Published in final edited form as:
Neurosci Lett. 2014 April 17; 565: 7–13. doi:10.1016/j.neulet.2014.01.055.

Role of GFAP in CNS injuries

Michael Brenner
Department of Neurobiology and the Civitan International Research Center, Center for Glial Biology in Medicine, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, Alabama

Abstract

The role of GFAP in CNS injury is reviewed as revealed by studies using GFAP null mice. In order to provide background information for these studies, the effects of absence of GFAP in the uninjured astrocyte is also described. Activities attributable to GFAP include suppressing neuronal proliferation and neurite extension in the mature brain, forming a physical barrier to isolate damaged tissue, regulating blood flow following ischemia, contributing to the blood-brain barrier, supporting myelination, and providing mechanical strength. However, findings for many of these roles have been variable among laboratories, pointing to the presence of unappreciated complexity in GFAP function. One complexity may be regional differences in GFAP activities; others are yet to be discovered.

Keywords

GFAP; astrocyte; CNS injury; reactive gliosis; ischemia; neurotrauma

Introduction

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is primarily expressed in astrocytes (reviewed in [1]). The evolution of a specific intermediate filament protein for astrocytes suggests that the protein plays a critical role, and its marked upregulation in CNS injury indicates that one of these is in injury damage control. This review exams the role of GFAP in CNS injuries; and to provide a foundation for this topic, also describes what is known about GFAP function in the uninjured state. Studies covered are largely limited to those investigating effects solely attributable to GFAP, and thus a substantial body of work examining the consequences of absence of both GFAP and vimentin [2] is not discussed, except when it enlightens findings obtained for GFAP alone.

Author contact information: Michael Brenner, PhD, Department of Neurobiology, tel,:(205) 934-1011, fax: (205) 975-6320, aaron@nrc.uab.edu. US Mail address: UAB/Neurobiology/CIRC 329, 1530 3rd Avenue South, University of Alabama at Birmingham, Birmingham, AL 35294-0021. Physical address (use for express mail): SRC R552, 1717 6th Avenue South, University of Alabama at Birmingham, Birmingham, AL 35294-0021.

A subtheme for this review arises from the remarkably discrepant results among laboratories for findings of GFAP functions. There has been a tendency for each laboratory to question (albeit tacitly) the competence of their competitors who present contrary observations, but the more likely explanation, and the one adopted here, is that these differences advertise complexities not yet appreciated, and provide an opportunity for deeper understanding of the astrocyte repertoire.

GFAP Overexpression

One approach to investigate the role of GFAP is to increase its expression as occurs during the reactive response to determine what other changes might occur, whereas another is to prevent GFAP upregulation, or to knock it out altogether. The former approach was undertaken by Messing et al. [3], who used a human GFAP transgene to increase GFAP expression in astrocytes, thus avoiding the confounding effects of an actual injury. Unexpectedly, chronically high GFAP levels proved lethal, and were accompanied by abundant deposition of GFAP-containing protein aggregates in astrocytes. These observations led to GFAP mutations being identified as the major cause of Alexander disease, a usually fatal neurodegenerative disorder characterized by astrocytic inclusions (reviewed in [4]). However, as the mutations appear to act by a gain of toxic function mechanism [4], this approach of chronic overexpression of GFAP does not provide information about the normal role of the protein in injury.

GFAP Null Mice

Suppression of GFAP expression was first accomplished by transfecting U251 astrocytoma cells with a GFAP antisense construct [5]. Whereas control U251 cells robustly extended processes when co-cultured with neurons, this response was almost completely absent in the transfected cells. Given the importance of astrocytic processes for guiding neuronal migration, inducing the blood-brain barrier, and ensheathing synapses, this requirement for GFAP for process extension suggested that a GFAP null mouse would be a dead mouse. Undeterred, four laboratories independently produced GFAP knockouts within a year of each other, and found them viable. Three of these groups, Gomi et al. [6], Pekny et al. [7] and McCall et al. [8], reported very similar findings of minimal effects of GFAP absence. All found normal development, growth, fertility and lifespan. All three also reported no difference from wild type in brain architecture, including unchanged numbers of neurons and astrocytes. No compensatory increase in any other intermediate filament was observed. The blood-brain barrier was found intact as judged by electron microscopy and exclusion of Evans blue and microperoxidase, the latter having a molecular weight of just 1862. Overt behavior and motor activity were normal.

The GFAP null line produced by the fourth group, Liedtke et al. [9], was also viable, but displayed some striking defects. Half the null mice older than 18 months developed hydrocephalus. Null mice over 18 months of age also had decreased levels of corpus callosum myelin, and 6 month old null mice had less myelination of the anterior column of the spinal cord, some non-myelinated axons in both the spinal cord and optic nerve, and myelinating, hyperplastic oligodendrocytes in the optic nerve. Significantly fewer total

blood vessels, especially of larger diameter, were found in the white matter of optic nerve and spinal cord at 4 months of age. Despite the aberrant morphology involving the optic nerve, there was no difference in its thickness, and visual evoked potentials in the visual cortex were normal. Use of ^{125}I-albumin revealed leakage of the blood-brain barrier in the lumbar spinal cord of mice over 1 year of age. These differences in tissue architecture in the spinal cord and optic nerve were apparently region specific, as no differences were seen in the cerebrum, brainstem, or cerebellum; although subsequently, Gimenez et al. [10] did report the presence of some disruption of myelin sheaths in the cerebellar white matter and granular layer of the Pekny et al. [7] GFAP null mouse. The other three laboratories may have missed observing the hydrocephalus because their mice were not followed beyond 14 months of age, but they also did not observe the changes in myelination, vascularization and blood-brain barrier that occurred much earlier in the Liedtke line. These analyses included ultrastructural study of the spinal cord, which found the diameter of blood vessels to be larger, rather than smaller than wild type [7,11], and of the optic nerve [8]. Thus the differences between the Liedtke line and those of the other groups arise from some unknown conditions interacting with the GFAP null. The basis for these different observations has not been pursued; doing so could provide a wealth of information about interacting partners of GFAP that regulate its roles in CNS development and function.

Astrocyte Processes

The state of astrocytic processes in the GFAP nulls is of particular interest given the prior finding in cell culture of their dramatic reduction in the absence of GFAP [5]. When these experiments were repeated using GFAP null primary astrocytes in place of transfected U251 cells, the results were not replicated [12]; instead, process extension by the cultured GFAP null astrocytes in response to neurons was indistinguishable from wild type. A subsequent analysis of GFAP null astrocytes cultured alone also showed no difference in morphology from wild type astrocytes [13]. In vivo, observations at the light microscope level of astrocytic processes in GFAP null mice found them to extend normally to and around blood vessels in the hippocampus [6], and ultrastructural examination of the hippocampus also found no differences from wild type [7,8]. However, in the optic nerve astrocytic processes were observed to be smaller than in the wild type [8], including those extending to the pial surface or to blood vessels. In the spinal cord, an ultrastructural study of the lateral funiculus [7] observed no difference in astrocyte process size, but an ultrastructural analysis in the anterior column of the cervical spinal cord at C7 revealed astrocyte processes to be short and club-like, and extracellular space increased [9]. Findings for the cerebellum of GFAP null mice mirror those of the spinal cord in inconsistency. Shibuki et al. [14] observed no differences in the structure of the cerebellum between GFAP null and wild type mice using detailed light and electron microscopic studies, but ultrastructural analysis by Gimenez et al. [10] found Bergmann glial processes were shorter and thinner, and more extracellular space was present. Glial coverage was incomplete of Purkinje cell soma, Purkinje dendrites, the vasculature, and the pial surface, and the endfeet did not appear to adhere as tightly to the pial surface. No explanation for this discrepancy is apparent. Gimenez et al. [10] did not specify the genetic background of their mice, but they likely were a mixed C57BL/6 and SJL as were the Shibuki et al. [14] mice, and Shibuki et al. [14] did not specify the age of

their mice, but they likely were young adults as were the 3 month old mice used by Gimenez et al. [10]. Perhaps more importantly, neither group stated the region of the cerebellum examined, raising the possibility that different lobes of the cerebellum, which are known to have different functions and to have evolved at different times, could also have different roles for GFAP. Thus overall, the observations concerning astrocyte processes suggest that the absence of GFAP strongly affects their formation in some CNS regions under some conditions, but is without consequence in others. Experiments specifically designed to investigate these regional differences should provide insights into the regulation of formation of astrocyte processes and the critical role these processes play in CNS functions.

The effect of GFAP absence on process extension following injury was investigated by Wilhelmsson et al. [15]. In order to observe the process of reactive gliosis in the absence of the confound of local traumatic tissue damage, they made an entorhinal cortex lesion and examined the downstream effects on astrocyte reactivity in the outer molecular layer of the hippocampal dentate gyrus. On the uninjured side, staining for glutamine synthetase revealed no difference from wild type in the appearance of astrocytic processes in mice doubly null for both GFAP and vimentin. However, on the injured side, the processes in the GFAP/vimentin double null appeared to be only about 50% as long as those of wild type, and those of the GFAP null about 80% as long. Dye-filling of astrocytes revealed no difference in the volume of tissue accessed by the reactive wild type and double null astrocytes, but the double null astrocytes had fewer thick, long processes, and those present were more tortuous. Thus the overall length of processes is not altered in reactive double null astrocytes in the dentate gyrus, but they are more irregular and less hypertrophic. Dye filling data for reactive astrocytes singly null for GFAP would be of considerable interest.

Growth Rate

Although they did not find an effect of the GFAP null on process extension in their co-culture experiments, Pekny et al. [12] did note that the growth rate of the null astrocytes was almost twice that of wild type. This was not confirmed by Xu et al. [16], however. These different results could possibly be due to the source of the astrocytes; Pekny et al. [12] used whole brains of P1 or P2 pups, whereas Xu et al. [16] used cerebral cortices of P0 pups; the genetic background for both was mixed C57BL/6 and 129 SV. In vivo there is no evidence for a growth rate difference--as noted above, several groups found equal numbers of astrocytes in GFAP null and wild type mice, and GFAP null astrocytes were found to proliferate to the same extent as wild type following a brain stab wound [11]. These data illustrate the difficulty of extrapolating cell culture studies to the animal, and raise again the possibility of regional differences in GFAP function.

Astrocyte-Neuronal Interactions and Adhesion

In addition to being used to study astrocyte process formation, co-culture of GFAP null astrocytes and neurons has also been used to examine effects on neurons. When GFAP null cortical astrocytes were co-cultured for 8 hours with DRG neurons isolated from E15 rat, neurite extension was the same as in the presence of wild type astrocytes [16]. However, when GFAP null cortical or spinal cord astrocytes were co-cultured instead for 7 days with

E14 mouse cortical neuroblasts, markedly increased neurite extension was observed [17,18]. This suggests that the source of neurons or duration of co-culture may be critical. Under the latter conditions, the number of βIII-tubulin positive neurons in the co-cultures was found to be about three times greater in the presence of GFAP null astrocytes than wild type [17]. This larger number of neurons was interpreted as due to greater neuronal survival, but could instead have resulted from increased differentiation or propagation. A difference in differentiation is suggested by a similar study in which the neural progenitors were neurospheres isolated from 4 day old mouse forebrain rather than neuroblasts isolated at E14 [19]. Perhaps due to this different source of progenitors, the finding that GFAP null astrocytes supported the presence of more βIII-tubulin stained neurons than wild type astrocytes was not replicated (neurite extension was not examined). However, when GFAP/vimentin double null astrocytes were used for the co-cultures, an increase in neurons was observed, and evidence was presented that this was caused by an increased rate of differentiation rather than proliferation or survival. The cultured double null astrocytes were found to be less active than the wild type in Notch signaling, an inhibitor of differentiation, which could account for their supporting a greater maturation of neurons.

A comparison of cultured GFAP null and wild type astrocytes for the levels of two extracellular matrix molecules that may regulate neurite extension and differentiation found that the level of laminin, which is a strong promoter of neurite outgrowth, was increased in the GFAP nulls, and that of chondroitin sulfate proteoglycan, which is inhibitory, was modestly decreased [18]. Another study, however, found no difference for these two extracellular matrix components between cultured wild type and GFAP null astrocytes [16]. This difference could be attributable to the astrocyte source, spinal cord for first report and cerebral cortex for the second. A dependence on cell type is clearly illustrated by an earlier finding that in mouse fibroblast L cells stably expressing GFAP laminin production was actually increased [20]. An evaluation of the effect of the GFAP null on neurite regrowth in vivo was performed using a spinal cord hemisection in GFAP null and GFAP/vimentin double null mice [21]. The GFAP/vimentin double null showed extensive sprouting of neurites from the uninjured side not seen in the wild type, and also behavioral improvement in traversing a grid walkway, but neither of these benefits was conferred by the GFAP null alone. In a different in vivo test of neurite extension, retinal cells isolated from wild type mouse pups were injected into the subretinal space of adult wild type, GFAP null, and GFAP/vimentin double null mice [22]. In the wild type eyes the injected retinal cells neither migrated nor extended neurites, whereas in double null mice they did both extensively. There was no neurite extension in the GFAP only null eyes and only limited migration, which did not reach statistical significance compared to wild type.

In summary, the absence of GFAP supports improved production of neurons and neurite extension under some conditions in culture. Since cultured neonatal astrocytes are believed to mimic reactive astrocytes in some respects, these findings suggest that the upregulation of GFAP that occurs in reactive gliosis may inhibit neurite growth and neuronal replenishment independently of producing a scar as a physical barrier. However, such an effect of the GFAP null was not observed in the few experiments performed in vivo. More extensive testing of different injury models in different CNS regions is warranted for better understanding of this potentially important injury-related role of GFAP.

Vimentin Polymerization

Vimentin and nestin are abundantly present in astrocyte progenitors, but as astrocytes mature, nestin becomes undetectable and vimentin is retained at lower levels in some cells and disappears from others [1]. Then both are upregulated with GFAP as part of the reactive response. Vimentin filaments are evident in astrocytes in the corpus callosum and hippocampus of wild type mice, but were found absent in GFAP null mice [23]. In addition, no vimentin filaments were found in the lateral funiculus of the cervical spinal cord of GFAP null mice [7]. However, vimentin filaments were present in cultured GFAP null astrocytes, or following a cortical stab wound [23]. These observations prompted the conclusion that vimentin requires a partner for polymerization, which can be GFAP in resting astrocytes and either GFAP or upregulated nestin in reactive ones. As apparent exceptions to this rule, vimentin filaments have been observed in uninjured GFAP null mice in the anterior column of the cervical spinal cord [9], in the spinal cord at T8 [24], in Müller cells in the retina [6], in the optic nerve [8], and in cerebellar Bergmann glia [6,10,14]. These different findings are perhaps the best indication of regional differences in the role of GFAP in astrocytes. Determining what these differences are will likely first require a better general understanding of the factors that control intermediate filament polymerization.

Long Term Potentiation and Depression

Despite the ultrastructure of the hippocampus being the same between GFAP null and wild type mice, McCall et al. [8] found that hippocampal long term potentiation was significantly increased in the null. However, no learning or memory correlate of altered long term potentiation has been reported; on the contrary, normal performance of the GFAP null was observed in the Morris water maze, which is considered a hippocampal dependent learning and memory task [7]. In examining the cerebellum, Shibuki et al. [14] found long term depression to be much weaker in the mutant than the wild type. Mutant mice also had impaired learning of a conditioned eye-blink response in which a tone was paired with a periorbital shock. No structural basis for the decrease in long term depression and conditioned learning was uncovered. At the light microscopic level, no difference was observed in the morphology of cerebellar Purkinje cells or Bergmann glia, including their processes, or in staining for vimentin, mGluR1, calbindin-D, inositol 1,4,5-triphosphate receptor type 1 or by Golgi impregnation. Electron microscopy found no difference in synaptic densities, structures, or ensheathment of Purkinje cells by Bergmann glia, including the spacing between glial processes and synapses between both climbing fibers and parallel fibers with Purkinje cells, and through use of immunogold, no difference in the intracellular localization of mGluR1, a major player in cerebellar neurotransmission. However, contrary to these negative structural data, Gimenez et al. [10] did find structural changes in the cerebellum of their GFAP null mice as described above, including shorter and thinner Bergmann glial processes and incomplete glial coverage of Purkinje dendrites. Other electrophysiological measurements by Shibuki et al. [14] were also negative. There was no difference in Purkinje cell excitatory postsynaptic potentials evoked by stimulating either climbing fibers or parallel fibers, and also no difference in paired pulse facilitation and depression, which are forms of short term plasticity. Normal Purkinje cell dendritic calcium spikes were evoked by climbing fiber inputs, and there was the normal one-to-one

relationship between climbing fibers and Purkinje cells. The deficiency in learning the conditioned eye-blink response was not accompanied by abnormalities in other motor tasks examined in the GFAP null mice, including analysis of gait, spontaneous activity, horizontal and vertical movements, rotorod performance, rope climbing and running through an elevated runway [14]. It is seductive to assume that the reduction in long term depression underlies the deficit in learning the conditioned eye-blink response, but abolishing long term depression has no effect on cerebellar motor tasks, including eyeblink conditioning [25]. Although they may thus not be causally linked, the results of Shibuki et al. [14] do indicate a role for GFAP in both long term depression and motor memory.

These effects of the GFAP null on long term potentiation and depression prompted Hughes et al. [26] to investigate whether the null mice differ in their glutamate transporters. They found that both glutamate uptake and the levels of the astrocytic glutamate transporters GLAST and GLT-1were decreased, unchanged or increased, depending on the brain region examined (cerebral cortex, hippocampus and cerebellum). A functional interaction was demonstrated in transfected COS7 cells, wherein the presence of GFAP increased D-aspartate transport by about 20%. These observations indicate that GFAP does indeed affect glutamate uptake, but as for process formation and vimentin polymerization, its effect is dependent on CNS region. A physical basis for defective GLAST trafficking in the absence of GFAP was provided by Sullivan et al. [27], who demonstrated an interaction between GFAP and GLAST by co-immunoprecipitation from brain homogenates. No such interactions were found for GLT-1 [27], but observations made by Li et al. [28] in GFAP/vimentin double nulls suggest a similar mechanism may be in play. They observed that although the total level of GLT-1 was similar between wild type and the double nulls, GLT-1 mediated glutamate transport was reduced by half. Together, these data suggest that GFAP facilitates extracellular glutamate clearance by participating in the trafficking or anchoring of glutamate transporters. The decrease in GLT-1 and GLAST levels often associated with injury [29] could possibly be due to sequestration of these transporters by the elevated GFAP levels in reactive astrocytes, preventing their transport to the plasma membrane.

Another way that astrocytes could affect synaptic physiology is through the provision of glutamine to neurons for synthesis of glutamate and GABA. Pekny et al. [30] found intracellular glutamine levels in cultured GFAP null and GFAP/vimentin double null astrocytes to be about 30% higher than in wild type astrocytes after 10 days in culture, and about twice as high as wild type after 20 days. The cause of this increase was not determined. As just noted, uptake of the glutamate substrate may actually be reduced in astrocytes lacking GFAP; and in a subsequent paper no difference was found in glutamine synthetase levels [15], although this does not rule out differences in activity of this highly regulated enzyme. A possible difference in glutamine release has not been investigated, but would be of considerable interest given the fundamental importance of the glutamate/glutamine cycle to neurotransmission.

Glial Scar Formation and Disease Response

The effect of absence of GFAP during a reactive response was examined following a needle stab wound through the frontal cortex [7]. The injury response appeared identical for both GFAP null and wild type mice, including formation of scar tissue and upregulation of vimentin. In follow-up studies that also included a knife wound to the dorsal funiculus of the spinal cord, no differences between wild type and GFAP null were observed for scar formation, vimentin upregulation or astrocyte proliferation [11]. Thus despite GFAP being a major constituent of the glia scar, a scar can effectively form in its absence. This is presumably due to the upregulation of vimentin that occurs during reactive gliosis, as evidenced by astrocytes lacking both GFAP and vimentin forming poorly developed glial scars [11]. Identical reactive responses of the GFAP null and wild type were also found for scrapie infection [6,31]. No differences were observed in vimentin upregulation, time of onset, symptoms or scrapie propagation. The GFAP null used by Gomi et al. [6] had β-galactosidase embedded in the GFAP gene, and both its upregulation and that of vimentin during Scrapie infection led these authors to conclude that “GFAP is not essential for the induction of reactive astrocytosis.”

In Alzheimer’s disease, astrocytes surrounding β-amyloid senile plaques are reactive and form a glial scar that walls off the plaques. To determine if this process was affected by the absence of GFAP, a β-amyloid peptide was injected into the CA3 region of hippocampal slices [16]. In a wild type slice, astrocytes extended processes parallel to the edges of the β-amyloid deposit that intertwined to form a distinct barrier, whereas the processes from GFAP null astrocytes were not well organized and failed to form a barrier. Cell culture studies also revealed a difference in adhesion properties between wild type and GFAP null astrocytes induced by β-amyloid peptide; wild type astrocytes adhered more to each other than to the β-amyloid peptide substrate, whereas the reverse was found for GFAP null astrocytes. Studies of the effect of the GFAP null on mouse models of Alzheimer’s disease would thus be of considerable interest.

Another disease with prominent glial scar formation is multiple sclerosis. Liedtke et al. [32] used the experimental autoimmune encephalomyelitis model of multiple sclerosis to determine if the myelination defects they observed in their GFAP null mice would result in a poorer outcome in a demyelinating disease. The immunized wild type and null mice had equal disease incidence, and equal scores for myelination, demyelination and immune cell infiltration. However, the null mice had slightly worse clinical scores. Lesion borders were poorly defined in the null, reminiscent of the findings noted above for β-amyloid deposits. This prompted the authors to suggest that the increased severity of experimental autoimmune encephalomyelitis in GFAP null mice was due to a defective glial scar permitting greater inflammatory toxicity, despite their observation of no quantitative difference in cellular inflammation. However, a marked increase in cellular inflammation was observed in a study of the effect of the GFAP null on progression of acute cerebral infection by *Staphylococcus aureus* and chronic infection by *Toxoplasma* [33]. For both infections the clinical signs, tissue damage and time to recovery were greater for the GFAP null than the wild type. This increased severity was attributed to poorer astrocyte process extension and organization in inflammatory loci.

In contrast to the greater susceptibility to inflammatory diseases in the absence of GFAP, GFAP null mice were found more resistant than wild type to induced neurotoxicity in the striatum [34]. Both lesion volume and loss of medium spiny neurons were reduced up to several fold in the GFAP null compared to wild type following striatal injection of either the mitochondrial poison 3-nitroproprionic acid or the excitotoxin quinolinic acid. This protection was correlated with a higher level of GDNF in the GFAP KO compared to wild type.

The above observations suggest that the contribution of GFAP to the injury response and glial scar formation may be context-dependent: no effect of the GFAP null was found for cutting injuries or scrapie; defective scar formation was found in mouse models of Alzheimer's disease, multiple sclerosis or infection; and protection against neurotoxicity was provided in the striatum.

Physical Trauma

Reasoning that GFAP may serve a role similar to keratins in providing tensile strength, Nawashiro et al. [35] compared wild type and GFAP null mice for the extent of traumatic brain injury produced by a weight drop device. An impact force just below the threshold that would produce serious injury in wild type mice resulted in acute death of most of the GFAP null mice, and the few survivors had hind limb paralysis. Histological examination revealed no difference in the injuries at the site of impact at the skull vertex, but found rupture of the veins exiting the upper cervical spinal cord of the null mice. Apparently the heads of the mice, cushioned on a foam support, underwent rapid displacement on impact, resulting in a shearing force in the neck similar to that occurring in shaken baby syndrome. When the heads of the mice were instead placed on a solid support, the GFAP null mice survived as well as the wild type. One interpretation of these results is that GFAP provides structural integrity to the vasculature through their enveloping endfeet.

The suggestion that astrocyte endfeet are structurally important is supported by studies in the eye that found fragility of the retina to shearing forces in GFAP/vimentin double null mice that was attributed to the absence of intermediate filaments in the Müller cell endfeet [36,37]. Although no difference was found for the GFAP null alone in these studies, they support a critical structural role for astrocytic endfeet, and raise the possibility that in other CNS regions loss of GFAP alone might compromise this role. For example, as noted above, ultrastructural studies of the cerebellum of GFAP null mice by Gimenez et al. [10] found Bergmann glial processes to incompletely cover the vasculature, and their endfeet to adhere less tightly to the pial surface. Astrocytic endfeet could contribute directly to vascular integrity as a structural element, or indirectly through its influence on other structural elements. A striking example of the latter is the finding that astrocytic laminins are required for differentiation and maintenance of vascular smooth muscle cells, and suppression of production of these laminins results in spontaneous intracerebral hemorrhagic stroke [38].

Ischemia

A different vascular role, regulation of blood flow, has been suggested for GFAP as a result of findings in a stroke model. Li et al. [28] observed about a 2-fold increase in infarct size in

GFAP/vimentin double null mice compared to wild type following permanent occlusion of the left middle cerebral artery, but no difference was observed for the GFAP single null. However, when the permanent middle cerebral artery occlusion was immediately followed by 15 minutes of transient carotid artery occlusion in GFAP null mice, Nawashiro et al. [39] found that within two minutes of onset of the transient carotid artery occlusion blood flow in the infarcted area was reduced to a greater extent in GFAP null mice than in wild type. Furthermore, the infarct volume measured at 48 hours was greater in GFAP null mice. This laboratory also found that transient carotid artery occlusion alone resulted in lower local cerebral blood flow in the GFAP null mice compared to wild type and higher intracranial pressure during reperfusion [40]. Astrocytic swelling was suggested as a possible explanation for these effects. Astrocytes are known to swell as a result of ischemia [41,42], and astrocytes lacking both GFAP and vimentin release less of the osmolyte taurine than wild type astrocytes, suggesting a defect in regulatory volume decrease [43]. However, the earliest time point in the studies showing astrocytic swelling following ischemia was 30 minutes, so it is not known if swelling occurs within the two minutes that Nawashiro et al. [40] detected reduced blood flow. Also, the impaired taurine release was seen in cultured astrocytes only if they lacked both GFAP and vimentin, but not if they lacked either alone. Most importantly, extracellular space has been inferred to be greater in GFAP null than wild type CNS following hypotonic stress, indicating that the null astrocytes swell less rather than more than the wild type [44], and thus suggesting that the decreased taurine release from GFAP/vimentin double null astrocytes reflects a reduced extent of swelling rather than a compromised ability to respond to it.

A different explanation for the greater reduction in blood flow in GFAP null mice following carotid artery occlusion draws on the observation described above that GFAP null mice are hypersensitive to vascular shear. Both these phenomena could be due to an altered association of astrocytic endfeet with the vasculature, such that both structural support and astrocytic signaling are defective. This suggestion is consistent with increased calcium in astrocytic endfeet being critical for astrocyte-vascular signaling (reviewed in [45]), and with the previously mentioned possibility that in at least some CNS regions both the number and diameter of astrocytic processes are reduced in GFAP null mice. Another indication of an altered interaction between GFAP null astrocytes and the vasculature is their reduced ability to induce formation of a permeability barrier in a cell culture model of the blood-brain barrier [46]. More detailed ultrastructural comparisons of astrocyte endfeet in wild type and GFAP null mice, as well as the strength of their attachment to the vascular and response to injury would be of interest.

Concluding Comments

Roles now attributed to GFAP in the CNS include suppressing neuronal proliferation and neurite extension in the mature brain, forming a physical barrier to isolate damaged tissue, participating in cerebellar motor learning, regulating blood flow following ischemia, contributing to the blood-brain barrier, supporting myelination, and providing mechanical strength. Findings for many of these functions, however, have been inconsistent among laboratories. Rather than being viewed as casting a shadow, these differences may be signaling that there is a richness and complexity in GFAP functionality that is yet to be

discovered. In several instances, such as vimentin polymerization and glutamate transport, regional differences in the role of GFAP are clearly evident, whereas in others this is suggested by comparisons of the protocols used. Astrocyte heterogeneity is well known (e.g., see [47]), but perhaps underappreciated when designing experiments and drawing conclusions from their results. Our own work has revealed unexpected regional differences in the use of regulatory elements that control GFAP transcription [48], suggesting regional differences in GFAP activities. However, for other discrepant findings the same CNS region was studied, indicating existence of other unrecognized variables. It is thus likely that continued pursuit of the role of GFAP in CNS injury will reveal far more than anticipated about the intricacies of the nervous system.

Acknowledgments

Writing of this review was supported by NINDS grants RO1NS39055 and P01NS42803 and a very patient wife.

References

1. Eng LF, Ghirnikar RS. GFAP and astrogliosis. Brain Pathol. 1994; 4:229–237. [PubMed: 7952264]
2. Pekny M, Wilhelmsson U, Pekna M. The dual role of astrocyte activation and reactive gliosis. Neurosci Lett. 2014 this issue.
3. Messing A, Head MW, Galles K, Galbreath EJ, Goldman JE, Brenner M. Fatal encephalopathy with astrocyte inclusions in GFAP transgenic mice. Am J Pathol. 1998; 152:391–398. [PubMed: 9466565]
4. Brenner, M.; Goldman, JE.; Quinlan, RA.; Messing, A. Astrocytes in (patho)physiology of the nervous system. In: Parpura, V.; Haydon, P., editors. Alexander disease: a genetic disorder of astrocytes. Vol. Chapter 24. Springer; New York, NY: 2009. p. 591-648.
5. Weinstein DE, Shelanski ML, Liem RK. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. J Cell Biol. 1991; 112:1205–1213. [PubMed: 1999469]
6. Gomi H, Yokoyama T, Fujimoto K, Ikeda T, Katoh A, Itoh T, Itohara S. Mice devoid of the glial fibrillary acidic protein develop normally and are susceptible to scrapie prions. Neuron. 1995; 14:29–41. [PubMed: 7826639]
7. Pekny M, Leveen P, Pekna M, Eliasson C, Berthold CH, Westermark B, Betsholtz C. Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally. Embo J. 1995; 14:1590–1598. [PubMed: 7737111]
8. McCall MA, Gregg RG, Behringer RR, Brenner M, Delaney CL, Galbreath EJ, Zhang CL, Pearce RA, Chiu SY, Messing A. Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology. Proc Natl Acad Sci USA. 1996; 93:6361–6366. [PubMed: 8692820]
9. Liedtke W, Edelmann W, Bieri PL, Chiu FC, Cowan NJ, Kucherlapati R, Raine CS. GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination. Neuron. 1996; 17:607–615. [PubMed: 8893019]
10. Gimenez YRM, Langa F, Menet V, Privat A. Comparative anatomy of the cerebellar cortex in mice lacking vimentin, GFAP, and both vimentin and GFAP. Glia. 2000; 31:69–83. [PubMed: 10816608]
11. Pekny M, Johansson CB, Eliasson C, Stakeberg J, Wallen A, Perlmann T, Lendahl U, Betsholtz C, Berthold CH, Frisen J. Abnormal reaction to central nervous system injury in mice lacking glial fibrillary acidic protein and vimentin. J Cell Biol. 1999; 145:503–514. [PubMed: 10225952]
12. Pekny M, Eliasson C, Chien CL, Kindblom LG, Liem R, Hamberger A, Betsholtz C. GFAP-deficient astrocytes are capable of stellation in vitro when cocultured with neurons and exhibit a reduced amount of intermediate filaments and an increased cell saturation density. Exp Cell Res. 1998; 239:332–343. [PubMed: 9521851]

13. Lepekhin EA, Eliasson C, Berthold CH, Berezin V, Bock E, Pekny M. Intermediate filaments regulate astrocyte motility. J Neurochem. 2001; 79:617–625. [PubMed: 11701765]
14. Shibuki K, Gomi H, Chen L, Bao S, Kim JJ, Wakatsuki H, Fujisaki T, Fujimoto K, Katoh A, Ikeda T, Chen C, Thompson RF, Itohara S. Deficient cerebellar long-term depression, impaired eyeblink conditioning, and normal motor coordination in GFAP mutant mice. Neuron. 1996; 16:587–599. [PubMed: 8785056]
15. Wilhelmsson U, Li L, Pekna M, Berthold CH, Blom S, Eliasson C, Renner O, Bushong E, Ellisman M, Morgan TE, Pekny M. Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. J Neurosci. 2004; 24:5016–5021. [PubMed: 15163694]
16. Xu K, Malouf AT, Messing A, Silver J. Glial fibrillary acidic protein is necessary for mature astrocytes to react to beta-amyloid. Glia. 1999; 25:390–403. [PubMed: 10028921]
17. Menet V, Gimenez YRM, Sandillon F, Privat A. GFAP null astrocytes are a favorable substrate for neuronal survival and neurite growth. Glia. 2000; 31:267–272. [PubMed: 10941153]
18. Menet V, Gimenez y Ribotta M, Chauvet N, Drian MJ, Lannoy J, Colucci-Guyon E, Privat A. Inactivation of the glial fibrillary acidic protein gene, but not that of vimentin, improves neuronal survival and neurite growth by modifying adhesion molecule expression. J Neurosci. 2001; 21:6147–6158. [PubMed: 11487638]
19. Wilhelmsson U, Faiz M, de Pablo Y, Sjoqvist M, Andersson D, Widestrand A, Potokar M, Stenovec M, Smith PL, Shinjyo N, Pekny T, Zorec R, Stahlberg A, Pekna M, Sahlgren C, Pekny M. Astrocytes negatively regulate neurogenesis through the Jagged1-mediated Notch pathway. Stem Cells. 2012; 30:2320–2329. [PubMed: 22887872]
20. Toda M, Asou H, Miura M, Toya S, Uyemura K. GFAP transfected cells produce laminin, leading to neurite outgrowth promotion. Neuroreport. 1994; 5:1969–1972. [PubMed: 7841387]
21. Menet V, Prieto M, Privat A, Gimenez y Ribotta M. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proc Natl Acad Sci USA. 2003; 100:8999–9004. [PubMed: 12861073]
22. Kinouchi R, Takeda M, Yang L, Wilhelmsson U, Lundkvist A, Pekny M, Chen DF. Robust neural integration from retinal transplants in mice deficient in GFAP and vimentin. Nat Neurosci. 2003; 6:863–868. [PubMed: 12845328]
23. Eliasson C, Sahlgren C, Berthold CH, Stakeberg J, Celis JE, Betsholtz C, Eriksson JE, Pekny M. Intermediate filament protein partnership in astrocytes. J Biol Chem. 1999; 274:23996–24006. [PubMed: 10446168]
24. Wang X, Messing A, David S. Axonal and nonneuronal cell responses to spinal cord injury in mice lacking glial fibrillary acidic protein. Exp Neurol. 1997; 148:568–576. [PubMed: 9417833]
25. Schonewille M, Gao Z, Boele HJ, Veloz MF, Amerika WE, Simek AA, De Jeu MT, Steinberg JP, Takamiya K, Hoebeek FE, Linden DJ, Huganir RL, De Zeeuw CI. Reevaluating the role of LTD in cerebellar motor learning. Neuron. 2011; 70:43–50. [PubMed: 21482355]
26. Hughes EG, Maguire JL, McMinn MT, Scholz RE, Sutherland ML. Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. Brain Res Mol Brain Res. 2004; 124:114–123. [PubMed: 15135219]
27. Sullivan SM, Lee A, Björkman ST, Meller SM, Sullivan RK, Poronnik P, Colditz PB, Pow DV. Cytoskeletal anchoring of GLAST determines susceptibility to brain damage. An identified role for GFAP. J Biol Chem. 2007; 282:2914–23.
28. Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, Nodin C, Stahlberg A, Aprico K, Larsson K, Yabe T, Moons L, Fotheringham A, Davies I, Carmeliet P, Schwartz JP, Pekna M, Kubista M, Blomstrand F, Maragakis N, Nilsson M, Pekny M. Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab. 2008; 28:468–481. [PubMed: 17726492]
29. Maragakis NJ, Rothstein JD. Glutamate transporters: animal models to neurologic disease. Neurobiol Dis. 2004; 15:461–473. [PubMed: 15056453]
30. Pekny M, Eliasson C, Siushansian R, Ding M, Dixon SJ, Pekna M, Wilson JX, Hamberger A. The impact of genetic removal of GFAP and/or vimentin on glutamine levels and transport of glucose and ascorbate in astrocytes. Neurochem Res. 1999; 24:1357–1362. [PubMed: 10555775]

31. Tatzelt J, Maeda N, Pekny M, Yang SL, Betsholtz C, Eliasson C, Cayetano J, Camerino AP, DeArmond SJ, Prusiner SB. Scrapie in mice deficient in apolipoprotein E or glial fibrillary acidic protein. Neurology. 1996; 47:449–453. [PubMed: 8757019]
32. Liedtke W, Edelmann W, Chiu FC, Kucherlapati R, Raine CS. Experimental autoimmune encephalomyelitis in mice lacking glial fibrillary acidic protein is characterized by a more severe clinical course and an infiltrative central nervous system lesion. Am J Pathol. 1998; 152:251–259. [PubMed: 9422542]
33. Stenzel W, Soltek S, Schluter D, Deckert M. The intermediate filament GFAP is important for the control of experimental murine Staphylococcus aureus-induced brain abscess and Toxoplasma encephalitis. J Neuropathol Exp Neurol. 2004; 63:631–640. [PubMed: 15217091]
34. Hanbury R, Ling ZD, Wuu J, Kordower JH. GFAP knockout mice have increased levels of GDNF that protect striatal neurons from metabolic and excitotoxic insults. J Comp Neurol. 2003; 461:307–316. [PubMed: 12746870]
35. Nawashiro H, Messing A, Azzam N, Brenner M. Mice lacking GFAP are hypersensitive to traumatic cerebrospinal injury. Neuroreport. 1998; 9:1691–1696. [PubMed: 9665584]
36. Lundkvist A, Reichenbach A, Betsholtz C, Carmeliet P, Wolburg H, Pekny M. Under stress, the absence of intermediate filaments from Muller cells in the retina has structural and functional consequences. J Cell Sci. 2004; 117:3481–3488. [PubMed: 15226376]
37. Verardo MR, Lewis GP, Takeda M, Linberg KA, Byun J, Luna G, Wilhelmsson U, Pekny M, Chen DF, Fisher SK. Abnormal reactivity of muller cells after retinal detachment in mice deficient in GFAP and vimentin, Invest. Ophthalmol Vis Sci. 2008; 49:3659–3665.
38. Chen ZL, Yao Y, Norris EH, Kruyer A, Jno-Charles O, Akhmerov A, Strickland S. Ablation of astrocytic laminin impairs vascular smooth muscle cell function and leads to hemorrhagic stroke. J Cell Biol. 2013; 202:381–395. [PubMed: 23857767]
39. Nawashiro H, Brenner M, Fukui S, Shima K, Hallenbeck JM. High susceptibility to cerebral ischemia in GFAP-null mice. J Cereb Blood Flow Metab. 2000; 20:1040–1044. [PubMed: 10908037]
40. Nawashiro H, Huang S, Brenner M, Shima K, Hallenbeck JM. ICP monitoring following bilateral carotid occlusion in GFAP-null mice. Acta Neurochir Suppl. 2002; 81:269–270. [PubMed: 12168322]
41. Garcia JH, Liu KF, Yoshida Y, Chen S, Lian J. Brain microvessels: factors altering their patency after the occlusion of a middle cerebral artery (Wistar rat). Am J Pathol. 1994; 145:728–740. [PubMed: 8080052]
42. Pantoni L, Garcia JH, Gutierrez JA. Cerebral white matter is highly vulnerable to ischemia. Stroke. 1996; 27:1641–1646. [PubMed: 8784142]
43. Ding M, Eliasson C, Betsholtz C, Hamberger A, Pekny M. Altered taurine release following hypotonic stress in astrocytes from mice deficient for GFAP and vimentin. Brain Res Mol Brain Res. 1998; 62:77–81. [PubMed: 9795147]
44. Anderova M, Kubinova S, Mazel T, Chvatal A, Eliasson C, Pekny M, Sykova E. Effect of elevated K(+), hypotonic stress, and cortical spreading depression on astrocyte swelling in GFAP-deficient mice. Glia. 2001; 35:189–203. [PubMed: 11494410]
45. Carmignoto G, Gomez-Gonzalo M. The contribution of astrocyte signalling to neurovascular coupling. Brain Res Rev. 2010; 63:138–148. [PubMed: 19948187]
46. Pekny M, Stanness KA, Eliasson C, Betsholtz C, Janigro D. Impaired induction of blood-brain barrier properties in aortic endothelial cells by astrocytes from GFAP-deficient mice. Glia. 1998; 22:390–400. [PubMed: 9517571]
47. Emsley JG, Macklis JD. Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. Neuron Glia Biol. 2006; 2:175–186. [PubMed: 17356684]
48. Yeo S, Bandyopadhyay S, Messing A, Brenner M. Transgenic analysis of GFAP promoter elements. Glia. 2013; 61:1488–1499. [PubMed: 23832770]

Highlights for Role of GFAP in CNS injuries

1. The response to CNS injury is altered in multiple ways in GFAP null mice.
2. There is considerable variability in findings of the effects of the GFAP null.
3. GFAP function likely differs depending on CNS region and other variables.