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Aggrecan expression, a component of the inhibitory interneuron perineuronal net, is altered following an early-life seizure

Paulette A. McRae [a,*], Esther Baranov [a], Shilpa Sarode [a], Amy R. Brooks-Kayal [b], Brenda E. Porter [a,c]

[a] Division of Neurology, Department of Pediatrics at The Children's Hospital of Philadelphia, Philadelphia, PA, USA
[b] Department of Pediatrics, Division of Neurology, University of Colorado, Denver School of Medicine and The Children's Hospital, Aurora CO, USA
[c] Department of Neurology, School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

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ABSTRACT

The perineuronal net (PN), a component of the neural extracellular matrix (ECM), is a dynamic structure whose expression decreases following diminished physiological activity. Here, we analyzed the effects of increased neuronal activity on the development of aggrecan, a component of the PN, in the hippocampus. We show aggrecan expression to be prominent around parvalbumin (PV) interneurons in the postnatal hippocampus. Moreover, after seizure induction in early life there was a significant increase in aggrecan expression in a region specific manner during the course of development. We conclude that increased neuronal activity leads to accelerated expression of PNs in the hippocampus that attenuates in the adult hippocampus. This study shows the dynamic nature of the PN component of the ECM and the role neuronal activity has in molding the extracellular milieu of inhibitory interneurons.

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Introduction

The perineuronal net (PN) is a complex extracellular structure, initially described by Camillo Golgi in 1893 as 'a thin envelope, with a reticular or continuous shape involving not only the cell bodies but also their branches' (for review see Celio et al., 1998; Spreafico et al., 1999). Today, we know that the PN surrounds synapses on the cell body, proximal dendrites, and the axon hillock of certain neurons in a lattice-like structure, and is therefore uniquely positioned to influence synaptic development and stabilization (Hockfield et al., 1990). PNs have apertures at points of synaptic contact (Bruckner et al., 1993) and this structure increases synaptic stability by decreasing extra-synaptic movement of receptors (Frischknecht et al., 2009).

Chondroitin sulfate proteoglycans, particularly members of the lectican family, are the major component of the PN (Yamaguchi, 2000). All members of the lectican family can form PNs, but aggrecan is the only lectican found almost exclusively in the PN expressed in adulthood (Matthews et al., 2002; Dino et al., 2006). Interestingly, the aggrecan containing PNs decrease following sensory deprivation during development (Sur et al., 1988; Guimaraes et al., 1990; Kind et al., 1995; Lander et al., 1997; McRae et al., 2007). However, once the mature synapses are established and ensheathed by an aggrecan containing PN, they are stable and subject to little reorganization in the adult (Hockfield et al., 1990; McRae et al., 2007).

The goal of this study was to understand the relationship between increased neuronal activity during development, as a result of seizure activity, and the aggrecan component of the PN. Early-life seizures result in persistent hyperexcitability in the hippocampus throughout adulthood as evidenced by increased seizure susceptibility (Holmes et al., 1988, 1998; Jensen et al., 1992; Dube et al., 2000; Zhang et al., 2004a). Alterations in inhibitory circuitry may have a role in initiating and maintaining the seizure prone condition of the brain (Sankar et al., 1997; Khazipov et al., 2004; Zhang et al., 2004b; Isaeva et al., 2006; Raol et al., 2006; Sanchez et al., 2007). Seizures have been shown to alter a variety of activity-dependent processes (Rakhade et al., 2007; Christensen et al., 2010). Therefore, the PN is a candidate structure to be altered following an early-life seizure, as it has been shown to primarily ensheath GABAergic cells and its development has been shown to be dependent on neuronal activity (Bruckner et al., 1993; Celio and Chiquet-Ehrismann, 1993; Wintergerst et al., 1996; Schuppel et al., 2002; Lander et al., 1997; McRae et al., 2007). We hypothesized that excessive neuronal activity, in the form of a seizure, during early postnatal development will accelerate aggrecan formation, ultimately resulting in a premature loss of plasticity.

* Corresponding author. The Children's Hospital of Philadelphia, Abramson Research Building, Room 509, 3516 Civic Center Boulevard, Philadelphia, PA 19104, USA.
E-mail address: mcraep@email.chop.edu (P.A. McRae).
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Materials and methods

Animals and experimental groups

The institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia approved all procedures used in this study. Sprague-Dawley rats, from Charles River (Wilmington, MA), were obtained from pregnant dams. Rats were housed together in standard plastic cages with corncob bedding and *ad libitum* access to food and water. Pregnant female rats were allowed to deliver naturally, and the day of birth was designated as postnatal day 0 (P0). Pups used for experiments were selected randomly from multiple litters at the desired age for experimentation and combined into experimental groups. A total of 30 litters, each yielding between of 4–10 male pups, were used for all experiments described in this study. Littermates were randomly assigned into either the control group or the experimental group.

Seizure induction

On P10, male rat pups were separated from their mother and injected intraperitoneally with saline (control group) or 2 mg/kg kainic acid (KA) (experimental group). The KA treated pups developed prolonged status epilepticus (SE) within 30–50 min after the KA injection, which continued for 2–3 h and resolved spontaneously. Both control and KA treated pups were separated from their mother for the same amount of time and the litters were returned to their home cages after the seizures subsided. Only pups that entered into stage five SE, which consisted of whole body convulsions and a loss of balance, as described by Racine (1972), were included in this study. All rats were weaned from the dam on P21. Stereology was performed on the dorsal hippocampus on P12 (control $n=6$, seizure $n=8$), P14 (control $n=5$, seizure $n=5$), P21 (control $n=7$, seizure $n=7$) or P60 (control $n=7$, seizure $n=6$).

Immunohistochemistry

Rats were deeply anesthetized with isofluorane and underwent transcardiac perfusion with 0.1 M PBS, followed by 4% phosphate-buffered paraformaldehyde, pH 7.4. The tissue was post-fixed overnight with 30% sucrose in phosphate buffer. Forty-micron sections were cut on a cryostat, and free-floating sections were incubated at 4 °C overnight in the primary antibodies Cat-315 (1:10; a gift from Dr. Russell Mathews, SUNY Upstate, Syracuse, NY) parvalbumin (1:400; Chemicon, Temecula, CA), somatostatin (1:50; Chemicon), or Neuropeptide Y (1:400; Abcam, Cambridge, MA) 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 or goat anti-rabbit secondary antibodies (Invitrogen, Carlsbad, CA) and 0.5% Triton X-100 in DMEM. 4' 6-Diamidino-2-phenylindole DAPI (Molecular Probes, Carlsbad, CA) with 0.5% Triton X-100 in DMEM was applied after the secondary antibody staining was complete. Sections were rinsed with phosphate buffer and mounted onto glass slides using Prolong Antifade mounting medium (Invitrogen).

Cell counts

Stained sections were visualized using a Zeiss Axioplan microscope (Thornwood, NY). Quantitative analysis of cells expressing aggrecan positive perineuronal nets and interneuron markers was performed at 10× and colocalization was verified at 20× and 40× magnifications. Stereological methods were employed using the fractionator method (Gundersen, 1986), which provides an unbiased estimate of the total number of cells. Systematically-randomly sampled (SRS) 40 μm thick sections throughout the hippocampus were evaluated. The SRS ranged from every 6th section in the immature rat to 10th section in the adult. The estimated number of cells was based on the following formula:

$$N = \Sigma Q \times 1 / \mathrm{ASF} \times 1 / \mathrm{SSF} \times 1 / \mathrm{TSF}$$

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

RT-PCR

Fresh frozen rat hippocampal tissue was homogenized using a sonicator. The Protein and RNA Isolation System (PARIS) kit (Ambion Inc., Austin, TX) was used to obtain RNA from the tissue. Concentrations of RNA were measured using a spectrophotometer (NanoDrop ND1000). One microgram of purified RNA was reverse transcribed using random hexamers and SuperScript II reverse transcription kit (Invitrogen, Carlsbad, CA). Approximately 1000 ng of sample concentrations underwent real-time PCR in a 384-well plate. Two master mixes were prepared using the Taqman Universal Master mix (Applied Biosystems, Branchburg, NJ): one with a probe for aggrecan (Acan Rn01477603_m1) (Applied Biosystems, Foster City, CA) and one with a probe for cyclophilin (Ppia Rn00690933_m1) (Applied Biosystems). Samples were assayed in triplicate to minimize pipetting error. The real-time PCR assay consisted of a cycle of 50 °C for 2 min, followed by a cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 60 s, and was carried out on a SDS 7900HT model thermocycler (Applied Biosystems). Cyclophilin was used as a housekeeping gene and all samples were quantified relative to its expression to help diminish loading errors. The data was then expressed as percent change relative to mean control values in the same run.

Statistical analysis

A one-way ANOVA was use to evaluate aggrecan positive PNs during the development of the hippocampus with the Bonferroni's multiple comparison post-test. Aggrecan positive perineuronal net expression changes 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. For the analysis of the RT-PCR data an unpaired *t*-test was used to evaluate aggrecan mRNA levels following a seizure relative to control mRNA levels. All values were normalized to cyclophilin. The null hypothesis states that there would be no changes in aggrecan protein expression or mRNA levels following a single seizure event early in life.

Results

Cat-315 development in the rat hippocampus

The aggrecan component of the PN was detected with the monoclonal antibody Cat-315. On postnatal day 12 (P12) (Fig. 1A–D) rarely Cat-315 containing PNs are seen throughout the hippocampus. At P14, Cat-315 expression remains low in the hippocampus (Fig. 1E–H), however, Cat-315 immunostaining begins to increase in the subiculum (Fig. 1H). Expression of Cat-315 in the subiculum continued to increase from P14 to P21 (Fig. 1H and L). On P21 Cat-315 positive PNs were observed throughout all regions of the hippocampus with the highest level of expression in the dorsal hippocampus and the subiculum (Fig. 1I). In all areas of the hippocampus the Cat-315 positive PNs surrounded a small subset of neuronal cell bodies and their proximal processes. In the CA3 region Cat-315 was predominately expressed on a subset of neurons between the stratum lucidum and the stratum oriens (Fig. 1J). In the granule cell layer of the DG Cat-315 was concentrated on a subset of cells interspersed with granule

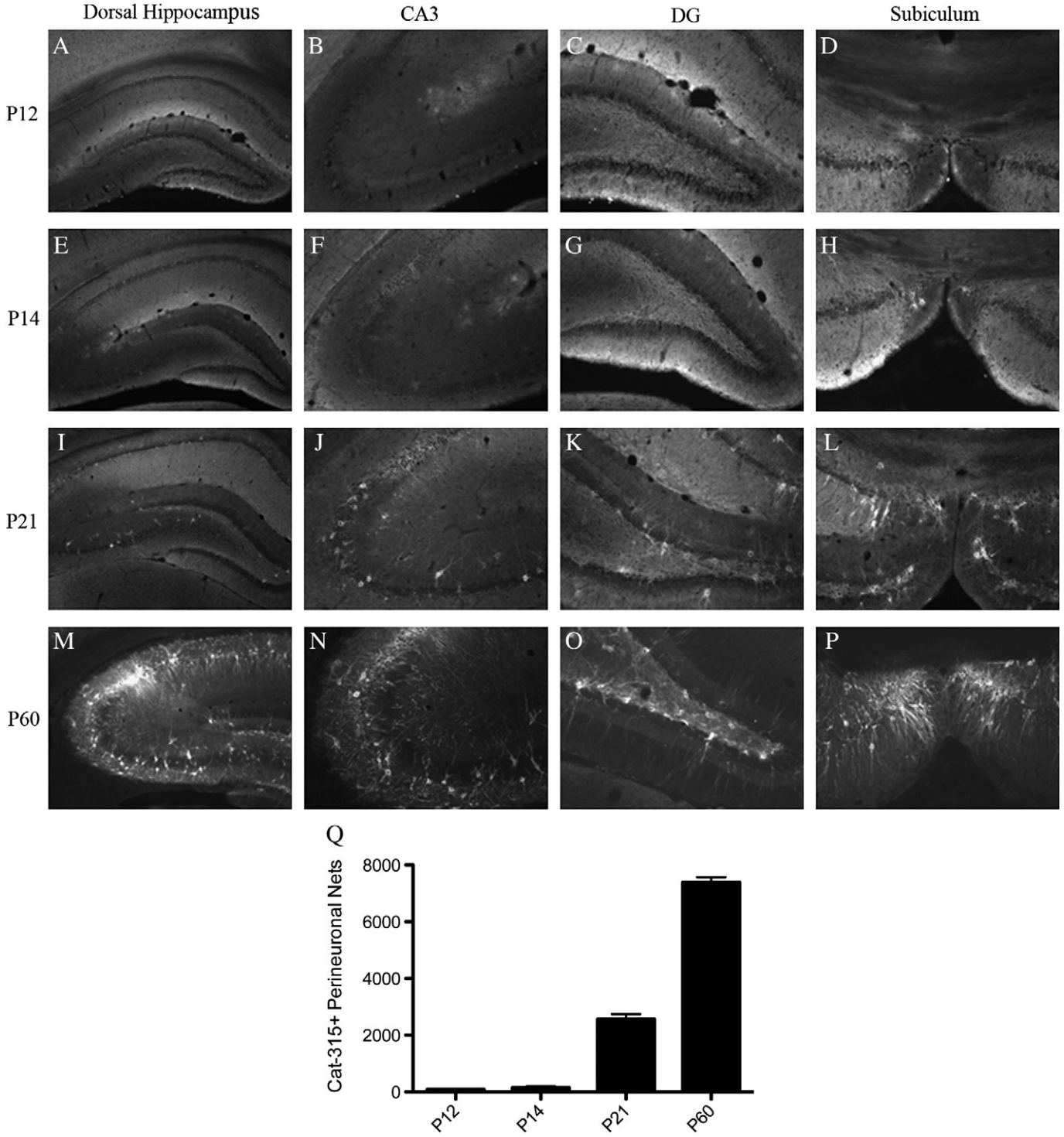

Fig. 1. Developmental time course of Cat-315-expressing perineuronal nets in the dorsal hippocampus. (A–D) Few aggrecan-expressing PNs, detected with Cat-315, are visible at P12, (E–H) by P14 Cat-315 is more highly expressed in the subiculum than other hippocampal regions, (I–L) by P21 Cat-315 visible in all hippocampal regions, and (M–P) by P60 adult expression levels of Cat-315 are reached. (A, E, I, and M) Low magnification images of the hippocampus, (B, F, J, and N) the CA3 region, (C, G, K, and O) the dentate gyrus and CA4 regions, and (D, H, L, and P) the subiculum. (Q) Graph of the number of Cat-315 positive PNs in the hippocampus during development. Results were analyzed using a one-way ANOVA followed by Bonferroni's post-hoc test.

cell bodies (Fig. 1K). Cat-315 was also expressed in the hilus or CA4 region of the hippocampus. By P60 Cat-315 in the hippocampus had reached adult levels of expression (Fig. 1M–P). In the adult animal the Cat-315 positive PNs were apparent around the cell body and more pronounced around the proximal dendrites (Fig. 1N–P) than in the developing hippocampus (Fig. 1A–H). In the DG of the adult rat, the dendrites of the granule cells were directed toward the molecular layer and Cat-315 staining coincided with this pattern of expression (Fig. 1O). There is a significant increase, using a one-way ANOVA, in the number of Cat-315 positive PNs from P12 through adulthood (P12 $n=6$; P14 $n=5$; P21 $n=7$; P60 $n=7$; $p<0.0001$; Fig. 1Q), with the Bonferroni's multiple comparison post-test showing significant increases occurred from P14 through P21 ($p<0.05$) and from P21 through P60 ($p<0.05$) (Fig. 1Q).

Cat-315 positive perineuronal nets are found primarily around inhibitory interneurons in the adult hippocampus

Prior studies have found the PN to be associated with inhibitory interneurons (Bruckner et al., 1993). We examined the relationship between GAD67 (Fig. 2A and D), a marker for the production of the inhibitory neurotransmitter GABA, and the aggrecan component of the PN (Fig. 2B and E). We found that the majority of Cat-315 immunoreactive PNs in the hippocampus primarily surround GAD67 expressing neurons in – DG 88%, CA4 62%, CA3 81%, CA1 75% and the subiculum 86% ($n=6$; Fig. 2C, F, and H). Conversely, only 48% of the GAD67 immunoreactive cells have a Cat-315 expressing PN surrounding them ($n=6$; Fig. 2G). This indicated that there is a subset of GAD67 interneurons with this specialized extracellular milieu.

Parvalbumin expressing interneurons are the major population of interneurons surrounded by the Cat-315 positive perineuronal net in the adult hippocampus

Because only a subset of GAD67 positive interneurons are surrounded by Cat-315 we carried out staining with specific interneuron markers including neuropeptide Y (NPY), somatostatin polypeptide (SOM), and the calcium binding protein parvalbumin (PV). Only 13% of the NPY immunostained cells were costained with Cat-315 ($n=6$; Fig. 3A–C and M). SOM showed a higher level of co-expression with Cat-315, with 24% of the cells expressing Cat-315 positive PNs ($n=6$; Fig. 3D–F and M). PV had the highest level of colocalization with Cat-315, where 87% of these cells were ensheated by PNs detected by Cat-315 ($n=7$; Fig. 3G–I and M). In all regions PV cells are highly associated with Cat-315 positive PNs—DG 83%, CA4 84%, CA3 93%, CA1 84% and the subiculum 90%. The PV staining extends along the neuronal processes was also enveloped by Cat-315 positive PNs (Fig. 3J–L).

Interestingly, the presence of PNs around SOM expressing cells has not been described previously. To further explore the relationship between SOM, PV and the PN in the adult hippocampus we used triple labeling for SOM, PV, and Cat-315. PV and SOM were simultaneously expressed in a subset of cells (Supplementary Fig. 1). We found that in all regions excluding the dentate gyrus, SOM expressing interneurons with a Cat-315 positive PN have a high level of association with PV—DG 0%, CA4 90%, CA3 63%, CA1 60% and the subiculum 100%. (Fig. 4A–C and E). The dentate had relatively few SOM immunoreactive cells with a PN as compared to other hippocampal regions (Supplementary Fig. 2). The fact that the majority of SOM cells ensheathed with a Cat-315 positive PN co-stain for PV further indicates that there is a strong relationship between PV and the aggrecan component of the PN.

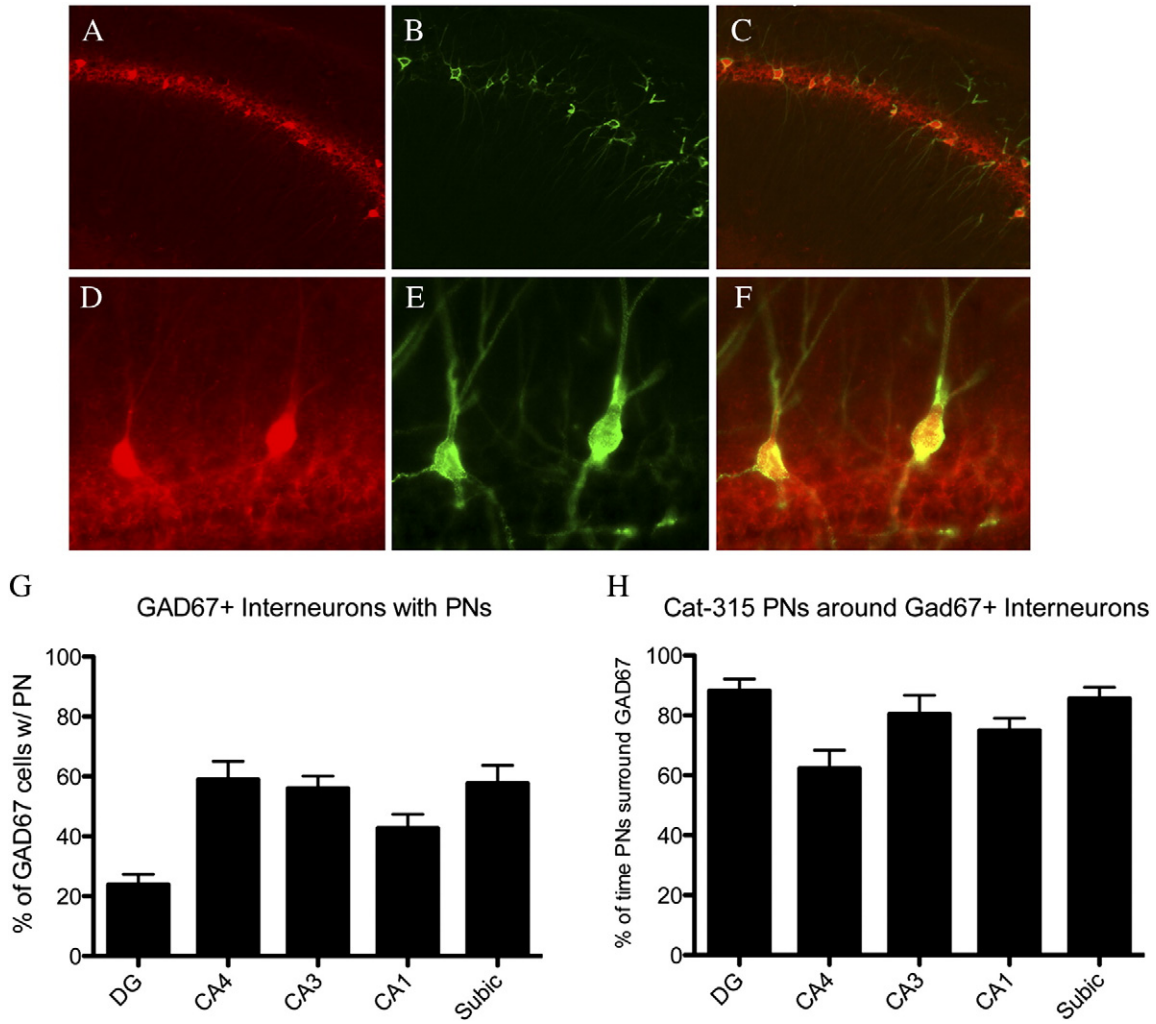

Fig. 2. Colocalization of Cat-315 and GAD67 positive interneurons in the hippocampus. (A) GAD67 staining in the CA1 pyramidal region, (B) Cat-315 in CA1, (C) overlay of GAD67 and Cat-315. (D–F) Higher magnification image of GAD67 and Cat-315. (D) GAD67 staining in CA1, (E) Cat-315 in the CA1, (F) Cat-315 expressing PN surrounds the GAD67 positive cells. (G) Graph demonstrating the percent of GAD67 positive cells surrounded by PNs in different regions of the hippocampus. (H) Graph showing the percent of Cat-315 positive PNs surrounding GAD67 interneurons.

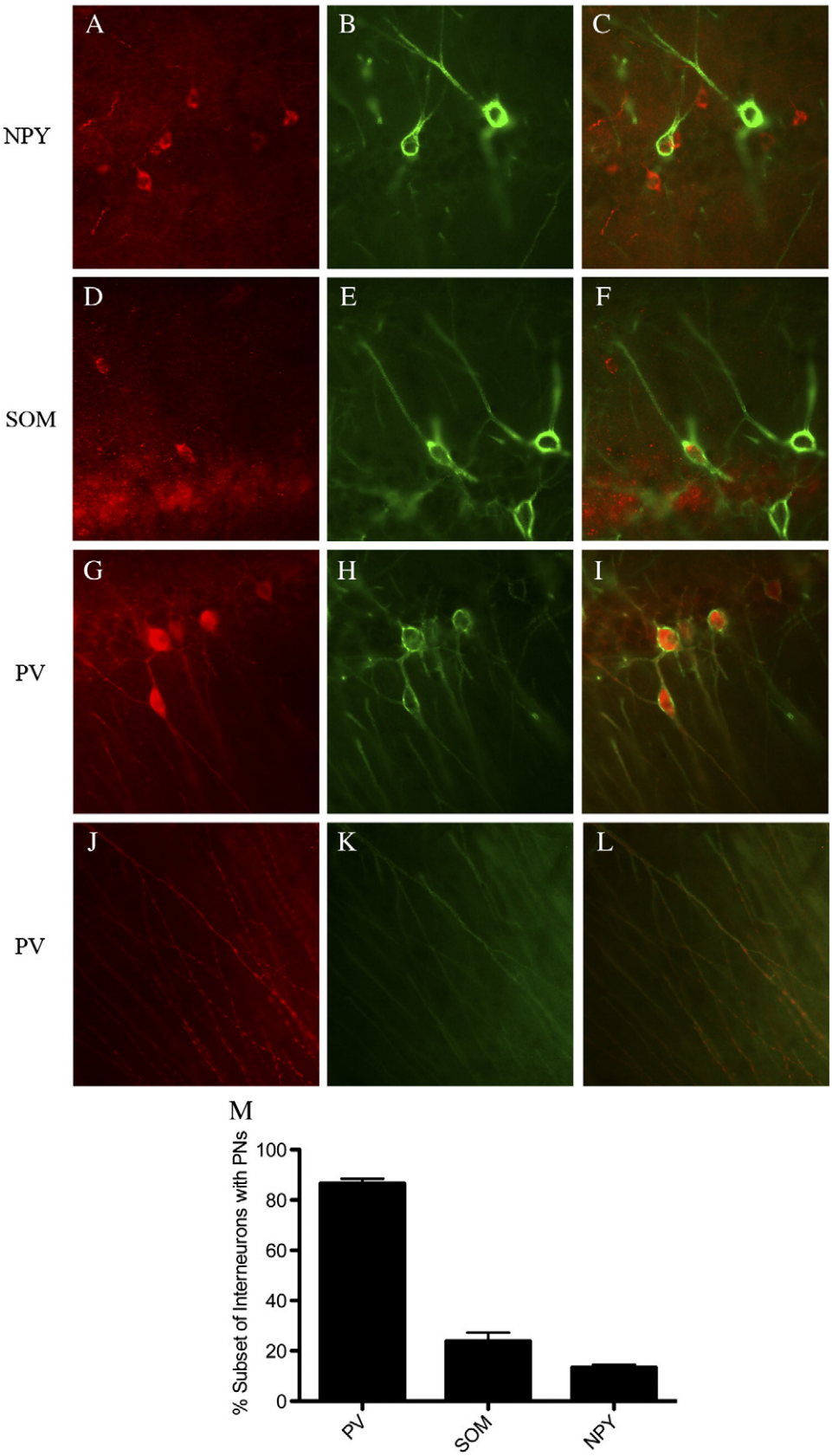

Fig. 3. Interneuron subtypes surrounded by Cat-315 positive perineuronal nets in the hippocampus. (A, D, G, and J) Interneuron subtype markers in the CA1 pyramidal region of the hippocampus. (B, E, H, and K) Cat-315 expression, (C, F, I, and L) overlay of interneuron subtype markers and Cat-315 positive PNs. (A–C) NPY expressing interneurons were rarely colocalized with PNs. (D–F) Somatostatin expressing interneurons occasionally colocalized with the PN. (G–I) Parvalbumin interneurons have a high level of colocalization with PNs, which extends along the processes of the parvalbumin cells (J–L). (M) Graph showing the percent of Cat-315 staining PNs colocalized with parvalbumin, somatostatin, and NPY.

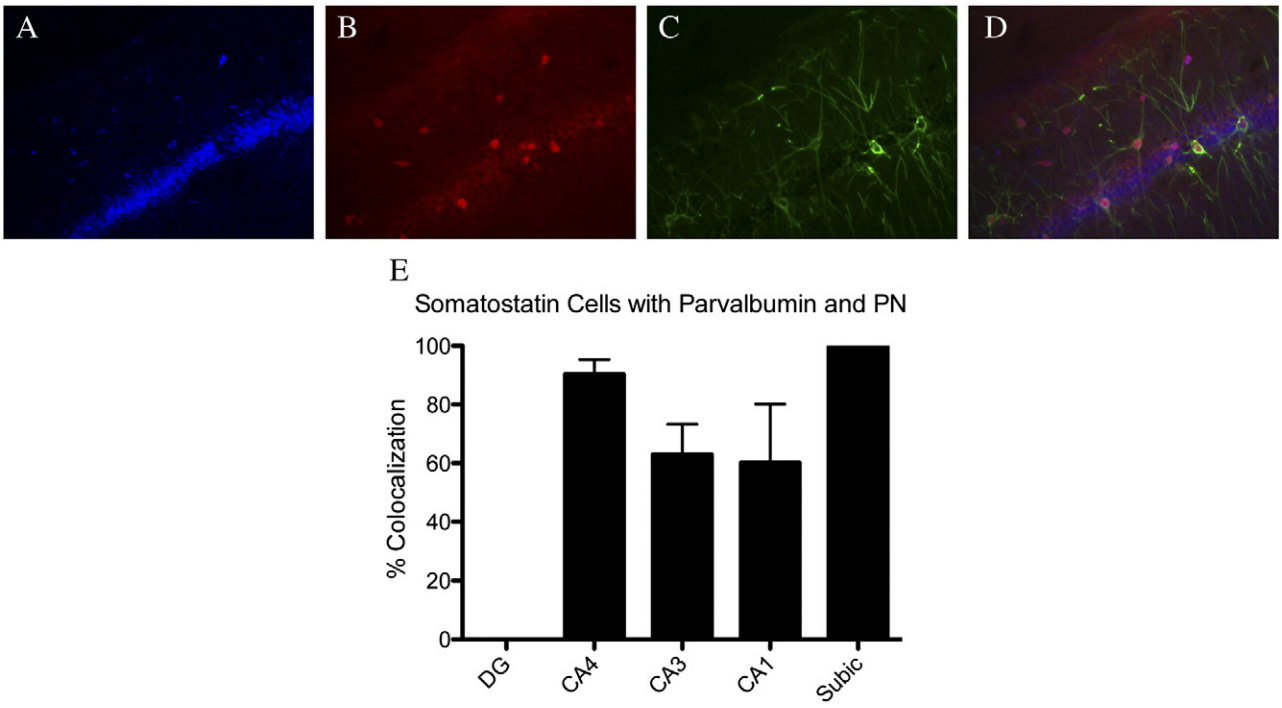

Fig. 4. The relationship between somatostatin, parvalbumin and Cat-315. (A) SOM staining in the CA1 pyramidal region, (B) PV staining in CA1, (C) Cat-315 staining in CA1 (D) overlay of SOM, PV, and Cat-315. There is overlap between some somatostatin and parvalbumin classified interneurons in the hippocampus. (E) A graph showing the percent of SOM positive cells with a Cat-315 PN that also express PV.

Seizures alter Cat-315 expression during development and cause aberrant perineuronal net expression in adulthood

To determine if increased neuronal activity during development would alter aggrecan expression in the postnatal hippocampus, we induced seizures on P10 with kainic acid (KA) and investigated the formation of the aggrecan component of the PN in the dorsal hippocampus on P12, P14, P21, or P60 (Fig. 5). On P12, 2 days following seizure induction, there was no change in the expression of PNs detected with Cat-315 (control $n=6$, seizure $n=8$; two-way repeated measures ANOVA: seizure $p=0.6542$; interaction $p=0.3490$; Fig. 5S). On P14 there was a significant increase in PNs around PV cells in several regions relative to control animals (control $n=5$, seizure $n=5$; seizure $p=0.0002$; interaction $p=0.0138$; Fig. 5T). The Bonferroni post-test confirmed an increase in the number of PNs surrounding PV expressing cells in the seizure group compared to controls in CA3 and the subiculum (CA3; $p<0.05$; Fig. 5G–L and T), (subiculum: $p<0.001$; Fig. 5M–R and T). On P14 there was a trend toward an increase of Cat-315 in the DG ($p>0.05$; Fig. 5A–F and T). By P21 the seizure induced increases in PN expression observed at P14 are no longer apparent (control $n=7$, seizure $n=7$; seizure $p=0.7642$; interaction $p=0.8420$; Fig. 5U). There was however, a trend toward a decrease in the percent of PV cells with PNs in the DG of seizure animals relative to control animals at both P21 and at P60 (control $n=7$, seizure $n=6$; seizure $p=0.0707$; interaction $p=0.3135$; Fig. 5V). At P60 there were no significant changes in Cat-315 expression across the hippocampus using a two-way repeated measures ANOVA (Fig. 5V).

Increased neuronal activity leads to a significant increase in Cat-315 expression around parvalbumin-positive cells early in the development of the dorsal hippocampus, which resolves by adulthood. Therefore, one explanation for the increase in Cat-315 expression is an increase in PV-positive cells early in development following a seizure. In order to evaluate this possibility we investigated the effect of seizures on the developmental expression of PV. The number of PV-positive interneurons increased nearly three-fold from P12 through adulthood (P60) (Table 1). Importantly, early-life seizure induction did not significantly alter the number of parvalbumin-expressing cells during postnatal hippocampal development in seizure animals relative to controls (Table 1).

Seizures lead to a change in aggrecan mRNA

To determine if the changes in Cat-315 staining after a seizure are due to a change in aggrecan translation, real-time PCR of *aggrecan* mRNA was performed. Quantification of *aggrecan* mRNA levels in the hippocampus following an early-life seizure revealed a significant increase of *aggrecan* mRNA on P12 ($p=0.0072$; Table 2). On P14 there was a trend toward increased *aggrecan* mRNA levels following the seizure, however, the difference was not statistically significant due to a high degree of variability within the groups ($p=0.3400$; Table 2).

Fig. 5. Effect of an early-life seizure on Cat-315-expressing perineuronal nets in the dorsal hippocampus. (A–R) P14 hippocampus following a saline injection (A–C, G–I, M–O) or a kainic acid induced seizure (D–F, J–L, P–R) on P10. The control dentate gyrus (A–C) has parvalbumin expressing cells, but no surrounding Cat-315-immunoreactive PNs. Following a seizure (D–F) the dentate gyrus shows a trend for more PNs surrounding parvalbumin cells. (G–I) Control animals express parvalbumin in the CA3 region of the hippocampus with few Cat-315 positive PNs. However, the seizure group (J–L) has a significant increase in Cat-315 expression around parvalbumin expressing cells in the CA3 region. In the subiculum of control animals (M–O) there is a modest level of colocalization between parvalbumin and Cat-315. The seizure group (P–R) shows a significant increase in the percent of parvalbumin cells surrounded by Cat-315. (S) At P12 PNs first appear in the subiculum around PV expressing cells, and there is no difference in Cat-315 levels following a seizure on P10. (T) By P14 there is a significant increase in the percent of parvalbumin cells surrounded by Cat-315 expressing PNs in CA3 and the subiculum. (U) At P21 the difference in PN expression between the control and seizure exposed animals is no longer apparent, with the dentate gyrus showing a nonsignificant decrease. (V) In adulthood, there is no difference in PN expression around parvalbumin-positive interneurons. Results were analyzed using a two-way repeated measures ANOVA followed by Bonferroni's post-hoc test.

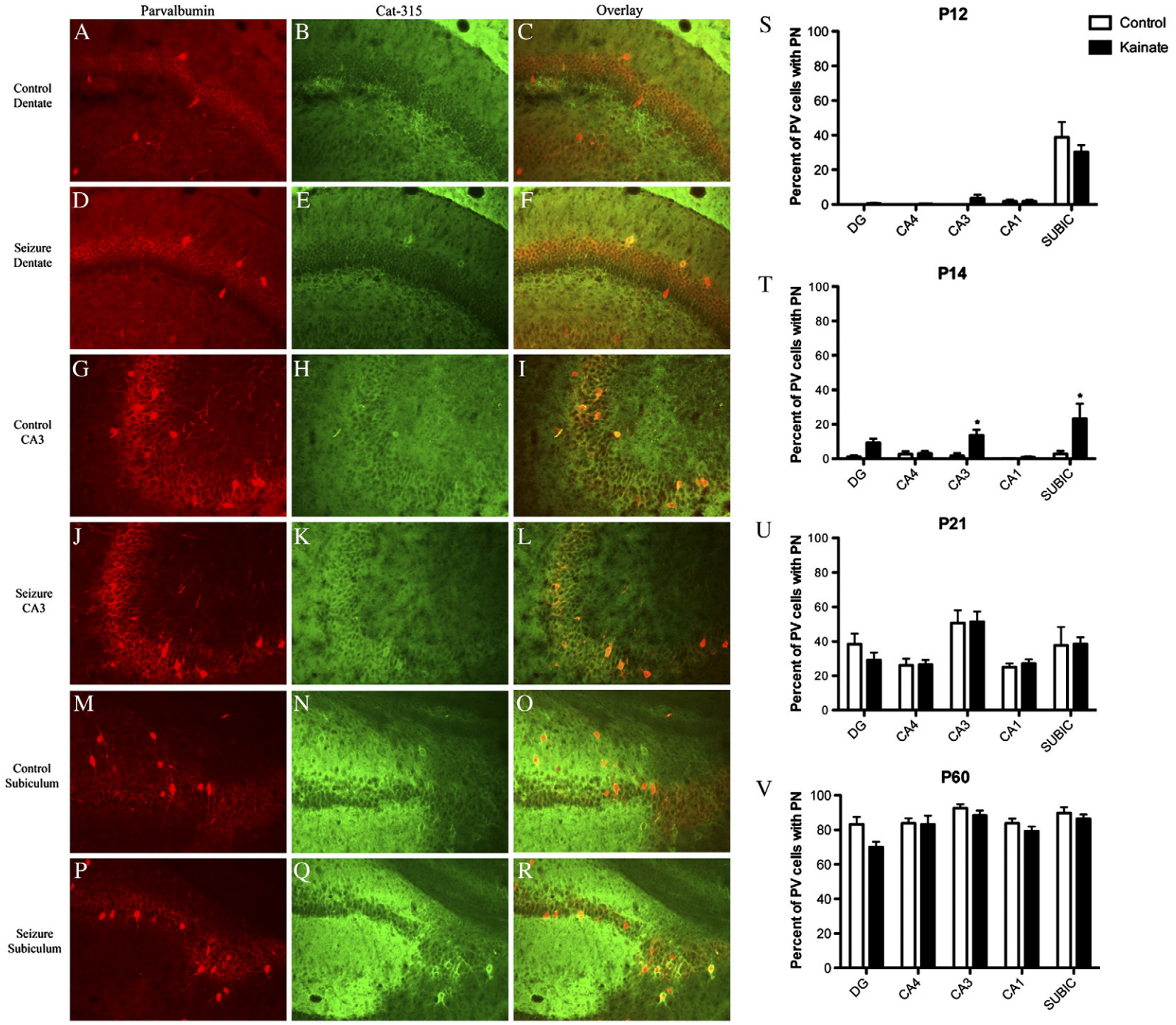
Parvalbumin
Cat-315
Overlay
A
B
C
Control Dentate
D
E
F
Seizure Dentate
G
H
I
Control CA3
J
K
L
Seizure CA3
M
N
O
Control Subiculum
P
Q
R
Seizure Subiculum
S
P12
Control
Kainate
Percent of PV cells with PN
100
80
60
40
20
0
DG
CA4
CA3
CA1
SUBIC
T
P14
*
*
U
P21
V
P60

Table 1
Parvalbumin-positive cells in the adult rat hippocampus.

Age	Control	Seizure	*p* value (Control vs. Seizure)	*n* (Control, Seizure)
	PV cells (±SEM)	PV cells (±SEM)		
P12	2708 ± 526	3580 ± 482	0.4584	5,7
P14	4224 ± 385	4092 ± 275	0.7879	5,5
P21	4754 ± 475	4884 ± 293	0.8198	7,7
P60	7870 ± 443	8880 ± 526	0.1729	6,6

Seizures were induced with kainic acid on P10. The number of parvalbumin labeled interneurons in the hippocampus was estimated based on stereological methods. There is no significant difference in the number of parvalbumin cells in the control animals compared to the seizure animals.

Aggrecan mRNA expression was not altered on P21 or in adulthood (P60) after a seizure on P10 (P21 $p = 0.9230$; P60 $p = 0.4123$; Table 2).

Discussion

In the present study, we report two novel findings regarding the aggrecan component of the PN. First, aggrecan is expressed in the hippocampus primarily around parvalbumin interneurons. Second, our data provides evidence that early-life seizures alter aggrecan expression in a complex and age dependant manner. To our knowledge, this is the first demonstration of increased neuronal activity during postnatal development altering the expression of aggrecan in the PN. These data suggest an important role for activity-dependent maturation of the aggrecan component of the PN in the hippocampus.

The expression of Cat-315 in the hippocampus begins postnatally, as it does in the visual, motor, and somatosensory systems (Hockfield and McKay, 1983; Sur et al., 1988; Kalb and Hockfield, 1988, 1990; McRae et al., 2007). In the present study we demonstrate that between P12 and P14 Cat-315 positive PNs are first found in defined regions of the hippocampus. By P21 Cat-315 is found throughout the hippocampus, reaching adult levels of expression prior to P60. This data is in accord with work done by Koppe et al. (1997) where they found that PNs detected with WFA in the hippocampus first appear after the second postnatal week and adult-like expression occurs after P21.

Postnatal expression of aggrecan in the visual and somatosensory systems coincides with close of the critical period and decreased synaptic plasticity (Sur et al., 1988; Hockfield et al., 1990; McRae et al., 2007). The hippocampus is a structure that remains relatively plastic throughout life. There are, however, numerous changes in the hippocampus during early postnatal development, including the appearance of aggrecan positive PNs, changes in the chloride gradient that result in the switch of the GABA neurotransmitter from excitatory to inhibitory, and changes in expression of excitatory and inhibitory receptor subunits (Ganguly et al., 2001; Khazipov et al., 2004; Zhang et al., 2004a,b; Ben-Ari et al., 2007; Tyzio et al., 2007, 2008). Further studies will be needed to determine the importance of developmental changes in the PN on the function of the hippocampus.

Table 2
Aggrecan mRNA levels evaluated using RT-PCR.

Age	Control	Seizure	*p* value (Control vs. Seizure)	*n* (Control, Seizure)
P12	1.000 ± 0.02601	1.311 ± 0.07449	0.0072***	7,6
P14	1.000 ± 0.1685	1.240 ± 0.1753	0.3400	6,7
P21	1.000 ± 0.09499	1.016 ± 0.1275	0.9230	5,5
P60	1.000 ± 0.08738	1.090 ± 0.06495	0.4123	8,10

Aggrecan mRNA in seizure animals relative to control animals. The expression was normalized to the housekeeping gene cyclophilin. Seizures were induced with kainic acid on P10. On P12 there is a significant increase in *aggrecan* mRNA levels in the hippocampus after a seizure.

The PN has been shown to largely envelope the cell surface of GABAergic nonpyramidal cells (Bruckner et al., 1993; Celio and Chiquet-Ehrismann, 1993; Wintergerst et al., 1996; Schuppel et al., 2002; McRae et al., 2007). We found that aggrecan staining primarily surrounded GAD67 expressing GABAergic interneurons. There was not 100% colocalization between Cat-315 and GAD67, which may imply that rarely PNs surround excitatory cells. Alternatively, there may be technical limitation with the antibody in detecting lower/subthreshold levels of GAD67 in a small population of interneurons. There are a plethora of GABAergic interneurons subtypes that can be classified based on their physiological, chemical and/or morphological properties. In our studies Cat-315 immunostaining predominantly surrounded PV expressing cells, consistent with prior work describing PNs stained with WFA surrounding PV expressing interneurons in primate and rodent hippocampus (Celio and Chiquet-Ehrismann 1993; Koppe et al., 1997), neocortex (Hartig et al., 1999), septum (Morris and Henderson, 2000), basal forebrain (Brauer et al., 1993), and the oculomotor system (Horn et al., 2003). SOM demonstrated the second highest level of colocalization. We further investigated these two interneuron markers and discovered that PV and SOM are colocalized in a subset of hippocampal interneurons which comprise the majority of SOM positive cells ensheathed with a Cat-315 positive PN.

Prior studies suggested that synaptic activity regulates Cat-315 expression (Lander et al., 1997; McRae et al., 2007) leading us to investigate if an early-life seizure alters Cat-315 expression. Kainic acid, a glutamate receptor agonist, induces status epilepticus (SE) and thus a state of increased neuronal excitation in the hippocampus. This increased activity led to a significant increase in Cat-315 expression 4 days after SE in CA3 and the subiculum. There also was a trend toward an increase in the number of PNs in the DG. The change in protein expression on P14 was preceded by an increase in *aggrecan* mRNA on P12. These findings suggest that early-life seizure activity results in enhanced transcription of Aggrecan that may, in part, contribute to the increase in PNs seen at P14. Whether changes also occur at a translational or post-translational level remains to be determined. PNs have been found to be neuroprotective in humans and rodents affected with Alzheimer's disease (Morawski et al., 2004, 2010); more work would need to be done to explore the possibility of a neuroprotective effect resulting from the transient increase in aggrecan expression following SE. It is important to note that the changes in the expression of both aggrecan protein and mRNA following an early-life seizure in the hippocampus are transient and resolve later in development. In contrast, in the visual cortex and somatosensory cortex following sensory deprivation during development, aggrecan expression is decreased and does not reach normal levels (Lander et al., 1997; McRae et al., 2007). The recovery of aggrecan expression could be due to the plastic nature of the hippocampus or it may be due to the use of an increased activity model instead of a decreased activity model. However, increased neuronal activity leading to increased aggrecan expression, in combination with previous work demonstrating that decreased neuronal activity suppressed aggrecan expression (Sur et al., 1988; Guimaraes et al., 1990; Kalb and Hockfield, 1990; Kind et al., 1995; Lander et al., 1997; McRae et al., 2007), supports the concept that neuronal activity can regulate PN development.

Changes in the extracellular matrix have been described during the development of epilepsy in adult animals. Neurocan, a brain specific chondroitin sulfate proteoglycan (CSPG) normally expressed in the immature brain but not in adults, is re-expressed following SE in adulthood (Kurazono et al., 2001; Matsui et al., 2002). Following KA induced convulsions in adult rats there is a decrease in phosphacan-positive PNs surrounding PV cells in the hippocampus, with little change in the number of PV cells present in most rats (Okamoto et al., 2003). These data in combination with the current results suggest that

enhanced excitability associated with seizures can alter extracellular matrix expression, but that the specific protein involved and direction of the change may be dependent on the age at which seizures occur.

The extracellular space is a dynamic and changing microenvironment composed of interconnected extracellular matrix molecules (ECM) that also associate with cell membranes. The ECM can be influenced by neuronal activity as well as having long lasting effects on neuronal function. We demonstrate in the hippocampus that aggrecan expression is dependent on appropriate neuronal activity, with abnormal increased synaptic activity leading to aberrant expression of the aggrecan component of the PN. Prolonged seizures in the immature brain do not result in major structural damage and there is minimal associated cell loss, a scenario very different from the remodeling of neural networks that occurs in the adult following SE (Holmes et al., 1988; Sankar et al., 1998; Yang et al., 1998; Raol et al., 2003; Zhang et al., 2004a). Indeed, in this study we also found no change in the number of PV cells, supporting the previously mentioned concept that cell loss is not a common sequelae of early-life seizure activity (Baram et al., 2002) as well as that the changes we observed in Cat-315 expression were likely not related to an increase or decrease in the PV expressing cells. The increased seizure susceptibility following an early-life seizure is likely the result of molecular changes instead of robust remodeling or cell loss. Further studies will be required to determine if alterations in extracellular matrix, particularly the PN component, following an early-life seizure, may be contributing to changes in excitability following early-life seizures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nbd.2010.05.015.

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