with poly-l-lycine (Peptide International, Louisville, KY, USA). The dishes were then placed in a 37°C humidified 5% CO2 incubator. For serum-free cultures that inhibit glia growth, the dissociated cells were plated at a density of 100,000 cells/ml in

Neurobasal media (Gibco) supplemented with B27 (Gibco). *In vitro* others have demonstrated that PNs develop over time and are only expressed in mature neuronal cultures (Brückner and Grosche, 2001; Dino et al., 2006; Miyata et al., 2005; Giamanco et al., 2010, 2012), therefore we used dissociated hippocampal cultures 30 DIV. Cultures were treated with the addition of a highly concentrated solution of KCl to a final concentration of 20 mM for six hours or two days or an equal volume of normal saline. Cells were fixed with 4% phosphate-buffered paraformaldehyde, pH7.4 for ten minutes and prepared for immunostaining at the end of six hours. After the two-day treatment the cultures were washed and cultured media from sister cultures was added. One week later the cells were fixed with 4% phosphate-buffered paraformaldehyde, pH7.4 for ten minutes and prepared for immunostaining.

### Statistical Analysis

Aggrecan, WFA, HAPLN1 and parvalbumin immunoreactivity were analyzed using a two-way repeated measures ANOVA comparing control and seizure animals across different regions of the hippocampus with a Bonferroni multiple comparison post-test run for all groups. Between 4–6 animals were used for each group. For the analysis of RT-PCR data a one-way ANOVA statistical test with a Bonferroni multiple comparison post-test run for all groups was used to evaluate *aggrecan*, *HAPLN1*, *HAS3* and *Parvalbumin* mRNA levels following a seizure relative to control mRNA levels. Between 6–10 animals used for each group. For the tissue culture KCl analysis a one-way ANOVA statistical test with a Bonferroni multiple comparison post-test run for all groups was used and results were compiled from three different experiments (each having a minimum of 500 DAPI positive cells counted). The null hypothesis states that there would be no changes in aggrecan, WFA, parvalbumin, HAPLN1 or HAS3 protein expression or mRNA levels following SE.

## Results

### Aggrecan expression decreases following SE

Aggrecan containing PNs prominently surround interneurons in the hippocampus (Fig. 1A,E,I,M). To evaluate whether a long seizure alters the expression of aggrecan, SE was induced with pilocarpine in adult rats and aggrecan expression was evaluated after an acute (48 hours post-SE), sub-acute (1 week post-SE), or chronic time point (2 months post-SE). The aggrecan component of the PN was detected with the monoclonal antibody Cat-315. Cat-315 detected PNs throughout the dorsal hippocampus and its expression was not altered 48 hours after SE induction (Fig. 1B,F,J,N). One week following SE, there was a decrease in aggrecan expressing PNs throughout the hippocampus (Fig. 1C,G,K,O). Two months after SE, aggrecan positive PN expression remained attenuated (Fig. 1D,H,L,P). The PNs in the dentate gyrus were the most sensitive to the effects of SE and demonstrated a substantial loss (Fig. 1C,D). Subiculum PNs were the most resistant to SE (Fig. 1O,P), but expression levels in the subiculum were still lower than control levels (Fig. 1M). These data indicate that SE is deleterious to aggrecan expressing PNs, reducing expression at sub-acute and chronic time points.

### There is a decrease in PNs around parvalbumin positive cells following SE

Next, we asked if SE alters the appearance of PNs that surround the parvalbumin-expressing inhibitory interneurons. The PN is a structure that normally surrounds the cell body and proximal processes of parvalbumin positive interneurons in the hippocampus (Fig. 2A–C). Nearly 80 percent of parvalbumin expressing cells in the hippocampus were ensheathed by this specialized matrix (Fig. 2G). Aggrecan expression around parvalbumin expressing cells did not change 48 hours after SE relative to control (Fig. 2G; two-way repeated measures ANOVA: $p=0.6644$; Bonferroni's post-hoc test $p>0.05$; DF=49). However, one week following SE, there was a significant decrease in aggrecan staining around parvalbumin cells in all regions of the hippocampus (Fig. 2H; two-way repeated measures ANOVA: $p=0.0013$; Bonferroni's post-hoc test $p<0.001$; DF=39). Two months post-SE all regions of the hippocampus displayed fewer aggrecan labeled PNs around parvalbumin immunoreactive cells. (Fig. 2I; two-way repeated measures ANOVA: $p=0.0002$; Bonferroni's post-hoc test CA3 and the subiculum $p<0.05$, CA1 $p<0.01$, DG and CA4 $p<0.001$; DG=39). We use wisteria floribunda agglutinin (WFA), another marker for PNs, and its expression was not altered at 48 hours post-SE, there was a significant decrease at one week and two months post-SE (Table 1; two-way ANOVA: $p=0.0881$, $p=0.0007$, $p=0.0001$ respectively; DF=49, 44, and 49 respectively).

### The integrity of the PN around parvalbumin positive cells is compromised following SE

At one week and two months after SE, in addition to having a loss in expression of PNs there was a simultaneous appearance of PNs with poor structural integrity (Fig. 3A,B). There was no change in the expression of degraded PNs 48 hours after SE (Fig. 3C; two-way repeated measures ANOVA: $p=0.4391$; Bonferroni's post-hoc test $p>0.05$; DF=49). There were significantly more degraded aggrecan labeled PNs one week following SE in all hippocampal regions except the dentate gyrus (Fig. 3D; two-way repeated measures ANOVA: $p=0.0004$; Bonferroni's post-hoc test CA4 $p<0.05$, CA3, CA1 and subiculum $p<0.001$; DF=34). Two months post-SE there were more degraded aggrecan positive PNs within all regions in the hippocampus, the subiculum was unchanged (Fig. 3E; two-way repeated measures ANOVA: $p=0.0003$; Bonferroni's post-hoc test DG and CA4 $p<0.001$, CA3 $p<0.05$, CA1 $p<0.01$; DF=39).

### Following SE parvalbumin positive cells increase at 1 week and are unchanged at 48 hours and 2 months

Importantly, the changes we observed in aggrecan expression were not associated with decreased parvalbumin expression. At 48 hours there was no change in the number of parvalbumin positive cells following SE (Table 2; Two-way ANOVA, $p=0.1218$; DF=49). One week following SE there was an increase in parvalbumin positive cells, but at two months there was no significant difference in the number of parvalbumin cells after SE (Table 2; two-way ANOVA, $p=0.0124$ and $p=0.0659$ respectively; DF=39 and 39 respectively). No differences in hippocampal *parvalbumin* mRNA expression levels between control and SE groups were detected at one week and two months post-SE (one-way ANOVA, $p=0.2890$; DF=38).

### The decrease in aggrecan protein expression precedes the decrease in *aggrecan* mRNA

To determine if the changes in aggrecan immunohistochemistry were associated with decreased transcription, we analyzed the expression of *aggrecan* mRNA using RT-PCR. There was a significant decrease in *aggrecan* mRNA (Table 3; one-way ANOVA, $p<0.0001$; DF=47). The Bonferroni post-hoc test showed no change in *aggrecan* mRNA levels 48 hours or one week following SE induction (Table 3; Bonferroni's post-hoc test $p>0.05$), with a significant decrease in *aggrecan* mRNA 2 months post-SE (Bonferroni's post-hoc test $p<0.0001$). This suggests that the chronic decreased expression of aggrecan in the PN is in part mediated by a late decrease in the transcription of *aggrecan* mRNA which does not account for PN loss prior to two months post-SE. These findings led us to investigate extracellular matrix components that stabilizing components of the PN.

### Hyaluronan and proteoglycan link protein 1 (HAPLN1) immunoreactivity decreases following SE and precedes the loss of aggrecan expressing PNs

To better understand why the PNs are poorly organized following SE, we investigated the expression of HAPLN1. HAPLN1 links aggrecan with hyaluronan, the most prominent component of the extracellular matrix (Mörgelin et al., 1994). In the adult hippocampus HAPLN1 expression was extensively colocalized with aggrecan, 92 % of HAPLN1 PNs were also aggrecan positive. A subset of aggrecan expressing PNs co-expressed HAPLN1 (11.6% of aggrecan expressing PNs in the dentate, 53.3% in CA4, 66.1% in CA3, 61.8% in CA1, and 60% in the subiculum). HAPLN1 cellular distribution closely mimics the pattern of aggrecan and was found around the cell body and proximal processes (Fig. 4A–C). Forty-eight hours post SE, HAPLN1 expression was significantly decreased (Fig. 4G; two-way repeated measures ANOVA: $p=0.012$; Bonferroni's post-hoc test CA4 and CA1 $p<0.0001$; DF=39). When HAPLN1 was present at 48 hours post-SE it was localized to the cell body (Fig. 4D) in comparison aggrecan was still expressed along the processes (Fig. 1J). One week after SE induction there was a significant decrease in HAPLN1 (Fig. 4E,H; two-way repeated measures ANOVA: $p=0.0023$; Bonferroni's post-hoc test CA1 $p<0.001$; DF=39). At two months HAPLN1 levels remained attenuated (Fig. 4F,I; two-way repeated measures ANOVA: $p=0.0003$; Bonferroni's post-hoc test CA4, CA3, CA1 $p<0.001$; DF=39). *HAPLN1* mRNA was significantly decreased at 2 months post-SE (Table 3; one-way ANOVA $p<0.0001$; Bonferroni's post-hoc test $p<0.0001$; DF=48). These data suggest that the process of epileptogenesis correlates with the loss of HAPLN1 protein and *HAPLN1* mRNA.

### Hyaluronan synthase 3 (HAS3) immunoreactivity decreases following SE and precedes the loss of aggrecan expressing PNs

To further investigate proteins capable of interacting with hyaluronan, we measured hyaluronan synthase 3 (HAS3) following SE. HAS3 is a membrane bound enzyme that both synthesizes and acts as a receptor for hyaluronan, immobilizing it on neuronal cell surfaces. The secretion and docking of hyaluronan forms a dense matrix sheath that is required for PN expression (Carulli et al., 2006; Galtrey et al., 2008; Kwok et al., 2010). Here we show that in the adult hippocampus, cells with aggrecan positive PNs express HAS3. However, HAS3 is also expressed in cells lacking an aggrecan positive PN (Fig. 5A,B,F and K,L,P). HAS3

expression decreased 48 hours post-SE (Fig. 5C,H,M,R) relative to the control (Fig. 5B,G). The loss of staining following SE was most apparent in CA3 (Fig. 5M,R). At both one week and two months following SE HAS3 immunoreactivity was markedly decreased (Fig. 5D–E, I–J, N–O, S–T). *HAS3* mRNA was not altered 48 hours post-SE, trended toward a decrease at one week, and was significantly decreased at 2 months (Table 3; one-way ANOVA $p=0.0007$; Bonferroni's post-hoc test $p<0.0001$; DF=44). Following SE there is a loss of HAS3 protein followed by the loss of *HAS3* mRNA. The loss of HAS3 protein appears to preceded the loss of aggrecan positive PNs.

### Neuronal activity *in vitro* alters the expression of multiple PN components

*In vivo* following SE we observed the loss of aggrecan, HAPLN1, and HAS3 along with a corresponding reduction in *aggrecan*, *HAPLN1*, and *HAS3* mRNA. To determine if neuronal activity could precipitate these effects *in vitro* we treated neuronal cultures with 20mM KCl for 6 hours or two days, with cultures fixed at 6 hours or one week respectively. Under control conditions aggrecan positive PNs were detected in neuronal cultures on the cell body and proximal neuronal processes (Fig. 6A1,A4,D1,D4). HAPLN1 expression was highly associated with aggrecan (Fig. 6A2,A4). HAS3 expression was associated with aggrecan, however it was also expressed in the soma and processes of cells lacking an aggrecan positive PN (Fig. 6D2,D4). HAPLN1 and HAS3 were expressed around the cell body and proximal processes in a similar pattern to aggrecan, therefore lack of co-expression along aggrecan positive processes was used as a parameter for abnormal HAPLN1 and HAS3 expression. After 6 hours of treatment with KCl the aggrecan component of the PN structure was still intact (Fig. 6B1,B4,E1,E4), HAPLN1 expression was significantly decreased and when visible only extended partially down the processes (Fig. 6B2,B4,G). HAS3 expression was also significantly decreased and localized to the cell body not extending down the processes (Fig. 6E2,E4,H). Following two days of KCl treatment aggrecan expression was diminished (Fig. 6C1,C4) and in some cases abolished (Fig. 6F1,F4). HAPLN1 expression was eliminated (Fig. 6C2,C4,G) while HAS3 expression remained restricted to the cell body (Fig. 6F2,F4,H). KCl treatment likely affects the general health of the neuronal cultures similar to the deleterious effects of SE, however the uniform size and expression of DAPI stained nuclei (Fig. 6A3,B3,C3,C3,E3,F3) and parvalbumin positive cells (data not shown) indicate that there are still viable cells even after the 2 day treatment. There was a significant decrease in HAPLN1 and HAS3 expression following 6 hours KCl treatment and two days of KCl treatment (One-way ANOVA, $p=0.0116$, $p<0.0001$ respectively). The Bonferroni post-test shows a significant decrease in HAPLN1 and HAS3 after KCl treatment for 6 hours $p<0.05$, $p<0.0001$ and two days $p<0.05$, $p<0.0001$ respectively; (DF=8 and 8 respectively). Taken together these data suggest that increased neuronal activity is sufficient to alter the expression of aggrecan in established PNs in culture. Similar to our *in vivo* findings HAPLN1 and HAS3 may be more acutely sensitive to excess neuronal activity since their expression was altered prior to changes in aggrecan.

## Discussion

In the present study, we demonstrate that the expression of PNs detected with the antibody Cat-315, a marker of aggrecan, and the lectin WFA decrease following SE and remained

diminished for at least two months. In addition to the loss of PNs in the hippocampus, there is an emergence of PNs with poor structural integrity, a phenotype that has not been reported previously. Furthermore, we show altered expression of both HAPLN1 and HAS3, two other net components, which may impact the stability of the PN. These effects are replicated *in vitro* with the addition of KCl to mature hippocampal neuron cultures. To our knowledge this is the first demonstration that SE, decreases aggrecan, HAPLN1, and HAS3 in the mature hippocampus.

### Aggrecan expression in the PN is dependent on normal neuronal activity in the adult

The PN component of the extracellular matrix (ECM) likely plays a role in synapse stabilization (Frischknecht et al., 2009). Previous work has demonstrated that synapses on GABAergic parvalbumin-positive cells are surrounded by PNs and that the PN may contribute to the ability of these cells to maintain high rates of action potential firing (Morris and Henderson, 2000). Interestingly, loss of tenascin-R and disruption of HNK-1 components of the PN caused diminished sensitivity to direct stimulation of inhibitory interneurons in CA1 (Saghatelyan et al., 2001). Loss of the PN could lead to disruption of inhibition, as the structure is primarily expressed around inhibitory cells, and may contribute to the increased excitation found in epilepsy.

The expression of the aggrecan component of the PN has been shown to require neuronal activity during development for normal expression based on studies where attenuated activity disrupted aggrecan formation (Sur et al., 1988; Kalb and Hockfield 1988, 1990; Hockfield et al., 1990; McRae et al., 2007). The same studies show that in the mature central nervous system once the net structure develops, activity is not required for maintenance of the PN and expression is unchanged following decreased activity paradigms. In contrast, here we demonstrate that SE leads to a persistent loss in aggrecan containing PNs around parvalbumin cells in the adult hippocampus. The decrease of aggrecan-positive PNs is detected in all sub regions of the dorsal hippocampus. At one week and two months post-SE all regions of the hippocampus displayed significantly less aggrecan labeled PNs around parvalbumin cells than control counter parts. Two months post-SE there is a modest level of recovery relative to the one week post-SE group, which is driven by slight increases of PN expression in CA3, CA1 and the subiculum (Bonferroni Post-test $p<0.01$, $p<0.01$, $p<0.05$ respectively). This recovery does not reach control levels, therefore, the decrease in PNs is long-lasting following SE. In addition, at one week and two months after SE, PNs lacking structural integrity are present. Using excess KCl as a simple paradigm for increased neuronal activity *in vitro* yields similar results with a decrease in aggrecan containing PNs and the presence of poorly formed PNs.

Importantly, the attenuation of aggrecan expression was not associated with the loss of parvalbumin positive interneurons. We demonstrate that parvalbumin levels in the dorsal hippocampus were not attenuated, and actually increased one week following SE induction. It is unclear why there was an increase in parvalbumin immunostaining at this time-point, the increase may be due to a transient increase in parvalbumin expression in the hippocampus. However, epileptic tissue is frequently associated with a loss of interneurons (for review see Dudek and Shao, 2003). Interneuron loss is most severe in the ventral/

temporal dentate gyrus of the hippocampus, which is often the only region to show a reduction in parvalbumin (Buckmaster and Dudek, 1997; Kobayashi and Buckmaster, 2003). One possible reason for our study showing no loss of parvalbumin cells is we focused on the dorsal hippocampus for our stereological counts as opposed to the ventral/temporal hippocampus. In fact a decrease in mRNA for GAD65, a global marker for interneurons, was more acute in slices form the temporal hippocampus than dorsal slices (Kobayashi and Buckmaster, 2003). However, our RT-PCR data included the entire hippocampus and we did not detect a change in *parvalbumin* mRNA levels following SE. Taken together, these data indicate that, depending on the location within the hippocampus SE may differentially affect interneurons, including parvalbumin immunoreactive cells.

We have demonstrated that aggrecan protein, detected with the antibody Cat-315, is decreased around parvalbumin cells in the hippocampus following SE. The loss of PNs we observe at the two month time point correlated with a decrease in *aggrecan* mRNA. But the decreased mRNA levels would not explain the loss of PNs at one week. One possibility is that there is in fact a decrease in aggrecan mRNA that we are unable to detect because our RT-PCR was done on whole hippocampal homogenate and not just the dorsal hippocampus. If there was a specific effect present only in the dorsal hippcampas our methods may not be sensitive enough to detect it. However this explanation would not account for the presence of PNs with poor structural integrity. To our knowledge, this is the first time that PNs with poor structural integrity have been described. Previously, decreases in *aggrecan* mRNA correlated with fewer PNs without the presence of PNs with poor structural integrity (McRae et al., 2007). Therefore, the decrease in *aggrecan* mRNA is not likely to be a major driving force behind the loss of PN structural compactness.

### Other components of the PN are altered by increased neuronal activity

To better understand the effects of epileptogensis on the PN and the presence of the degraded PN we investigated two other PN components HAPLN1 and HAS3. The PN is thought to provide structural stability at synapses as well as an organizational scaffold for the surrounding extracellular space. Hyaluronan is the most abundantly expressed component of the ECM, and molecules that bind hyaluronan help organize the extracellular space. Lecticans, the family of chondroitin sulfate PGs (CSPGs) to which aggrecan belongs, have a large globular N-terminal capable of binding hyaluronan, and a C-terminal domain that can bind other ECM and cell-surface molecules. This unique ability of lecticans to bind hyaluronan, allows them to serve as a molecular bridge between ECM and cells, giving them a prominent role in organizing the extracellular space (Yamaguchi, 2000).

HAPLN is thought to stabilize interactions between lecticans and the hyaluronan backbone of the PN, contributing to its compact lattice structure. A study by Carulli and colleagues (2010) supported the importance of HAPLN in PN distribution by exploring HAPLN1 knockout animals. They found that knockout animals have decrease PN production, resulting in residual PN formation around the cell somata with no coverage of proximal dendrites. Here we show that HAPLN1 protein and mRNA decrease following SE, which may contribute to the appearance of PNs with an abnormal aggrecan pattern of expression.

HAS3 has been shown to have a dual role, including promoting the synthesis of hyaluronan and acting as a receptor for hyaluronan on neurons (Kwok et al., 2010). Members of the HAS family are expressed before the PN develops making it a candidate for initiating PN development and expression. All cells with a PN express one of three HASs (Carulli et al., 2006, 2007; Galtrey et al., 2008), with HAS3 being most commonly co-expressed with aggrecan expressing PNs (Kwok et al., 2010). In HEK cell lines co-expression of HAS3, HAPLN1 and aggrecan was necessary for the formation of compact PNs in the extracellular matrix (Kwok et al., 2010). The ability to bind and dock hyaluronan in the adult extracellular space is required for PN expression and aggrecan, HAPLN1, and HAS3 are capable of doing this (Carulli et al., 2006).

During development, high levels of hyaluronan keep the ECM permissive for axonal outgrowth and cell motility, because of its ability to provide large hydrated spaces. In adults hyaloronan is expressed at lower levels and the ECM is less soluble. Hyaluronan insolubility in the adult is likely due to interaction with lecticans through their hyaluronan-binding domain. In the mature brain, aggrecan binds hyaluronan and forms insoluble aggregates within the extracellular space (Rauch, 2004). These insoluble aggregates ultimately seem to play an important role in the decreased plasticity and motility found in the mature nervous system (Rauch, 2004). Interestingly, seizures have been shown to increase hyaluronan in the hippocampus, both *in vivo* and *in vitro*, and this upregulation is believed to play a role in mossy fiber sprouting (Perosa et al., 2002a,b Bausch, 2006).

Here we show that 48 hours after SE HAPLN1 and HAS3 decreased, prior to the decrease in aggrecan immunostaining within the PNs. It is possible that the decrease in these two important PN stabilizing proteins contributed to the appearance of aggrecan expressing PNs with poor structural integrity. In human and animal models of TLE hyaluronan was increased (Perosa et al., 2002a,b Bausch, 2006). The increase in hyaluronan and the decreases in aggrecan, HAPLN1, and HAS3 we describe suggest an increase in unbound hyaluronan, likely contributing to increased neurite outgrowth and synaptic plasticity after SE.

## Changes in aggrecan, HALPN1, and HAS3 protein precede changes in their respective mRNAs

All of the decreases in protein expression, aggrecan, HAPLN1 and HAS3 preceded the decrease in mRNA. This suggests an additional mechanism exists for altering protein production/expression prior to the observed decrease in mRNA levels. One possibility is reduced or suppressed translation impacting the production of functional proteins. Another possibility is that there are alterations in post-translational modifications to the proteins altering their ability to contribute to the PN structure. There is evidence for post-translational modifications taking place in the production of aggrecan (Matthews et al., 2002), HAPLN1 (Roughley et al., 1982), and HAS3 (for review see Tammi et al., 2011; Goentzel et al., 2006). Alterations in post-translational modifications of these proteins may lead to diminished or non-functioning proteins. An additional possibility is that the proteins are produced and fully functional but due to alterations in other components remain diffuse in the matrix unable to form a proper PN.

The mechanism for the loss of HAPLN1, and HAS3 at 48 hours, prior to any detectable change in aggrecan suggests they are undergoing changes in protein translation, production or degradation earlier than aggrecan. We cannot however rule out that or our methods for detecting changes in HAPLN1 and HAS3 expression are more sensitive than aggrecan. These data suggests either suppressed translation, altered post-translation modifications, or protein degradation contributes to the loss of the PNs in addition to suppressed transcription at two months. Future studies are needed to further clarify the mRNA independent changes in aggrecan, HAPLN1, and HAS3 prior to two months post-SE.

### Functional implication of PN loss in the progression of epileptogenesis

Interestingly, SE in the developing and mature brain has opposing effects on the aggrecan component of the PN. Our previous work showed that SE induced before PN development leads to aggrecan-positive PN expression earlier in the dorsal hippocampus with normal levels of expression in the adult (McRae et al., 2010). However, as the current study demonstrates, SE induced in the adult affects multiple components of the PN and leads to a persistent decrease in aggrecan-positive PNs as well as the emergence of PNs with diminished structural integrity and compactness. One difference between the adult and developing brain's response to SE is the adults tend to develop frequent spontaneous seizure and robust mossy fiber sprouting while SE induction early in life, leads to infrequent spontaneous seizures and modest mossy fiber sprouting (Jensen et al., 1992; Dube et al., 2000; Zhang et al., 2004a,b; Raol et al., 2006). It is possible that the transient increase of aggrecan early in development contributes to the milder synaptic rearrangement while the loss of aggrecan and the PN in the adult may provide a permissive extracellular environment for the remodeling of neural networks that coincides with more severe epilepsy.

SE induction in adult rats led to changes in the PN throughout the dorsal hippocampus. There was some level of recovery of PN expression by two months in all regions except the dentate gyrus and CA4. Fewer PNs on parvalbumin interneurons in the adult dentate gyrus may leave interneurons more susceptible to synaptic remodeling. Interestingly, parvalbumin basket cells, the primary cell with a PN within the dentate gyrus display reduced synaptic input and output following pilocarpine induced SE (Zhang and Buckmaster 2009). Two months following SE the parvalbumin cells in the dentate had the lowest PN expression and the highest level of degraded PNs, which might contribute to their reduced synaptic input.

Here, we describe changes in components of the PN, following SE in adulthood. Decreases in aggrecan containing PNs around parvalbumin cells throughout the dorsal hippocampus occurred concurrent with a loss of structural integrity in some of the remaining PNs. Decreases in HAPLN1 and HAS3 protein expression occurred prior to the loss of aggrecan, likely contributing to the deterioration of the matrix by decreasing aggrecan's ability to bind hyaluronan. Taken together the data suggest that following SE changes in the PN may contribute to a more permissive extracellular environment within the hippocampus providing a space conducive for the progression of epileptogenisis.

## References


Bausch SB. Potential roles for hyaluronan and CD44 in kanic acid-induced mossy fiber sprouting in organotypic hippocampal slice cultures. Neurosci. 2006; 143(1):339–50.

Borges K, McDermott DL, Dingledine R. Reciprocal changes of CD44 and GAP-43 expression in the dentate gyrus inner molecular layer after status epilepticus in mice. Exp Neurol. 2004; 188(1):1–10. [PubMed: 15191797]

Brückner G, Brauer K, Hartig W, Wolff JR, Rickmann MJ, Derouiche A, Delpech B, Girard N, Oertel WH, Reichenbach A. Perineuronal nets provide a polyanionic, glia-associated form of microenvironment around certain neurons in many parts of the rat brain. Glia. 1993; 8:183–200. [PubMed: 7693589]

Brückner G, Grosche J. Perineuronal nets show intrinsic patterns of extracellular matrix differentiation in organotypic slice culture. Exp Brain Res. 2001; 137:83–93. [PubMed: 11310175]

Buckmaster PS, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. J Neurophysiol. 1997; 77(5):2685–2696. [PubMed: 9163384]

Carulli D, Pizzorusso T, Kwok JC, Putignano E, Poli A, Forostyak S, Andrews MR, Deepa SS, Glant TT, Fawcett JW. Animals lacking link protein have attenuated perineuronal nets and persistent plasticity. Brain. 2010; 133(Pt 8):2331–47. [PubMed: 20566484]

Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K, Strata P, Fawcett JW. Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol. 2006; 494(4):559–77. [PubMed: 16374793]

Cummings D, Wilcox K, Dichter M. Calcium-dependent paired-pulse facilitation of miniature EPSC frequency accompanies depression of EPSCs at hippocampal synapses in culture. J Neurosci. 1996; 16:5312–5323. [PubMed: 8757244]

Dino MR, Harroch S, Hockfield S, Matthews RT. Monoclonal antibody Cat-315 detects a glycoform of receptor protein tyrosine phosphatase beta/phosphacan early in CNS development that localizes to extrasynaptic sites prior to synapse formation. Neuroscience. 2006; 142:1055–1069. [PubMed: 16989954]

Dube C, Chen K, Eghbal-Ahmadi M, Brunson K, Soltesz I, Baram TZ. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. Ann Neurol. 2000; 47:336–344. [PubMed: 10716253]

Dudek FE, Shao LR. Loss of GABAergic Interneurons in Seizure-induced Epileptogenesis. Epilepsy Curr. 2003; 3(5):159–161. [PubMed: 15902313]

Frischknecht R, Heine M, Perrais D, Seidenbecher CI, Choquet D, Gundelfinger ED. Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. Nat Neurosci. 2009; 12:897–904. [PubMed: 19483686]

Galtrey CM, Kwok JC, Carulli D, Rhodes KE, Fawcett JW. Distribution and synthesis of extracellular matrix proteoglycans, hyaluronan, link proteins and tenascin-R in the rat spinal cord. Eur J Neurosci. 2008; 27(6):1373–90. [PubMed: 18364019]

Giamanco KA, Matthews RT. Deconstructing the perineuronal net: Cellular contributions and molecular composition of the neuronal extracellular matrix. Neuroscience. 2012; 218:367–84. [PubMed: 22659016]

Giamanco KA, Morawski M, Matthews RT. Perineuronal net formation and structure in aggrecan knockout mice. Neuroscience. 2010; 170(4):1314–27. [PubMed: 20732394]

Goentzel BJ, Weigel PH, Steinberg RA. Recombinant human hyaluronan synthase 3 is phosphorylated in mammalian cells. Biochem J. 2006; 1;396(2):347–54.

Gombos Z, Spiller A, Cottrell GA, Racine RJ, McIntyre Burnham W. Mossy fiber sprouting induced by repeated electroconvulsive shock seizures. Brain Res. 1999; 844(1–2):28–33. [PubMed: 10536258]

Guimaraes A, Zaremba S, Hockfield S. Molecular and morphological changes in the cat lateral geniculate nucleus and visual cortex induced by visual deprivation are revealed by monoclonal antibodies Cat-304 and Cat-301. J Neurosci. 1990; 10:3014–3024. [PubMed: 1697900]

Gundersen HJ. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. J Microsc. 1986; 143:3–45. [PubMed: 3761363]

Hockfield S, Kalb RG, Zaremba S, Fryer H. Expression of neural proteoglycans correlates with the acquisition of mature neuronal properties in the mammalian brain. Cold Spring Harb Symp Quant Biol. 1990; 55:505–514. [PubMed: 2132834]

Jensen FE, Holmes GL, Lombroso CT, Blume HK, Firkusny IR. Age-dependent changes in long-term seizure susceptibility and behavior after hypoxia in rats. Epilepsia. 1992; 33:971–980. [PubMed: 1464280]

Kalb RG, Hockfield S. Molecular evidence for early activity-dependent development of hamster motor neurons. J Neurosci. 1988; 8:2350–2360. [PubMed: 3249230]

Kalb RG, Hockfield S. Induction of a neuronal proteoglycan by the NMDA receptor in the developing spinal cord. Science. 1990; 250:294–296. [PubMed: 2145629]

Kind PC, Beaver CJ, Mitchell DE. Effects of early periods of monocular deprivation and reverse lid suture on the development of Cat-301 immunoreactivity in the dorsal lateral geniculate nucleus (dLGN) of the cat. J Comp Neurol. 1995; 359:523–536. [PubMed: 7499545]

Kind PC, Sengpiel F, Beaver CJ, Crocker-Buque A, Kelly GM, Matthews RT, Mitchell DE. The Development and Activity-Dependent Expression of Aggrecan in the Cat Visual Cortex. Cereb Cortex. 2012 Feb 23.10.1093/cercor/bhs015

Kobayashi M, Buckmaster PS. Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. J Neurosci. 2003; 23(6):2440–2452. [PubMed: 12657704]

Kurazono S, Okamoto M, Sakiyama J, Mori S, Nakata Y, Fukuoka J, Amano S, Oohira A, Matsui H. Expression of brain specific chondroitin sulfate proteoglycans, neurocan and phosphacan, in the developing and adult hippocampus of Ihara's epileptic rats. Brain Res. 2001; 898:36–48. [PubMed: 11292447]

Kwok JC, Carulli D, Fawcett JW. In vitro modeling of perineuronal nets: hyaluronan synthase and link protein are necessary for their formation and integrity. J Neurochem. 2010; 1;114(5):1447–1459.

Lander C, Kind P, Maleski M, Hockfield S. A family of activity-dependent neuronal cell-surface chondroitin sulfate proteoglycans in cat visual cortex. J Neurosci. 1997; 17:1928–1939. [PubMed: 9045722]

Matsui F, Kawashima S, Shuo T, Yamauchi S, Tokita Y, Aono S, Keino H, Oohira A. Transient expression of juvenile-type neurocan by reactive astrocytes in adult rat brains injured by kainate induced seizures as well as surgical incision. Neuroscience. 2002; 112:773–781. [PubMed: 12088737]

Matthews RT, Kelly GM, Zerillo CA, Gray G, Tiemeyer M, Hockfield S. Aggrecan glycoforms contribute to the molecular heterogeneity of perineuronal nets. J Neurosci. 2002; 22:7536–7547. [PubMed: 12196577]

McRae PA, Baranov E, Sarode S, Brooks-Kayal AR, Porter BE. Aggrecan expression, a component of the inhibitory interneuron perineuronal net, is altered following an early-life seizure. Neurobiol Dis. 2010 Sep; 39(3):439–48. [PubMed: 20493259]

McRae PA, Rocco MM, Kelly G, Brumberg JC, Matthews RT. Sensory deprivation alters aggrecan and perineuronal net expression in the mouse barrel cortex. J Neurosci. 2007; 27:5405–5413. [PubMed: 17507562]

Miyata S, Nishimura Y, Hayashi N, Oohira A. Construction of perineuronal net-like structure by cortical neurons in culture. Neuroscience. 2005; 136(1):95–104. [PubMed: 16182457]

Mörgelin M, Heinegård D, Engel J, Paulsson M. The cartilage proteoglycan aggregate: assembly through combined protein-carbohydrate and protein-protein interactions. Biophys Chem. 1994; 50(1–2):113–28. [PubMed: 8011926]

Okamoto M, Sakiyama J, Mori S, Kurazono S, Usui S, Hasegawa M, Oohira A. Kainic acid-induced convulsions cause prolonged changes in the chondroitin sulfate proteoglycans neurocan and phosphacan in the limbic structures. Exp Neurol. 2003; 184:179–195. [PubMed: 14637091]

Morris NP, Henderson Z. Perineuronal nets ensheath fast spiking, parvalbumin-immunoreactive neurons in the medial septum/diagonal band complex. Eur J Neurosci. 2000; 12(3):828–838. [PubMed: 10762312]

Perosa SR, Porcionatto MA, Cukiert A, Martins JR, Passeroti CC, Amado D, Matas SL, Nader HB, Cavalheiro EA, Leite JP, Naffah-Mazzacoratti MG. Glycosaminoglycan levels and protoeglycan expression are altered in the hippocampus of patients with mesial temporal lobe epilepsy. Brain Res Bull. 2002a Sep; 15;58(5):509–16.

Perosa SR, Porcionatto MA, Cukiert A, Martins JR, Amado D, Nader HB, Cavalheiro EA, Leite JP, Naffah-Mazzacoratti MG. Extracellular matrix components are altered in the hippocampus, cortex, and cerebrospinal fluid of patients with mesial temporal lobe epilepsy. Epilepsia. 2002b; 43(Suppl 5):159–161. [PubMed: 12121313]

Raol YH, Zhang G, Lund I, Porter BE, Maronski M, Brooks-Kayal AR. Increased GABA(A) receptor alpha one subunit expression in hippocampal dentate gyrus after early-life status epilepticus. Epilepsia. 2006; 47(10):1665–73. [PubMed: 17054689]

Rauch U. Extracellular matrix components associated with remodeling processes in brain. Cell Mol Life Sci. 2004; 61:2031–2045. [PubMed: 15316653]

Roughley PJ, Poole AR, Mort JS. The heterogeneity of link proteins isolated from human articular cartilage proteoglycan aggregates. J Biol Chem. 1982; 25;257(20):11908–14.

Saghatelyan AK, Dityatev A, Schmidt S, Schuster T, Bartsch U, Schachner M. Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R. Mol Cell Neurosci. 2001; 17(1):226–240. [PubMed: 11161481]

Shibley H, Smith BN. Pilocarpine-induced status epilepticus results in mossy fiber sprouting and spontaneous seizures in C57BL/6 and CD-1 mice. Epilepsy Res. 2002; 49(2):109–20. [PubMed: 12049799]

Sur M, Frost DO, Hockfield S. Expression of a surface-associated antigen on Y-cells in the cat lateral geniculate nucleus is regulated by visual experience. J Neurosci. 1988; 8:874–882. [PubMed: 3346725]

Tammi RH, Passi AG, Rilla K, Karousou E, Vigetti D, Makkonen K, Tammi MI. Transcriptional and post-translational regulation of hyaluronan synthesis. FEBS J. 2011; 278(9):1419–28. [PubMed: 21362137]

Treiman DM, Walton NY, Kendrick C. A progressive sequence of electroencephalographic changes during generalized convulsive status epilepticus. Epilepsy Res. 1990 Jan-Feb;5(1):49–60. [PubMed: 2303022]

Wilcox K, Dichter M. Paired pulse depression in cultured hippocampal neurons is due to a presynaptic mechanism independent of GABAB autoreceptor activation. J Neurosci. 1994; 14:1775–1788. [PubMed: 8126570]

Yamaguchi Y. Brevican: a major proteoglycan in adult brain. Perspect Dev Neurobiol. 1996; 3:307–317. [PubMed: 9117262]

Yamaguchi Y. Lecticans: organizers of the brain extracellular matrix. Cell Mol Life Sci. 2000; 57:276–289. [PubMed: 10766023]

Yuan W, Matthews RT, Sandy JD, Gottschall PE. Association between protease-specific proteolytic cleavage of brevican and synaptic loss in the dentate gyrus of kainate-treated rats. Neurosci. 2002; 114:1091–1101.

Zhang G, Raol YH, Hsu FC, Coulter DA, Brooks-Kayal AR. Effects of status epilepticus on hippocampal GABAA receptors are age-dependent. Neurosci. 2004a; 125:299–303.

Zhang G, Raol YS, Hsu FC, Brooks-Kayal AR. Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. J Neurochem. 2004b; 88:91–101. [PubMed: 14675153]

Zhang W, Buckmaster PS. Dysfunction of the dentate basket cell circuit in a rat model of temporal lobe epilepsy. J Neurosci. 2009 Jun; 17;29(24):7846–56.

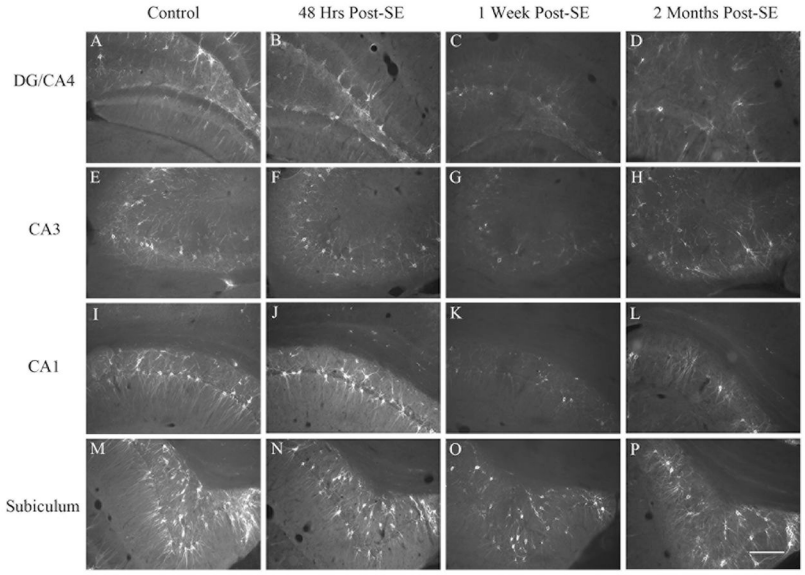


**Figure 1. Status epilepticus decreases expression of aggrecan positive PNs in the rat dorsal hippocampus**

PN expression was observed in the dentate gyrus/CA4 (A–D), CA3 (E–H), CA1 (I–L), and subiculum (M–P) under control conditions (A, E, I, M) or following pilocarpine induced SE. Aggrecan expression did not differ from control 48 hours post-SE (B, F, J, N). One week post-SE there was a decrease in the expression of Cat-315 in all regions of the hippocampus, (C, G, K, O), which remained attenuated two months following SE (D, H, L, P). Scale bar, 200 μm.

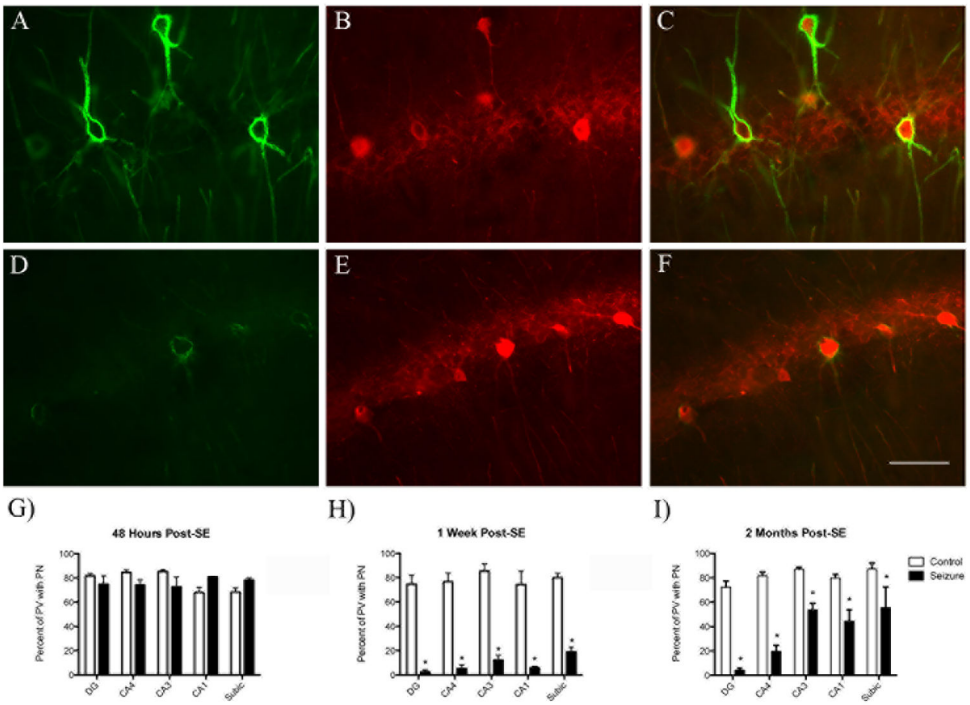


**Figure 2. Status epilepticus alters the quantity of Aggrecan expressing PNs surrounding parvalbumin interneurons in the rat dorsal hippocampus**

In the control CA1 region of the hippocampus aggrecan PNs (green) encapsulated parvalbumin cells (red) (A–C). Two months post-SE there were parvalbumin cells lacking the PN (D–F). Stereological counts of aggrecan positive PNs with normal morphology (G–I) and were performed at 48 hours (G), one week (H), and two months (I) post-SE. There was no change in aggrecan expressing PNs 48 hours after SE (G). There was a significant decrease in aggrecan PNs one week following SE in all regions of the hippocampus Bonferroni's post-hoc test $p<0.001$ (H). There was a significant decrease in aggrecan PNs two months post-SE in all regions of the hippocampus, Bonferroni's post-hoc test CA3 and the subiculum $p<0.05$, CA1 $p<0.01$, DG and CA4 $p<0.001$ (I). Error bars represent the standard error of the mean. Scale bar, 50 μm.

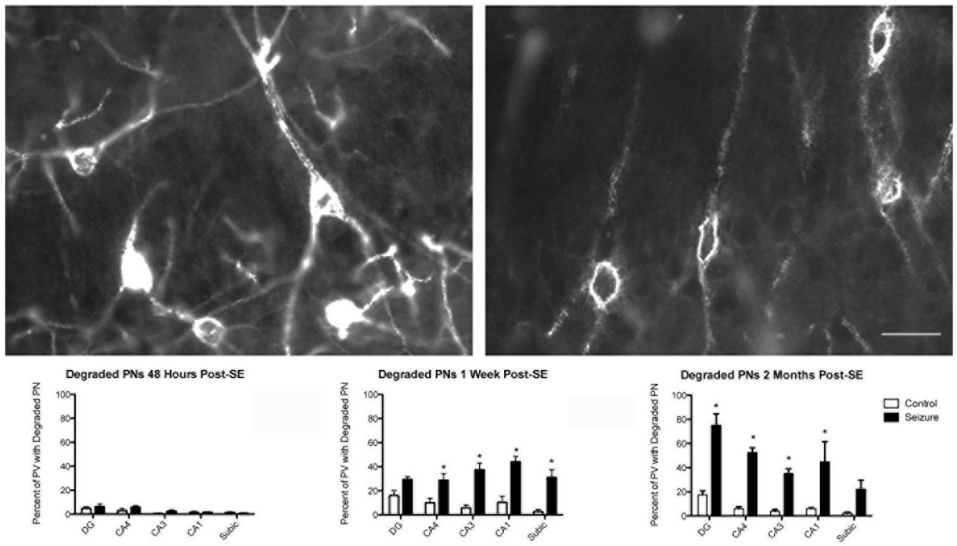


**Figure 3. Status epilepticus alters the quality of aggrecan expressing PNs surrounding parvalbumin interneurons in the rat dorsal hippocampus**

The PN normally ensheathes the soma and proximal processes (A), however after SE the structural integrity of the PN was compromised (B). Stereological counts of aggrecan positive PNs with poor integrity (degraded) (C–E) were performed at 48 hours (C), one week (D), and two months (E) post-SE. There were few degraded PNs 48 hours after SE (C). There was a significant increase in degraded PNs in all regions excluding the dentate gyrus at one week, Bonferroni's post-hoc test CA4 $p<0.05$, CA3, CA1 and subiculum $p<0.001$ (D). There was a significant increase in degraded PNs in all regions excluding the subiculum two months post-SE, Bonferroni's post-hoc test DG and CA4 $p<0.001$, CA3 $p<0.05$, CA1 $p<0.01$ (E). Error bars represent the standard error of the mean. Scale bar, 40 μm.

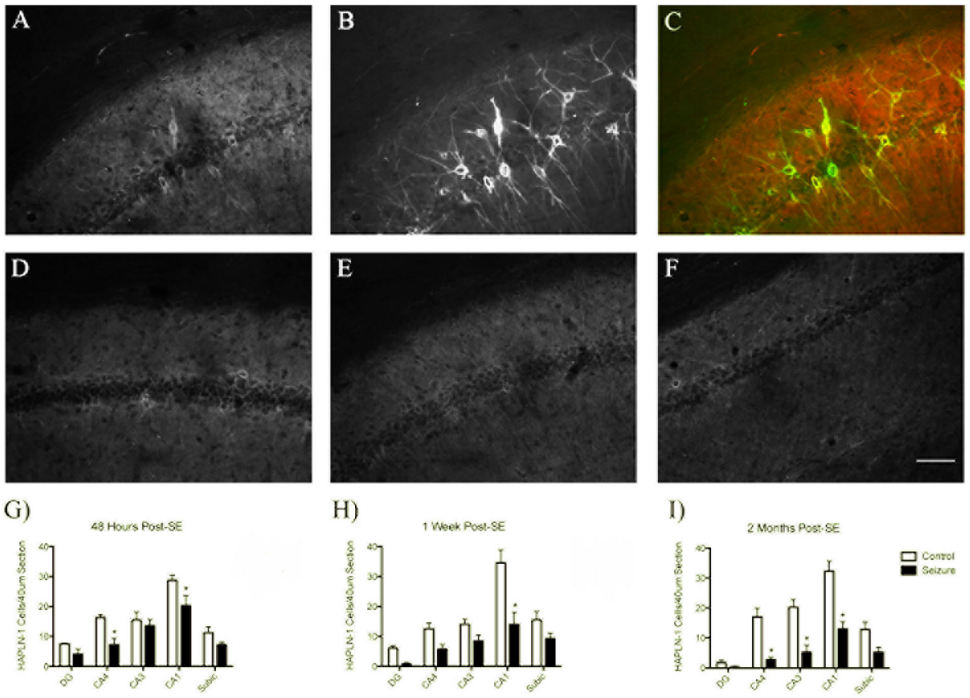


**Figure 4. HAPLN1 expression decreases in the rat dorsal hippocampus following status epilepticus**

In the control CA1 region HAPLN1 (A) and aggrecan (B) colocalize (C, HAPLN1 is red, Cat-315 is green). HAPLN1 expression decreased in the CA1 region 48 hours (D), one week (E) and two months (F) following SE. HAPLN1 expression significantly decreased 48 hours post-SE, Bonferroni's post-hoc test CA4 and CA1 $p<0.0001$ (G), and remains attenuated one week post-SE, Bonferroni's post-hoc test CA1 $p<0.001$ (H), and two months post-SE, Bonferroni's post-hoc test CA4, CA3, CA1 $p<0.001$ (I). Error bars represent the standard error of the mean. Scale bar, 100 μm.

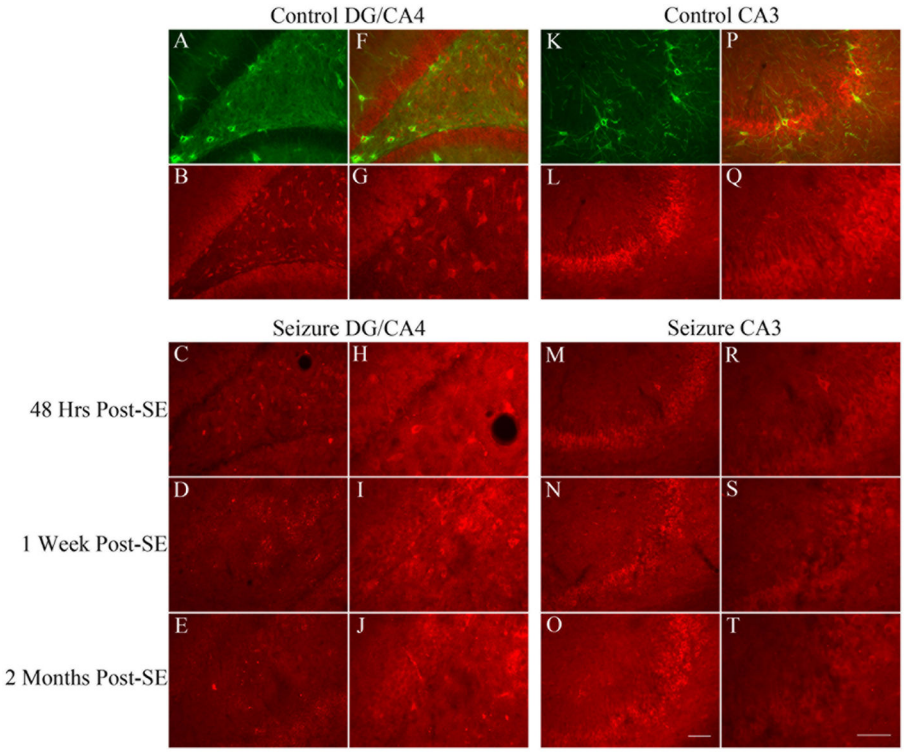


**Figure 5. HAS3 expression is decreased in the rat dorsal hippocampus following status epilepticus**

In the DG/CA4 region aggrecan (green) colocalizes with HAS3 (red) (A,B,F). Aggrecan colocalized with HAS3 in CA3 (K,L,P). There was a decrease in HAS3 expression 48 hours post-SE in DG/CA4region (C,H) and CA3(M,R). There was a more pronounced decrease in HAS3 1 week post-SE in the DG/CA4 and CA3 regions (D,I; N,S). HAS3 expression remained suppressed at 2 months in the DG/CA4 and CA3 regions (E,J; O,T). High magnification images (G–J and Q–T). Scale bar, 100 μm (A–F, K–P). Scale bar 50 μm (G–J, Q–T).

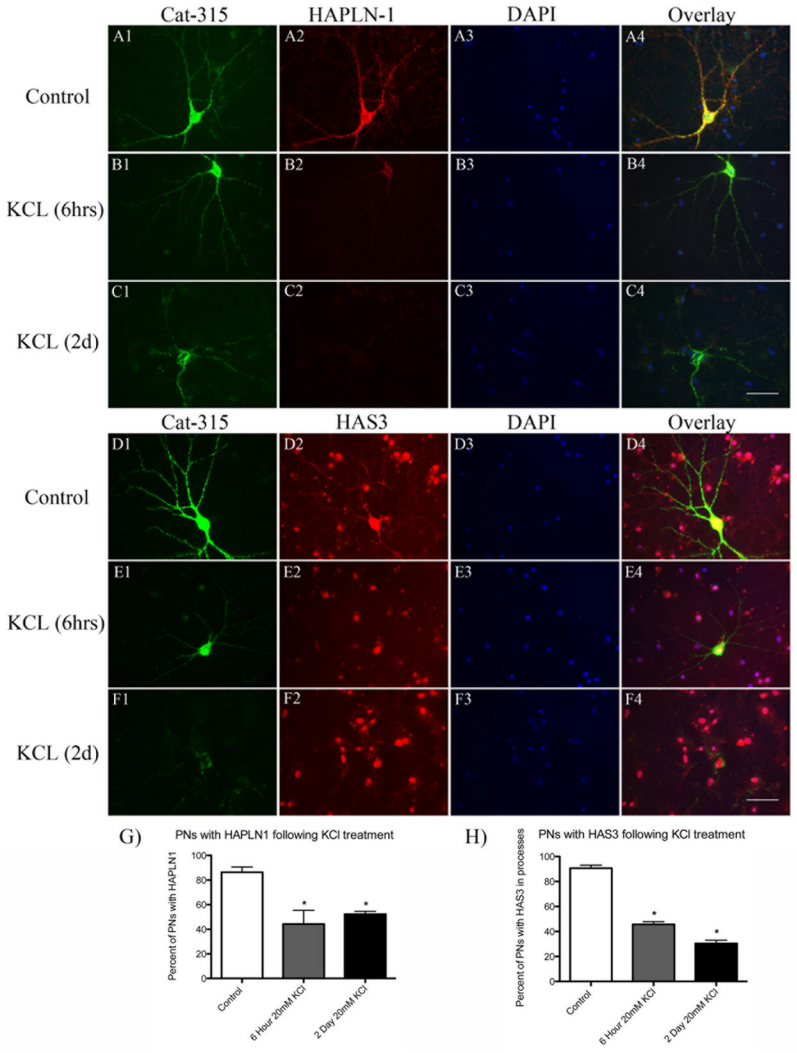


**Figure 6. Increased neuronal activity in-vitro decreases aggrecan, HAPLN1 and HAS3 expression**

Saline (A1-A4, D1-D4) or 20 mM KCl was added to hippocampal neurons at 30 DIV for 6 hours (B1-B4, E1-E4) or 2 days (C1-C4, F1-F4). In the control condition aggrecan expression was found in the cell body and along the processes of hippocampal neurons (A1, A4, D1, D4), 6 hours after the addition of the addition of KCl aggrecan was still expressed (B1, B4, E1, E4). HAPLN1 expression was significantly decreased throughout the cell (B2, B4, G) and HAS3 expression was limited to the soma and no longer expressed in the processes (E2, E4, H). However, following 2 days of KCl treatment, with cultures fixed at one week, aggrecan expression decreased (C1, C4, F1, F4) while HAPLN1 (C2, C4, G) and HAS3 (F2, F4, H) levels remain depressed. DAPI expression (A3, B3, C3, D3, E,3, F3). The Bonferroni post-test shows a significant decrease in HAPLN1 after KCl treatment for 6 hours ($p<0.05$) and two days ($p<0.05$) and HAS3 after 6 hours ($p<0.0001$) and two days ($p<0.0001$). Scale bar, 50 μm.

*Eur J Neurosci*. Author manuscript; available in PMC 2014 June 16.

**Table 1**

Stereological counts of WFA Positive Perineuronal Nets in the Dorsal Hippocampus

| | 48 Hours | | 1 Week | | 2 Months | |
|---|---|---|---|---|---|---|
| **Region** | **Control n=6** | **Seizure n=4** | **Control n=5** | **Seizure n=4** | **Control n=6** | **Seizure n=4** |
| **Dentate** | 100±11.1 | 55.6±10.2 | 100±14.5 | ***29.5±9.6**** | 100±9.4 | ***18.8±3.6***** |
| **CA4** | 100±15.0 | 79.6±20.2 | 100±14.0 | 50.4±15.8 | 100±11.4 | ***31.5±11.8**** |
| **CA3** | 100±14.6 | 100.4±22.6 | 100±12.9 | 63.6±13.7 | 100±11.1 | ***27.0±3.4***** |
| **CA1** | 100±12.5 | 60.2±13.2 | 100±7.0 | 88.5±10.8 | 100±9.1 | 77.2±14.7 |
| **Subiculum** | 100±16.9 | 76.7±7.1 | 100±18.8 | 83.3±22.1 | 100±30.4 | 47.6±7.8 |
| **P-Value** | | 0.0881 | | **0.0007** | | **0.0001** |

Percent of WFA positive PNs in seizure animals relative to control animals. Status epilepticus was induced with Pilocarpine and WFA counts were conducted at 48 hours, 1 week, and 2 months post-SE. Values shown are ± the standard error of the mean. There was a significant decrease in WFA immunolabeled PNs at one week and 2 months post-SE (One-way ANOVA; p=0.0007 and p=0.0001 respectively). The Bonferroni post-hoc test showed a significant decrease in the dentate gyrus at one week, and in the dentate gyrus, CA4 and CA3 at two months (* p<0.05, ** p<0.01, ***p<0.0001).

**Table 2**

Stereological counts of Parvalbumin Positive Cells in the Dorsal Hippocampus

| | 48 Hour | | 1 Week | | 2 Months | |
|---|---|---|---|---|---|---|
| **Region** | **Control n=6** | **Seizure n=4** | **Control n=5** | **Seizure n=4** | **Control n=4** | **Seizure n=4** |
| **DG** | 876±132 | 846±138 | 486±44 | 643±48 | 527±170 | 396±55 |
| **CA4** | 732±75 | 655±131 | 546±77 | 711±29 | 799±103 | 479±143 |
| **CA3** | 1101±142 | 892±306 | 981±180 | 1231±185 | 1007±77 | 864±98 |
| **CA1** | 2983±294 | 2067±723 | 1872±184 | ***2583±202**** | 2567±103 | 1591±285 |
| **Subic** | 1938±157 | 965±230 | 1075±148 | 1364±220 | 938±273 | 751±165 |
| **P-Value** | | 0.1218 | | **0.0124** | | 0.0659 |

Status epilepticus was induced with Pilocarpine. Parvalbumin immunolabeling was evaluated 48 hours, 1 week, or 2 months post-SE. The number of parvalbumin labeled interneurons in the hippocampus was estimated based on stereological methods ± the standard error of the mean. There was no significant decrease in the number of parvalbumin cells in the control animals compared to the seizure animals. There was a transient increase in parvalbumin 1 week post-SE (Two-way repeated measures ANOVA; p=0.0124) the Bonferroni post-hoc test showed a significant decrease in CA1 (* p<0.05).

**Table 3**

Real Time PCR for *Aggrecan*, *HAPLN1*, *HAS3* mRNA

| | ***Aggrecan*** | | | ***HAPLN1*** | | | ***HAS3*** | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***n*** **CT, SE** | **Control** | **Seizure** | ***n*** **CT, SE** | **Control** | **Seizure** | ***n*** **CT, SE** | **Control** | **Seizure** |
| **48 Hrs** | 10,7 | 1.00±0.34 | 1.44±0.56 | 10,8 | 1.00±0.23 | 0.55±0.17 | 9,7 | 1.00±0.22 | 0.83±0.23 |
| **1 Week** | 8,7 | 1.00±0.08 | 0.97±0.11 | 8,7 | 1.00±0.07 | 1.00±0.08 | 7,6 | 1.00±0.09 | 0.61±0.08 |
| **2 Mths** | 7,9 | 1.00±0.09 | ***0.57±0.04****** | 7,9 | 1.00±0.10 | ***0.60±0.47****** | 7,9 | 1.00±0.13 | ***0.33±0.03****** |
| **P-Value** | | | **<0.0001** | | | **<0.0001** | | | **0.0007** |

*Aggrecan, HAPLN1, and HAS3* mRNA in seizure animals relative to control animals. Status epilepticus was induced with Pilocarpine and mRNA levels were evaluated 48 hours, 1 week, or 2 months post-SE. Values shown are ± the standard error of the mean. There was a significant decrease in *aggrecan*, *HAPLN1*, and *HAS3* mRNA (One-way ANOVA; $p<0.0001$, $p<0.0001$, $p=0.0007$ respectively). The Bonferroni post-hoc test showed a significant decrease at 2 months for *aggrecan*, *HAPLN1*, and *HAS3* mRNA (*** $p<0.0001$).