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Extracellular matrix alterations in brains lacking four of its components

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Abstract

The organization of the brain extracellular matrix appears to be based on aggregates of hyaluronan and proteoglycans, connected by oligomeric glycoproteins. Mild phenotypical consequences were reported from several mouse strains lacking components of this matrix such as neurocan, brevican, tenascin-R and tenascin-C. To further challange the flexibility of the extracellular matrix network of the brain, mice lacking all four brain extracellular matrix molecules were generated, which were found to be viable and fertile. Analysis of the brains of one month old quadruple KO mice revealed increased protein levels of fibulin-1 and fibulin-2. Histochemical analysis showed an unusual parenchymal deposition of these fibulins. The quadruple KO mice displayed also obvious changes in the pattern of deposition of hyaluronan. Further, an almost quadruple knock out like extracellular environment was noticed in the brains of triple knock out mice lacking both tenascins and brevican, since these brains had strongly reduced levels of neurocan.

Keywords: neurocan, brevican, tenascin, fibulin, hyaluronan, extracellular matrix, perineuronal nets, mouse, brain, aggrecan

1. Introduction

The cells of the central nervous system and their projections are embedded in an unique extracellular matrix. In contrast to most other tissues, this matrix does not contain fibrillar collagens; rather it is composed of hyaluronan and a variety of secreted glycoproteins and proteoglycans. The abundance of hyaluronan binding proteoglycans in nervous tissue suggests that the organization of the extracellular matrix is based mainly on aggregates of central hyaluronan filaments with associated proteoglycans, which can be interconnected further by oligomeric glycoproteins, such as tenascins [1]; [2]; [3]; [4]; [5]. Hyaluronan binding proteoglycans of the lectican family are structurally characterized by a globular hyaluronan binding N-terminal domain and a globular C-terminal domain, separated by an extended mucin-like central region carrying oligosaccharides and the glycosaminoglycan chains. All four mammalian members of the lectican family have been identified in the brain. While brevican and neurocan are both expressed predominantly in nervous tissue and aggrecan occurs predominantly in cartilage, versican is the most ubiquitously expressed family member [4]. A fifth lectican, dermacan, with closest homology to versican has recently been observed in zebrafish [6].

The presence of proteoglycan aggregates consisting of hyaluronan filaments densely substituted with lecticans is implicated by the observation of several distinct link proteins in the brain [7]; [8]; [9]. Cartilage link protein, the first identified and best characterized link protein, has been shown to stabilize the binding of aggrecan to hyaluronan and to enhance the formation of dense proteoglycan aggregates in cartilage [10]. Cartilage link protein has also been detected in brain, especially during early brain development, a stage when aggrecan is barely present, but neurocan and versican are abundent [7]; [11]; [12]. In accordance with this temporal expression pattern it has been demonstrated that cartilage link protein is also able to interact with neurocan and versican [13]; [14]. With maturation of the brain cartilage link protein and neurocan are replaced by brain specific link proteins and brevican [7]; [8]; [9]; [12]. Also more evident later in development is aggrecan [12]; [15], which is likely to be predominantly expressed by neurons [15], while other lecticans and tenascins appear to be mainly products of cells of the glial lineage [16]. Together, these

observations would predict that throughout life proteoglycans of the lectican family and link proteins are significant components of the brain extracellular matrix.

Aggregates of hyaluronan, lecticans and link proteins can further be interconnected by oligomeric proteins. The trimeric glycoprotein tenascin-R has been demonstrated to interact with the C-terminal domain of all four mammalian lecticans. A particular high affinity has been observed for brevican [1], while the structural details of the interaction have recently been elucidated for aggrecan [17]. The related hexameric glycoprotein tenascin-C, expressed in rodent brain preferentially during development, has a strong affinity for the C-terminal domains of aggrecan, versican and neurocan, and a somewhat lower affinity for brevican [18].

Both tenascin genes and all four lectican genes have been previously eliminated in the mouse. Versican and aggrecan null mutations are embryonic and perinatally lethal, respectively [19]; [20], and their importance for brain development and function will have to be determined by more specific targeting approaches. In contrast, mice lacking neurocan or brevican are viable and fertile and show no gross anatomical alterations of the central nervous system [21]; [22]. Major apparent effects on the anatomy of the brain were also not observed in mice lacking tenascin-C or tenascin-R [23]; [24]. Thus, each of the four components of the brain extracellular matrix network, neurocan, brevican, tenascin-C and tenascin-R, has no crucial life sustaining function.

To challange the flexibility of the extracellular matrix network of the brain even further, mice lacking multiple of these brain extracellular matrix molecules were generated. Finally, animals lacking all four molecules, neurocan, brevican, tenascin-C and tenascin-R, turned out to be viable and fertile. The ability of the quadruple KO mice to overcome to the loss of the two proteoglycans might have been predictable, since they are overlapping in their interactions with versican and aggrecan and an overlap in the distribution with these lecticans is even evident in the normal brain matrix. On the other hand, the molecules, which have been shown to overlap in their interactions with tenasin-C and R, are essentially fibulin-1 and fibulin-2 [25]; [26]. Fibulin-1 and 2 represent an anaphylatoxin-domain containing subgroup of the fibulin

family of extracellular matrix proteins [27]. Both interact with aggrecan and with versican [25]; [26], but have so far not been implicated in brain parenchymal extracellular matrix structure and function. Western blot and immunohistochemical analysis of the brains of one month old quadruple KO mice revealed an aberrant interstitial deposition of fibulin-1 and 2. This suggests a broad structural flexibility of the brain extracellular matrix, while the basic concept of its organisation is maintained.

Materials and methods

Generation of mice and brain homogenates

Multiple KO-mouse strains were generated by crossings of inbred 129sv single KO-mouse strains, which were all kind gifts from Reinhard Faessler (Martinsried) and produced by homologous recombination of the respective constructs in R1 embryonic stem cells as described [28]. Homogenates of their brains were prepared in 5 volumes of ice cold 150 mM NaCl, 20 mM TRIS-HCl, pH 8, (TBS), with 5 mM NEM, 5 mM EDTA, 5 mM benzamidinium chloride, and 1 mM PMSF with a Dounce homogenizer. These homogenates were further sonicated for 20 seconds with a Branson sonifier, 50%, level 2, using the special microtip. Homogenates, rather than soluble extracts, were analysed, because changes in the composition of the brain might alter the extractability of its components considerably. From adult rat brain, for example, only 15 % of the hyaluronan content is water extractable, while 90 % is water extractable from one week old rat brain [29]. The protein concentration in the homogenates was determined with the BCA system (Pierce) after solubilisation by supplementation with 50 % of the volume with 6% sodium dodecylsulfate (SDS), 30 % glycerol, and 0.1875 M Tris/HCl, pH 6.8, (3 X SDS-PAGE buffer), followed by a 1:20 dilution with H_2O.

SDS-PAGE and Western blotting

The homogenate samples were adjusted with 1 X SDS-polyacrylamide gelelectrophoresis (SDS-PAGE) buffer containing 20 mM dithiothreitol to a concentration of 3.3 g/l and 50 μg samples were separated by SDS-PAGE. Blots were performed in Tris/glycine buffer containing 10 % methanol for 1h at 100V using the

Bio-Rad mini gel system. For immunodetection proteins were transfered to polyvinyldifluoride membranes (Amersham), which were blocked overnight with 5% dry milk in TBS with 0.1% Tween 20 (TBST), incubated 90 minutes with the respective antiserum (see below), and 90 minutes with a horseradish peroxidase-linked donkey anti rabbit antibody (Jackson) and developped with the ECL plus detection system (Amersham). Primary incubations were performed with antisera against neurocan and brevican [30]; an antiserum raised against purified rat tenascin-R recombinantly expressed in 293-EBNA cells [18] (a kind gift from Anders Aspberg, Lund); antisera against tenascin-C, fibulin-1 and fibulin-2, (kind gifts from Rupert Timpl and Takako Sasaki, Martinsried) and antisera against fibronectin and neurofilament-M (Chemicon).

Immunohistochemical analysis

Brains for histochemical analysis, which correlated positively with the Western blot results, were all derived from one month old animals and fixed either in 4% paraformaldehyde in 150 mM NaCl, 20 mM Na/phosphate pH 7,4 (PBS), in BOUIN-fixative or in Methacarn over night, dehydrated in ethanol and xylene, and embedded in paraffin and sectioned at 10 μm. After rehydration, immunohistochemistry was performed, in which sections were blocked in 5% normal goat serum/ 1% BSA in PBS for 1h and incubated over night with 1:200 in blocking buffer diluted antisera. After washing with PBS and 1h incubation with Cy3 labeled goat anti rabbit antibodies diluted 1:250 in PBS, the sections were mounted in Vectashield (Vector laboratories).

Reagents for immuno- and affinityfluorescence

Immunofluorescence stainings were performed with antiserum against neurocan [30]; affinity purified antibodies against fibulin-1 and fibulin-2 (kind gifts from Rupert Timpl and Takako Sasaki, Martinsried); an antiserum against heparan sulfate proteoglycan (a kind gift from Gerd Klein, Tuebingen) and an antiserum against aggrecan (a kind gift from Dick Heingard, Lund). Affinityfluorescence stainings with neurocan-GFP fusion protein in a single step incubation with concentrated, serum free 293HEK cell conditioned medium, 1:5 diluted in 2% normal goat serum in PBS, as described [31].

Results

Brains of quadruple KO mice contain more fibulin-1 and fibulin-2

Mice lacking one of the four brain extracellular matrix components, neurocan, brevican, tenascin-R or tenascin-C were interbred to obtain a mouse strain lacking all four molecules. These mice were viable, fertile, and the gross anatomy of their brains showed no obvious abnormalities. Western blot analysis of whole brain homogenates of these mice and of wild type mice confirmed the absence of all four proteins (Fig. 1). While fibronectin, a common interstitial extracellular matrix protein and identified ligand of tenascin-C [32]; [33], could be detected in similar amounts in the brains of the quadruple KO mice and wild type controls (Fig. 1), clearly more fibulin-1 and fibulin-2 was present in the brains of the quadruple KO mice as compared to wild type controls (Fig. 1).

In quadruple KO mice fibulins are deposited interstitially

Immunohistochemically, fibulin-1 was located predominantly in blood vessel basement membranes in wild type mice (Fig. 2A, C). This pattern is similar to that of heparan sulfate proteoglycans, basement membrane molecules usually observed in association with blood vessels [34] (Fig. 2 B). In quadruple KO mice fibulin-1 was not predominantly associated with blood vessels, although their presence and distribution in brain appeared normal, since no change in the heparan sulfate proteoglycans distribution could be observed (Fig. 2D, E, F). Fibulin-1 immunoreactivity was generally increased in the quadruple KO mice troughout the brain parenchyma with particularly prominent deposition in and along the corpus callosum, in the fimbria, and in the dentate gyrus.

In accordance with the Western blot results the immunohistochemical analysis also showed a significant increase in the deposition of fibulin-2 in the quadruple KO mice, particularly in the corpus callosum and in the adjacent zones of the cortex and hippocampus (Fig. 3). In contrast to the microvascular deposition pattern of fibulin-1, the deposition pattern of fibulin-2 in wild type mouse brains was restricted to larger blood vessels, which remained unaltered in the quadruple KO animals (Fig. 3).

Quadruple KO mice have an altered hyaluronan deposition pattern

While tenascins and fibulins appear to be alternative ligands for the C-terminal domains of lecticans, hyaluronan is clearly the predominant ligand of their N-terminal domains. Therefore, possible changes in the deposition of hyaluronan in the brains of the mutant mice were examined. For this analysis a recently developed direct hyaluronan detection tool, a fusion protein of the neurocan hyaluronan binding domain and of the green fluorescent protein (GFP), was used [31]. Distinct differences were observed in the hyaluronan staining pattern between wild type and quadruple KO animals, in particular in the corpus callosum and adjacent part of the hippocampus (Fig. 4). Hyaluronan appeared more evenly and continuously distributed throughout the hippocampus and organized into fibrillar structures in the corpus callosum in wild type animals, while in the quadruple KO mice the deposition appeared punctuate and less organized (Fig. 4). Moreover, hyaluronan-containing pericellular extracellular matrix depositions enclosing somata of individual neural cell bodies, also known as perineuronal nets [35], could clearly be observed in wild type mice (Fig. 4A, C), while they were difficult to identify in quadruple KO mice, although staining for aggrecan (Fig. 4E, F) and with the perineuronal nets marker Wisteria floribunda lectin (results not shown) revealed that these structures are present in these mice.

Brevican/tenascin-C/R triple KO mouse brains contain less neurocan

While the fibulins could be shown to interact with the C-terminal domain of aggrecan, of versican and even of brevican [25]; [26], neither fibulin-1 nor 2 interacted in those in vitro studies with neurocan [26]. To see whether the apparent replacement of tenascins by fibulins would affect the deposition of neurocan, Western blot analysis of whole brain homogenates of brevican/tenascin-C/R triple KO mouse brains were performed. This analysis showed that neurocan was almost absent in the brains of these mice (Fig. 5A). This was also evident in immunohistochemical analysis of triple KO mice, which showed little or no neurocan deposition in the hippocampus, usually one of the brain structures with the most intense neurocan immunofluorecence in wild type mice (Fig. 5B, C). Thus, one month old brevican/tenascin-C/R triple KO-mice could be considered to be phenotypically almost quadruple KO mice. In the light of these findings it was not surprising that the increased deposition of fibulin-1 and

fibulin-2 was also observed in the brevican/tenascin-C/R triple-KO mice, by Western blot of whole brain homogenates (Fig. 5D,E) and by immunohistochemistry, in a pattern similar to that observed in the quadruple KO mouse brains (results not shown).

Discussion

Crossbreeding of the respective single KO strains revealed that mice lacking four major brain extracellular matrix molecules, neurocan, brevican, tenascin-C and tenascin-R, are still viable and fertile. These molecules represent core components of current models of the structural organization of the brain extracellular matrix, which is considered to constitute a network of hyaluronan, proteoglycans and oligomeric glycoproteins [2]; [3]; [4], [5].

Deposition of fibulins

The most apparent observation in the quadruple KO mice was an aberrant deposition of fibulin-1 and 2 in the brain parenchyma. Although fibulin-1 has been immunochemically identified in and shown to be secreted by neurons [36]; [37], until now fibulins have not been implicated in brain parenchymal extracellular matrix structure and function. In contrast, the involvement of fibulin-1 in structure and function of blood vessel basement membranes is evident from the phenotype of fibulin-1 KO mice, which suffer from bleedings in the perinatal period [38]. Since fibulin-1 and 2 bind preferentially to versican and aggrecan, the two mainly non-neuronal members of the lectican proteoglycan family [26], they appear to be functionally mainly destinated for non-neuronal extracellular matrices.

Observations made by electron microscopy indicate that dimeric fibulin-2 molecules are like tenascin-C and R able to interconnect hyaluronan aggregates by interacting with the C-terminal domains of two proteoglycan molecules simultanously [17]; [26] (Fig. 6). By rotary shadowing electron microscopy it was shown that fibulin-1 is also able to oligomerize, although non-covalently, into multimers [39] (Fig. 6). Additional alternative ways of oligomerisation can be considered for fibulin-1, such as

association with other extracellular matrix molecules, like fibronectin and laminin, which are able to multimerize [39] (Fig. 6). Therefore, it appears that in the quadruple KO brains both fibulins could crosslink and organize hyaluronan proteoglycan aggregates in a similar manner as tenascins in wild type mice (Fig. 6).

A change in the ultrastructural distribution of fibulin-1 and 2 has previously been observed during heart development [40]. Both fibulins colocalize with versican in the mainly unstructured extracellular matrix of the embryonic endocardial cushion tissue, while they were associated with fibrillar structures in the adult heart valve. Similarly, in the course of healing of skin wounds, alteration in the histochemical pattern of fibulin-1 and 2 staining were observed, which were found to be uniformly distributed in the granulation tissue [41]. Both investigations show an interstitial deposition pattern of fibulins in tissues, which are in a transient state destinated for remodeling. Thus, beside their involvement in blood vessel basement membrane structure and function, fibulin-1 and 2 appear to have also a role in the deposition of transient extracellular matrices, which might be structurally related to the matrix in the brain of the quadruple KO mice.

Deposition of proteoglycans and glycosaminoglycans

In the quadruple KO animals the neuronal lecticans neurocan and brevican are not present. Versican has been identified as a significant constituent of the normal brain extracellular matrix in other species [42]. However, attempts to quantitate levels of high molecular weight proteoglycans such as versican by Western blot gave inconclusive results, even by using tissue extracts, which are suitable for chondroitinase digestions of proteoglycans, but might not be as reliable as homogenates due to variabilities in the efficiency of their extraction. Judged by immunofluorescence intensity, aggrecan deposition did not appear to be significantly altered and was apart from the hippocampal CA1/2 junction (shown in Fig. 4) mainly evident in areas rich in perineuronal nets, as previously reported for rat brain [15].

Due to its smaller size, neurocan levels could be compared by Western blots of whole brain homogenates. Such blots and immunohistochemical analysis revealed a strong decrease of neurocan deposition in the brains of brevican/tenascin-R/C triple KO-

mice compared to wild type mice. This observation corroborates data of interaction studies, which could not demonstrate an interaction of neurocan with fibulin-1 and fibulin-2 [26], indicating that fibulin-1 and 2 are not able to compensate for the loss of tenascin-C and R in all respects. Other known interactions of neurocan, for example with neural cell adhesion molecules [43] or heparan sulfate chains [44]; [45] are apparently not be able to compensate the loss of the tenascins.

The interaction of neurocan and of the other lecticans with hyaluronan might be interdependently regulated by the presence of appropriate link proteins. An indication for such an interdependence is the observed reduction of brain link protein 2 in brevican KO mice [9]. The decreasing expression of cartilage link protein, so far the only link protein, which has been shown to support the binding of neurocan, after postnatal day 10 might render neurocans interaction with hyaluronan in brains of older mice less effective [7]. The observation that the hyaluronan staining pattern in the quadruple KO mice is different, although hyaluronan binding molecules of the lectican family are still present in brain, might partially be caused by a mismatch of the available lecticans and link proteins.

Although the different staining pattern reflects most probably alterations in the deposition of hyaluronan, it has to be pointed out that all currently used staining techniques for hyaluronan require streches of this polysaccharide, which are freely accessible for an interaction with the detection reagent. Thus, the observed differences in the hyaluronan staining pattern should also reflect differences in its accessibility. Differences in the availability of hyaluronan for interactions with other molecules in the parenchymal matrix could, for example, change the ability of the extracellular matrix to support or restrict neurite growth and cellular migrations. The expression of the hyaluronan binding molecule CD44 has been implicated in axonal pathfinding and sprouting [46]; [47] and has been found associated with infiltrating polymorphonuclear leukocytes, activated T-cells and astrocytes, and glioma cells [47]; [48]; [49]; [50]. Moreover, the tumorigenic potential of glioma cells depends considerably on the activity of their hyaluronidases [51], and therefore also on the accessibility of hyaluronan for these enzymes. The quadruple KO mice will be an interesting opportunity to analyze the influence of the extracellular matrix on cellular

migrations in tumor and inflammation models and may give clues about potentials for therapeutic interventions.

Conclusion

The fibulin-enriched matrix observed in the brains of the neurocan, brevican, tenascin-C and tenascin-R quadruple KO mice is apparently able to support the basic, structural requirements for nervous tissue development and maintenance of its gross anatomy. The matrix might be similar to matrices, which are deposited in other tissues in transitory stages or in cases of tissue repair, after a disruption of the normal structure. Interestingly, the increased presence of fibulins, molecules with the ability to interact with the C-terminal domains of lecticans, indicates that even this matrix, apparently assembled by chance, obeys basic concepts of the organisation of the extracellular matrix in the brain: aggregates of hyaluronan and lecticans interconnected by oligomeric glycoproteins (Fig. 6).

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Figure legends:

Figure 1: Western blots of extracellular matrix components in wild type and quadruple KO animals

Whole brains of three 10 day (q, w) or two 30 day (Q, W) old mice were pooled, homogenized, separated by SDS-PAGE and transferred to PVDF membrane. Stainings were performed with neurocan (A), brevican (B), tenascin-C (C), tenascin-R (D), fibronectin (E), fibulin-1 (F) or fibulin-2 (G) antisera. Each lane of the 6 % (A-D), 5 % (E, G) or 8 % (F) acrylamide gels was loaded with 50 μg of protein. In panels E-G two different preparations of homogenates of brains of each kind were used. Arrows in E and G point to the bands corresponding to the size of the unprocessed protein. W,w: wild type mice; Q,q: quadruple KO mice

Figure 2: Distribution of fibulin-1 and perlecan in wild type and quadruple KO mouse brains. Immunohistochemical staining of one month old wild type (A, B, C) and quadruple KO mice (D, E, F). Consecutive saggital sections of the hippocampal area were stained with purified anti-fibulin-1 antibodies (A, C, D, F) and anti-heparan sulfate proteoglycan antiserum (B, E), the latter showing an area equivalent to the left half of (A) and (D), respectively. To reveal labeling of the microvasculature in wild type mice, figures A and C had to be much stronger enhanced than figures D and F, respectively. Without different enhancements, the choroid plexus (black arrows in C and F) would show about equal staining intensity. Note the prominent deposition of fibulin-1 (lighter areas indicate higher fibulin-1 immunofluorescence) along the corpus callosum (star in D), extending into the rostral migratory stream (white arrow in F), the inner surface of the granule cell layer in the dentate gyrus (arrowheads in D), and within the fimbria (diamond in F). Bar: 100 □m

Figure 3: Distribution of fibulin-2 in wild type and quadruple KO mouse brains. Immunohistochemical stainings of the brain of one month old wild type mice (A, C, D), and neurocan/ brevican/ tenascin-R/C quadruple KO-mice (B, E) with affinity purified antibodies. Lighter areas indicate higher fibulin-2 immunofluorescence. Pictures A, B, C, and E were photographed with the same exposure time and the images were enhanced in parallel, while (D) shows a higher enhancement of (C). Note

the maintenance of the labeling of larger vascular structures (small arrows) along the borders of dentate gyrus and fimbria (diamond in E) in the mutant animal, which shows prominent fibulin-2 deposition along the corpus callosum (star in B) extending into the rostral migratory stream (bold arrow in E). Bar: 100 μm.

Figure 4: Affinityhistochemical detection of hyaluronan and immunohistochemical detection of aggrecan in wild type and quadruple KO mouse brains.

A & B: Stratum oriens (so), pyramidale (sp), radiatum (sr) and lacunosum moleculare (slm) of the hippocampal CA1/2 field and the adjacent corpus callosum (star) and cerebral cortex (cx) of sagital brain sections of one month old wild type mice (A), or quadruple KO-mice (B), stained with a neurocan-GFP fusion protein. C & D: Higher magnifications of the areas of stratum oriens and corpus callosum indicated in A & B, respectively. Note the pericellular deposition of hyaluronan within the stratum oriens of the wild type animals (bold arrow in C). E & F: Immunohistochemical detection of aggrecan in brains of one month old wild type mice (E), or quadruple KO-mice (F) in the same area as above revealing the presence of perineuronal nets (small arrows) in both mouse strains. Bar: A, B, E, F: 50 μm; C, D: 12.5 μm.

Figure 5: Western blot and immunohistochemical analysis of KO-mice

A: Western blot with homogenate samples, each prepared from two brains of 30 day old mice. All lanes of the 5 % polyacrylamide gels were loaded with 50 μg of protein. Except for the negative control (N), three different preparations of homogenates of brains of each kind were used. The blot was developed with anti-neurocan antiserum and redeveloped with anti-neurofilament-M antibody (N-M). W: wild type mice; B: brevican KO mice; T: brevican/tenascin-R/C triple KO mice; N: neurocan KO control. B & C: Immunohistochemical stainings of hippocampi of one month old wild type mice (B), and brevican/ tenascin-R/C tripple KO-mice (C). (lighter areas indicate higher neurocan immunofluorescence) Bar: 100 μm. D & E: Western blots with homogenates of total brain of 30 day old mice developed with anti-fibulin-1 (D) and anti-fibulin-2 (E) antiserum and redeveloped with anti-neurofilament-M antibody (N-M). Each lane of the 8 % (d) or 5 % (e) polyacrylamide gels was loaded with 50 μg of

protein. W: wild type mice; B: brevican KO mice; T: brevican/tenascin-R/C tripple KO mice; Q: quadruple KO mice.

Figure 6: Cartoon indicating potential molecular interactions between brain extracellular matrix molecules of wild type and quadruple KO mice.
The cartoon indicates individual components within the brain extracellular matrix of wild type (left) and quadruple KO mice (right) and their proposed organization: aggregates assembled of hyaluronan, lecticans and link proteins are interconnected by oligomeric glycoproteins. The identity of the homologous terminal domains of the four lecticans, aggrecan, brevican, neurocan and versican (with a size corresponding to the versican-V2 variant) is pointed out at the bottom. In addition to its intermediate size neurocan was distinguished from brevican and versican by a lighter coloration of its central, mucin-like glycosaminoglycan attachment region. Note that (as normally seen in one month old mice) many neurocan molecules are proteolytically processed. (For clarity only the fragments of neurocan are indicated, although other lecticans are proteolytically processed as well.) In quadruple KO mice hyaluronan proteoglycan aggregates could be formed and interconnected by fibulins in a similar manner as by tenascins. Fibulin-2 has been shown to form covalently linked dimers via the central anaphylatoxin domain. Fibulin-1 has been shown to oligomerize into rossette-like oligomers, although the terminal globular domain in the center of such structures has not been identified and may be depicted wrong. 'Fibulin-1-binding molecule' indicates molecules, which can bind fibulin-1 and are able to multimerize, like for example fibronectin or laminins

Fig. 1

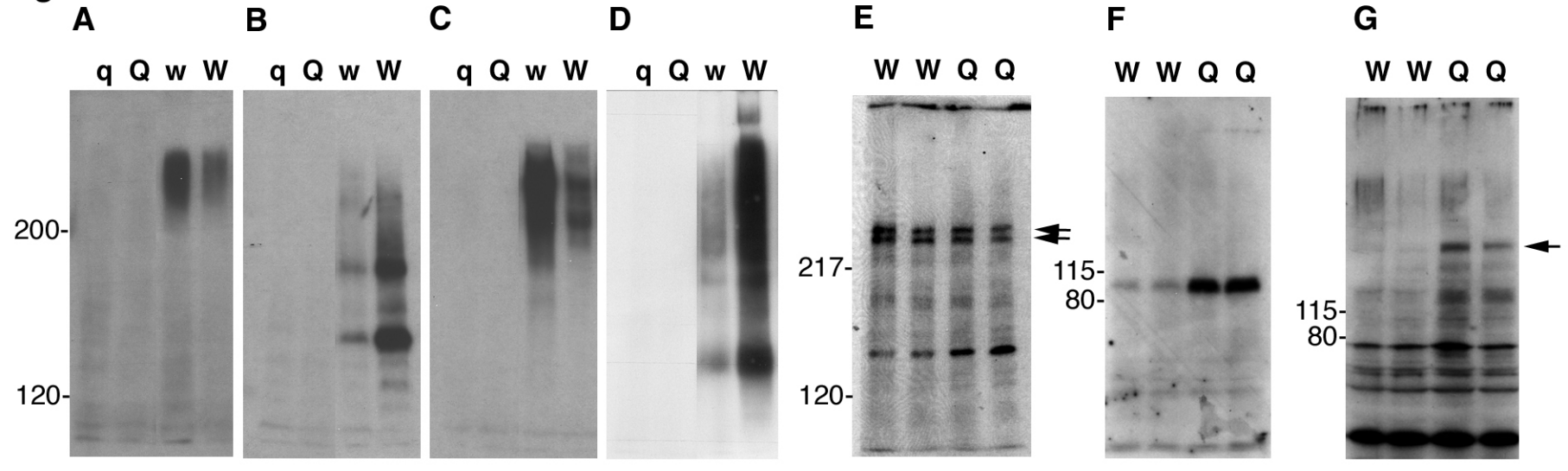
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Fig. 2

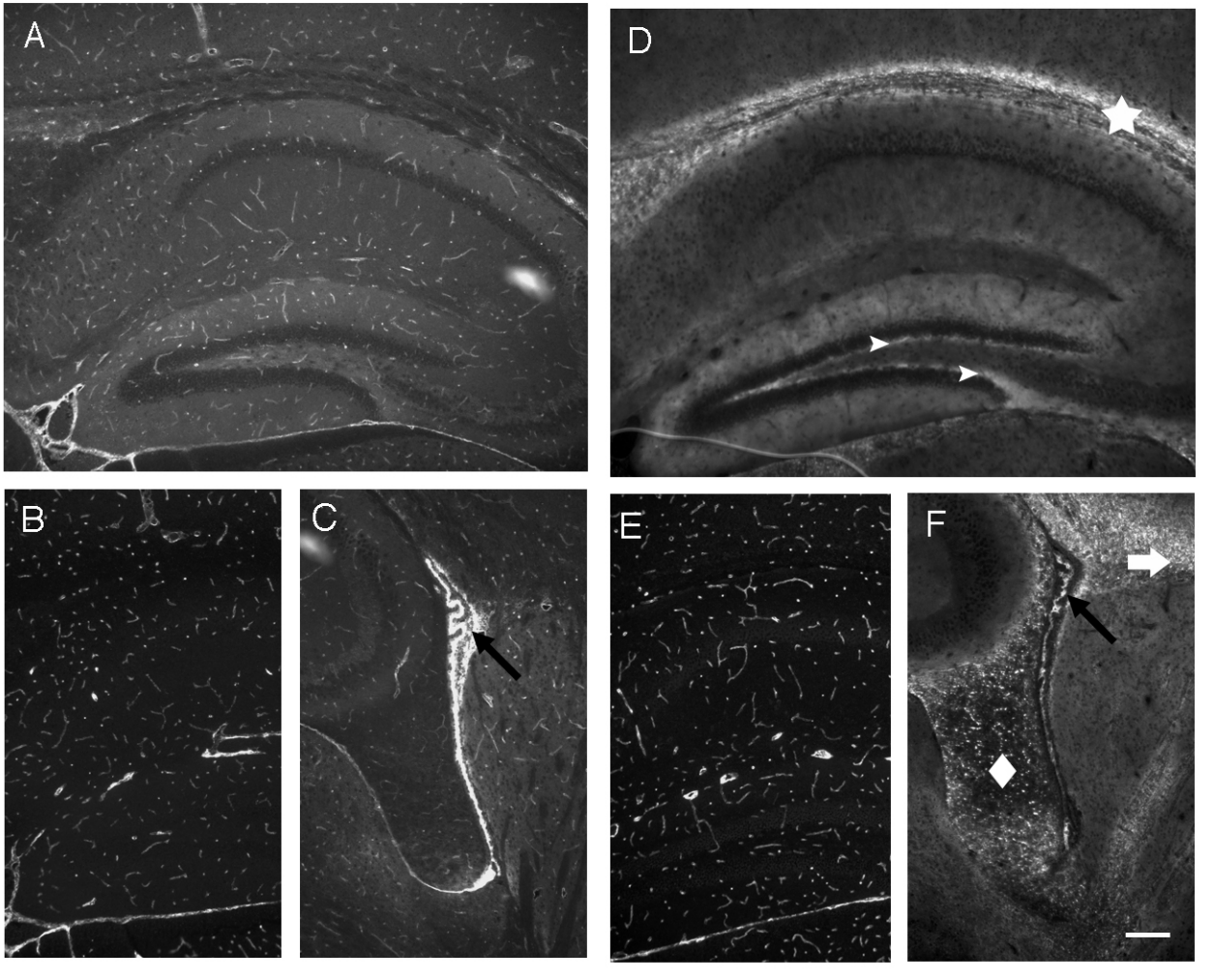


Fig. 3

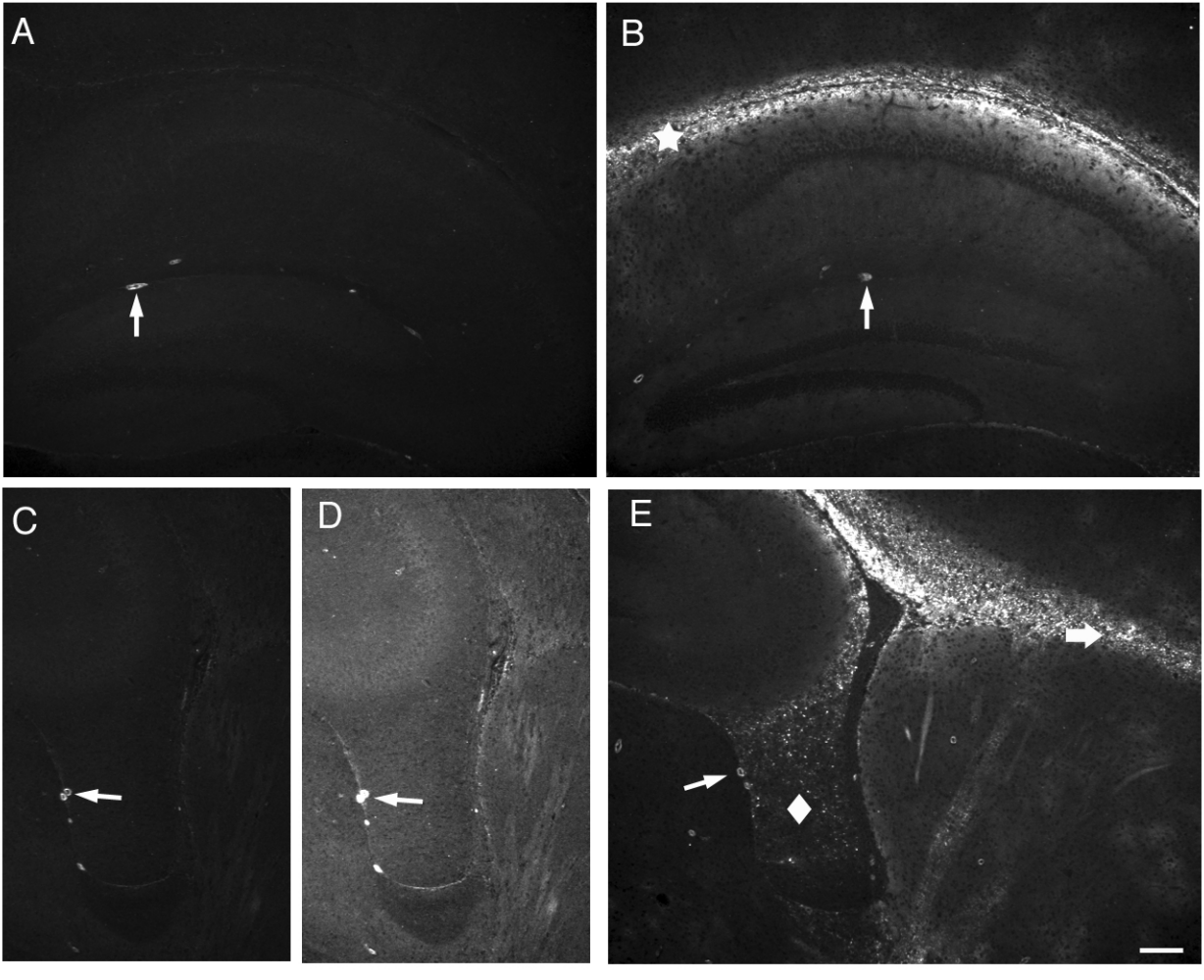


Fig. 4

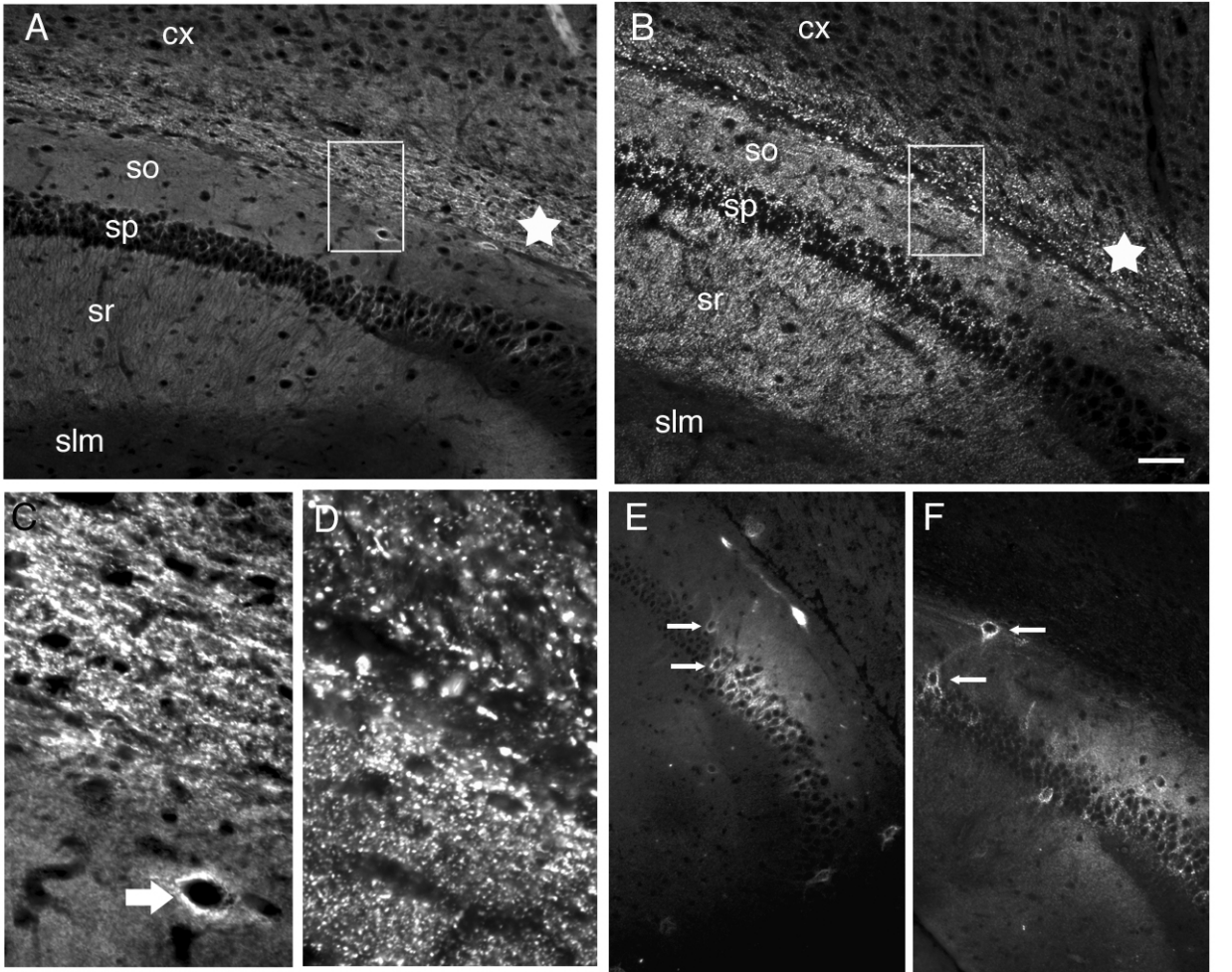


Fig. 5

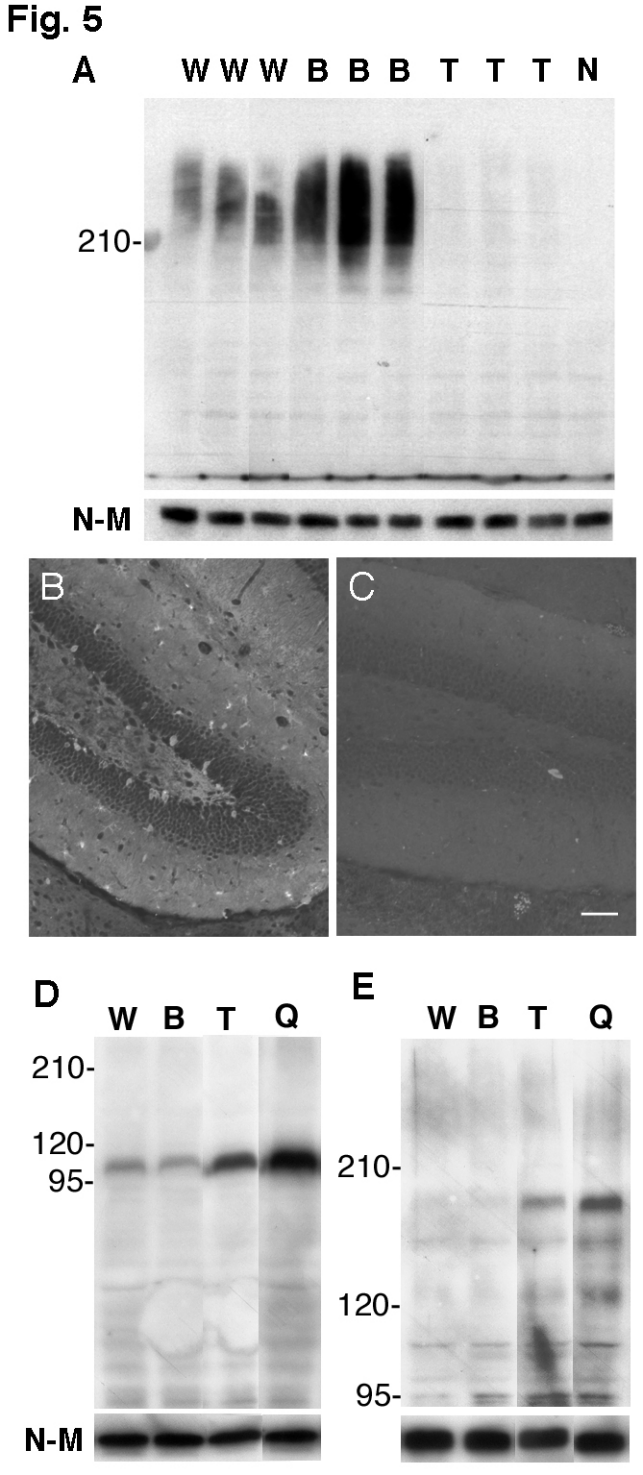
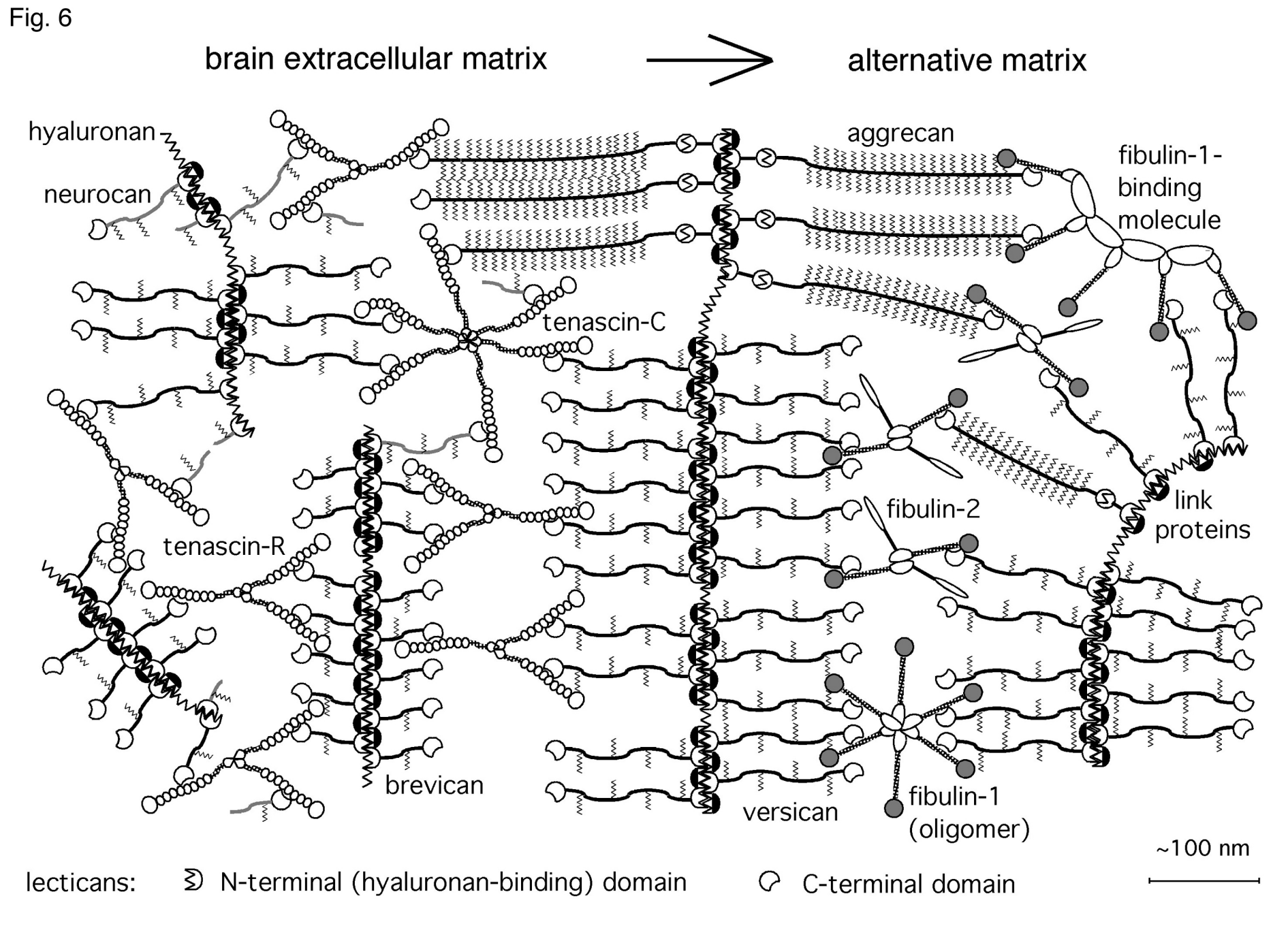


Fig. 6


brain extracellular matrix
alternative matrix
hyaluronan
neurocan
aggrecan
fibulin-1-binding molecule
tenascin-C
tenascin-R
fibulin-2
link proteins
brevican
versican
fibulin-1 (oligomer)
~100 nm
lecticans:
N-terminal (hyaluronan-binding) domain
C-terminal domain