I. INTRODUCTION

Neuroglia are a highly heterogeneous population of nonexcitable cells scattered throughout the CNS and responsible for a wide range of housekeeping functions including the formation of myelin. The main function of neuroglia is to maintain the homeostasis of the nervous tissue and to control, protect, and support the function of neurons. Viewed from this perspective, neuroglia respond to injury to mount defensive reaction and restore homeostasis, but when malfunctioning, neuroglia can also constitute the primary pathogenic element (249). In the CNS, astrocytes, oligodendrocytes, NG2 cells and ependymal cells are derived from the ectoderm and constitute the macroglia (248). Microglia, on the other hand, originate from the mesodermal/myeloid tissue and are regarded as the macrophages of the CNS (190).

Astrocytes, also known as astroglia, received their name from their characteristic starlike appearance. Astrocytes are the most abundant cells in the CNS. Astrocytes are not uniform; their functions and morphology differ largely depending on their location, subtypes, and the developmental stage. The most prevalent are the protoplasmic astrocytes of the gray matter. The protoplasmic astrocytes exhibit short and largely branched tertiary processes and are in close proximity to neuronal synapses. The fibrous astrocytes are typically found in the white matter and exhibit long unbranched processes. Radial glia, that are present in the periventricular space and play a major role in neuronal migration during development, are another example of astrocyte heterogeneity, and in the adult brain they are present as Müller cells of the retina or Bergmann glia of the cerebellum (77a, 188a, 248).

Reactive astrogliosis, a response of astrocytes seen in a multitude of neurological disorders, can be defined as constitutive, graded, multistage, and evolutionarily conserved defensive astroglial reaction (245). Astrocyte reactivity and reactive astrogliosis accompany many pathological situations that affect the CNS, such as trauma, ischemic damage, neuroinflammation, or neurodegeneration. Compared with nonreactive astrocytes, reactive astrocytes show altered expression of many genes and exhibit distinct morphological and functional features (63, 66, 94, 175, 220, 223, 246, 260). The concept of reactive astrogliosis and its molecular and cellular definition is still incomplete, and we are only starting to understand its multifaceted functions in disease pathogenesis and in the process of recovery from CNS injuries (17, 34, 52, 146, 210, 221) (FIGURE 1).
Before even considering the reasons why the reactive response of astrocytes in a disease context might have evolved, we have to acknowledge one important fact. For all the organs in the body except for the brain, we are able to provide a solid connection between the function of individual cellular players and the function of the organ itself. Such a picture might be incomplete, and perhaps some of the interpretations might not be completely true and will be revised in the future, but it is still a relatively solid foundation on which we can interpret what might be going on in a pathological context. In contrast, the brain remains the only organ where we are still unable to connect the function at the level of individual cells and their networks to some of the major functions at the level of the whole organ, e.g., thinking, memory, emotions, etc. The existence of such a “functional gap” asks for caution when it comes to our interpretation of a particular cellular response in a disease context, such as astrocyte activation and reactive astrogliosis, that accompany a particular neuropathology.

II. STARTING WITH A HEALTHY BRAIN

To be able to understand the function of reactive astrocytes, i.e., astrocytes in a disease context, it might be best to start with the appreciation of the functions of astrocytes in normal unchallenged CNS, i.e., the brain, spinal cord, and retina. Astrocytes are abundant throughout the CNS (FIGURE 2), and the protoplasmic astrocytes divide the whole gray matter of the brain and spinal cord into distinct domains, with blood vessels, neurons, and synapses contained within these domains (37, 38, 83, 86, 124, 158). The fibrous astrocytes in the white matter are in physical contact with oligodendrocytes and play a crucial role in myelination and support of the white matter (reviewed in Ref. 141). The membrane of astrocyte processes is often in a position also with other cell types in the CNS, such as neurons, endothelial cells, or pericytes, and this forms the basis for extensive cell-cell communication and processes, such as recycling of neurotransmitters or nutrition of neurons (181, 200, 215). Based on these functions, astrocytes were for decades considered to be cells assisting and nurturing neurons (113). However, astrocyte functions in unchallenged CNS go far beyond assistance and support (170, 247). Astrocytes were shown to control formation, maintenance, function, and removal of neuronal synapses (45, 68, 168, 228, 237, 238); help to control blood flow (104, 120, 153, 207, 276); and together with pericytes regulate the blood-brain barrier (1, 11, 17, 133). A single protoplasmic astrocyte in the human brain accommodates within its domain as many as two million neuronal synapses (161). The specialized foot processes of perivascular astrocytes are in physical

FIGURE 1. Reactive astrogliosis is a response of activated astrocytes seen in many neurological diseases. It is at least partially disease specific. In most situations, it can be viewed as a defensive reaction counteracting acute stress, restoring the CNS homeostasis and limiting the tissue damage. Persisting reactive astrogliosis can be maladaptive and lead to inhibition of neural plasticity and other regenerative responses.
contact with the endothelial cells; these astrocyte endfeet completely ensheathe CNS capillaries, and astrocytes secrete factors that control formation, maintenance, function, and repair of the blood-brain barrier (244). It is possible that all astrocytes participate in the astrovascular interface with at least one astrocyte process being in contact with a blood vessel (244), and through their endfeet regulate the transport of water (89) and other substances between the intravascular space and brain parenchyma (5). It is very tempting to view an astrocyte as a cell that integrates the functions of different cellular elements of the CNS. This concept has recently been supported by experiments in which mouse brain cortex was partially humanized by transplanted human astrocytes (90). When human glial progenitor cells were grafted into mouse brain, they developed into human-like astrocytes, became gap-junction-coupled to mouse astrocytes, and showed some humanlike physiological phenotypes including fast propagation of calcium signals, enhanced long-term potentiation, associated with improved learning and memory of the recipient mice (90). These findings support the notion that human and rodent astrocytes are different (161), and this difference is preserved when the cells are placed into host tissue of another species such as human astrocytes being able to influence a broad range of brain functions in mice.

Yet another level of complexity emerges when we stop thinking about astrocytes as individual cells and start to apply similar criteria that we use when we characterize neuronal networks, i.e., to think of them as elements within a network. Astrocytes are interconnected through gap junctional coupling into a network that allows communication between individual astrocytes (70, 233). The astrocyte network might possess unique features that could be compared with a matrixlike system spread throughout the CNS, able to constantly sense the environment, interact with other cellular elements, and mount appropriate responses (FIGURES 3, 4, and 5).

A further increase in the complexity is caused by a substantial heterogeneity of astrocytes (6, 147, 273), which can perhaps be compared with the heterogeneity of neurons. In sharp contrast to neuronal classification aided by the possibility of defining neurons by their anatomical location, axonal arrangement, the type of neurotransmitter used, their electrophysiological responses, etc., we have yet to classify astrocytes. Molecular classification of astrocytes under physiological conditions as well as disease-activated astrocytes is now being attempted by a number of research groups (21, 39, 138, 165, 226, 271, 273), and will constitute the basis for their functional classification. The latter will in turn help us to form and test the unifying hypotheses about the function of astrocytes in a disease context.

In vitro systems allowing maintenance and experimentation on primary cultured astrocytes, alone or in cocultures with other CNS cells, have been used for decades (26, 149, 203) (FIGURE 6A). They have been essential for understanding the function of astrocytes in health including their function in metabolism, neurotransmitter recycling, and calcium signaling and to mimic various disease situations such as injury, hypoxia, or neurodegenerative diseases (2, 3, 51, 65, 76, 128, 157, 206, 230, 265). Traditional two-dimensional culture systems for astrocytes, however, have a number of limitations that include highly reduced morphological complexity of astrocytes upon their transfer in vitro and undesired baseline reactivity of astrocytes upon in vitro culture (128, 186). Some of these limitations can now be overcome with the use of newly developed methods for astrocyte preparation (73) and three-dimensional cell culture systems (186) (FIGURE 6B). In contrast to artificial reactivity of astrocytes in response to two-dimensional culture environ-
ment with enforced planar tiling of adjacent astrocytes and profound biochemical signs of cellular stress, the three-dimensional culture environment leads to minimal astrocyte reactivity under basal conditions, thus making the whole range of astrocyte activation available for experimental manipulation, e.g., in studies assessing treatment-induced activation of astrocytes, alone or in cultures with other cells (186). The three-dimensional astrocyte culture systems are already proving their usefulness for assessing physiological and pathophysiological responses of astrocytes (186, 187) and may also become valuable for the identification of clinically relevant targets and leads targeting astrocytes.

III. ASTROCYTE REACTIVITY AND REACTIVE ASTROGLIOSIS

Keeping in mind the above-mentioned gap in our understanding of the functions of astrocytes in the context of the functions of the healthy CNS, let’s try to address two questions: when and where do we see astrocyte activation and...
why has it evolved? There are many molecular and some morphological features of reactive astrocytes and reactive astrogliosis, i.e., astrocytes responding to an injury or other pathological processes in the CNS. Of those, upregulation of glial fibrillary acidic protein (GFAP), the main constituent of astrocyte intermediate filaments (IFs, sometimes also referred to as nanofilaments), has most commonly been used as a hallmark of reactive astrocytes in both human pathology and in experimental studies using various mammalian models (67). The expression of glutamine synthetase (GS), an enzyme essential for glutamate conversion into glutamine by astrocytes, is also altered in a number of neurological conditions such as brain hypoxia and ischemia, epilepsy, and chronic neurodegenerative processes. The alterations in GS expression are brain region specific, and the GS expression does not positively correlate with the expression of GFAP (197). Thus, whereas GS serves as an astrocyte marker, it does not appear as a good marker of reactive astrogliosis. The critical involvement of GS in normal brain development and function, demonstrated by an early mortality of both GS-deficient mice (92) and humans (87), points to the role of reduced GS expression and function in the pathogenesis of at least some CNS disorders.

Immunohistochemical analyses of the brain, spinal cord, or retinal tissue show upregulation of GFAP and other signs of astrocyte reactivity and reactive astrogliosis in a whole range of neuropathologies, such as neurotrauma; focal brain ischemia; brain hemorrhage; perinatal asphyxia; acute, subacute, or chronic CNS infections; epilepsy; primary or secondary CNS tumors; retinal ischemia; diabetic retinopathy; Alzheimer’s disease (AD), Parkinson’s disease; multiple sclerosis; Batten disease (BD); or amyotrophic lateral sclerosis (ALS, known also as Lou Gehrig’s disease). Cytokines, such as transforming growth factor (TGF)-α, ciliary neurotrophic factor (CNTF), interleukin (IL)-6, leukemia inhibitory factor (LIF), and oncostatin M, trigger astrocyte activation in a rodent brain (15, 101, 118, 188, 225, 263). The effect on astrocyte activation may be mediated via the gp-130/activator of transcription 3 (STAT3) signaling pathway, phosphorylation, and nuclear translocation of STAT3 in astrocytes (225) as well as indirectly through the effects of these molecules on other cell types such as microglia, neurons, or endothelial cells. Signaling mediated by β1-integrin has the opposite effect on astrocyte activation and is required to promote their acquisition of a mature, nonreactive state (194). Thus alterations in STAT3 or β1-integrin-mediated signaling may be involved in eliciting specific aspects of reactive astrogliosis after injury. Inositol 1,4,5-trisphosphate (IP₃)-dependent Ca²⁺ signaling and the downstream functions of N-cadherin in astrocytes are required for normal reactive astrogliosis and neuroprotection after brain injury (110). Whereas epidermal growth factor receptor (EGFR) is not expressed in nonreactive adult astrocytes, it is detected in reactive astrocytes and in activated microglia/macrophages, and EGFR activation has been implicated in astrocyte transition from nonreactive to the reactive state (69, 183). EGFR ligands stimulate the secretion of chondroitin sulfate proteoglycans, and inhibition of EGFR signaling enhances axonal regeneration and improves functional recovery after CNS injury (69, 123, 218). Astrocyte polarity and directional migration seem to play a crucial role in astrocyte ability to react to injury: astrocytes depleted of the small RhoGTPase Cdc42, a key regulator of cell polarization, show impaired recruitment to the stab wound lesion despite their upregulation of GFAP and hypertrophic response (192). On a morphological level, reactive astrogliosis ranges from subtle to moderate to very prominent, with the prominent one being often accompanied by glial scarring, a phenomenon known by generations of neuropathologists (221). Glial scarring has been traditionally connected to astrocytes, but most recently, pericytes were proposed to be key contributors to glial scars (82).
In response to injury, a proportion of reactive astrocytes proliferate, and this increases the number of astrocytes at the lesion site (33, 193, 220). When such astrocytes are isolated and cultured in vitro, some are capable of forming neurospheres that contain self-renewing and multipotent cells (32, 127). This “stem cell response” of astrocytes in the adult mammalian brain is injury dependent, as it is induced only by “invasive” injury, such as stab wound or brain ischemia, but not by “noninvasive” injury, such as chronic amyloidosis or induced neuronal death (217). The signal that acts directly on astrocytes and is both necessary and sufficient to elicit the stem cell response of astrocytes is sonic hedgehog, conceivably derived from the cerebrospinal fluid and plasma (217). The proliferative response of astrocytes after injury seems to be specific for astrocytes in the juxtavascular space and depends only partially on Cdc42 (16). Contrary to what was previously believed, repeated examination of the same area in the injured mouse brain cortex by live imaging suggested that astrocytes do not migrate towards the injury side (16). Most astrocytes in the injured cortex become hypertrophic, upregulate GFAP, but stay within their tiled domains with only a limited overlap between domains of neighboring astrocytes (16, 260), while subsets of astrocytes become polarized or proliferate, the latter typically found in close association with blood vessels (16). Thus the reaction of cortical astrocytes to injury shares some similarities with astrocytes during development, which proliferate but remain in their original positions not showing any signs of migration (77, 236). It was proposed that these proliferating blood vessel-associated astrocytes regulate migration and proliferation of glial scar forming pericytes (16, 82). Moreover, during development, astrocyte precursors migrate from the ventricular zone in a strictly radial fashion that resembles the columnar distribution of cortical projection neurons (189) and form well-defined and stable domains throughout the brain and spinal cord (236). There is no evidence of secondary tangential migration of astrocytes in healthy brain or after injury (236). It was proposed that this region-specific astrocyte allocation throughout CNS allows astrocytes to act as repositories of spatial information for development and region-specific control of the CNS functions (236). The above would strengthen the concept of an astrocyte as an integrating element of the CNS.

With respect to the primary lesion (which can range from local to generalized), reactive astrocytes form a border between the lesion and the surrounding CNS tissue (FIGURE 7A), or are present throughout the lesion (FIGURE 7B). The former can be exemplified by reactive astrogliosis around focal ischemic lesions, reactive astrogliosis in larger neurotrauma, or by the presence of reactive astrocytes around amyloid plaques in AD. Thus, at least in some situations, reactive astrocytes seem to constitute a border between a focal lesion and the tissue around it. This aspect of reactive astrogliosis might have evolved to demarcate the lesion and temporarily or permanently separate it from the surrounding tissue (191, 252). Such a sequestering of a lesion might favor quick clinical stabilization and thus allow survival but, as will be discussed further, might affect the quality and quantity of the regenerative response later on (214).

Only recently, we have started to unravel to what extent astrocyte reactivity, reactive astrogliosis, and correspond-
ing changes in the astrocyte network are disease specific or have disease-specific effects. Indeed, the available evidence suggests that reactive astrogliosis in different neuropathologies has both common and unique cellular and molecular characteristics, that are qualitative as well as quantitative (271). Barres and co-workers (271) compared gene expression profiles in reactive astrocytes isolated from mouse brains in two injury models: ischemic stroke and bacterial endotoxin-induced global neuroinflammation and found that at least 50% of the injury-altered gene expression was injury type specific (271). As mentioned above, the “stem cell response” of astrocytes is also dependent on the invasive nature the injury (217).

IV. EXPERIMENTAL MANIPULATION OF REACTIVE ASTROGLIOSIS IN CNS TRAUMA AND FOCAL BRAIN ISCHEMIA

Given the critical role of astrocytes in both the control and support of various cell types and diverse functions of the CNS, it is not surprising that elimination of astrocytes in developing mouse brain led to an early death of the animals (56). Several experimental approaches have been used to either eliminate reactive astrocytes or to prevent them from becoming fully reactive.

Sofroniew’s group (36) generated a transgenic mouse model in which herpes simplex virus thymidine kinase was placed under the control of the GFAP promoter. Administration of gancyclovir to such mice in conjunction with neurotrauma led to the elimination of dividing GFAP-positive cells that predominantly constitute the dividing subpopulation of reactive astrocytes. The results obtained from this mouse model showed that the dividing subpopulation of reactive astrocytes has a positive role in the acute repair process limiting the extent of neurodegeneration and allowing the repair of the blood-brain barrier, and suggested that astrocyte activation and reactive astrogliosis are important for the acute response after brain or spinal cord trauma (36, 71, 222). The above transgenic mouse model also helped to establish that most, if not all, adult mouse neural stem/progenitor cells (NSC/NPSs) at least transiently express GFAP (75, 137). The expression of IF proteins GFAP and nestin and a host of other markers is shared between reactive astrocytes and NSC/NPSs (47, 62, 193, 226, 242). In fact, it was proposed that some reactive astrocytes have stem cell properties and the potential to differentiate into neurons or oligodendrocytes (32, 217).

Highly interesting data came from experiments in which reactive astrogliosis around focal lesions was manipulated by ablation of the transcription factor STAT3, a member of the Janus kinase-STAT family, and a transducer of signals for many cytokines and growth factors, such as IL-6, LIF, and CNTF (112, 225, 266). GFAP or nestin promoter-driven ablation of STAT3 in astrocytes led to the inhibition of astrocyte migration and lesion demarcation together with increased infiltration by CD11b positive inflammatory cells, associated with larger lesions and more prominent functional impairment (95, 163, 256). In contrast, following spinal cord lesions, mice with nestin promoter-driven ablation of Socs3, a negative feedback molecule of STAT3 (74, 151), showed increased phosphorylation of STAT3, increased astrocyte migration, more prominent contraction of the lesion area, and better functional recovery compared with wild-type mice (163). These results point to important

**FIGURE 7.** Reactive astrocytes in neuropathologies. A: reactive astrocytes around CNS injury were visualized with antibodies against GFAP, a component of the astrocyte intermediate filament system (red). In situations such as neurotrauma or focal brain ischemia, astrocytes surround the lesion area with processes of some astrocytes characteristically extended towards the lesion. [From Pekny and Pekna (176).] B: reactive astrocytes in an epileptic focus from a patient who suffered from pharmacologically intractable epilepsy were visualized with antibodies against GFAP (red). Reactive astrocytes are typical components of epileptic lesions (258) and here they are spread throughout the epileptic focus. (Courtesy of Drs. Pekny, Wilhelmsson, Rydenhag, and Malmgren, University of Gothenburg.)
functions of reactive astrocytes and STAT3 signaling in CNS injury and suggest that the demarcation of the lesion area by reactive astrocytes evolved to sequester a potentially toxic lesion environment from the rest of CNS (95, 163, 191, 252), even though this might restrict regenerative responses at a later stage (214).

Another attempt to elucidate the function of reactive astrocytes was to use genetic ablation in mice of the IF proteins, the upregulation of which had been considered the hallmark of reactive astrogliosis. Mice deficient for GFAP (GFAP<sup>−/−</sup>) were generated independently in four laboratories (81, 134, 148, 174). While one laboratory reported spontaneous demyelination in the CNS (134), the other three did not observe any overt CNS pathology in unchallenged GFAP<sup>−/−</sup> mice (81, 148, 174). Similarly, unchallenged vimentin-deficient mice (Vim<sup>−/−</sup>) did not show any CNS defects (50). This outcome is understandable in the light of what we now know about the IF system of a cell, namely, that it is critical in situations connected with acute, subacute, or chronic cellular stress (173). Also, since the IFs in reactive astrocytes are composed of GFAP, vimentin, and nestin, and in some reactive astrocytes also synemin (107, 180, 226); genetic ablation of GFAP or vimentin still allows IFs to be formed (in the former case composed of vimentin, nestin and in some astrocytes of synemin, in the latter case of GFAP). However, combined deficiency of GFAP and vimentin in GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> mice leads to complete absence of IFs in reactive astrocytes (172), since in the absence of both GFAP and vimentin, neither nestin (64) nor synemin (107) is able to self-polymerize or co-polymerize into IFs. GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> mice show reduced reactive gliosis and glial scar formation, a slower healing process with increased loss of neuronal synapses after neurotrauma (172, 262), and decreased resistance of the CNS tissue to severe mechanical stresses (142, 243). While astrocytes around the CNS lesion in GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> mice are comparable to those of wild-type mice with respect to their number and the volume they access (262), they do not develop the typical reactive phenotype characterized by the thickening (hypertrophy) of their main cellular processes (260, 262) (FIGURE 8A AND B).

This shows that IF upregulation is an important part of astrocyte activation and reactive astrogliosis, albeit not their proliferation in response to traumatic brain injury, and suggests a positive role for reactive astrocytes in the acute phase of neurotrauma (175, 176). The same conclusion was made by exposing the GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> mice to focal brain ischemia (induced by the transection of the middle cerebral artery), a mouse model of ischemic stroke, which resulted in larger infarction compared with wild-type mice (132). The underlying cellular and molecular mechanisms remain incompletely understood, although the astrocyte IF system has been linked to astrocyte motility (131); viscoelastic properties, which might affect cell migration (139); vesicle trafficking (184, 185, 241); activation of Erk and c-fos (156); the efficiency of glutamate transport and astrocyte gap junctional communication (132), both of which play an important role in CNS trauma or ischemia (135); response to hypoosmotic and oxidative stress and neuroprotective properties (55, 58); reconstruction of blood-brain barrier (177); MHC class II molecule presentation (241); and interaction with microglia (125, 143). The functions of astrocytes in healthy CNS and in acute and late stages of neurological diseases are exemplified in FIGURE 8C.

V. REACTIVE ASTROGLIOSIS IN NEURODEGENERATIVE DISEASES

In several neurodegenerative diseases including AD and BD, reactive astrogliosis is very prominent. In AD, reactive astrocytes intimately associate with amyloid plaques, but the functional implication of this phenomenon was unclear. On one hand, activation of astrocytes was often viewed as part of the neuroinflammatory neurotoxic response (152, 264); on the other hand, it was shown that in brain slices from AD mice, astrocytes migrated and degraded amyloid plaques (263) in an apolipoprotein E-dependent manner (121). Whether this is the case in human AD brain tissue remains to be determined. Whereas both microglia and astrocytes around amyloid plaques show increased immunoproteasome expression (164), amyloid deposition is associated with increased proliferation of microglia but not reactive astrocytes (109, 217). When GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> mice that exhibit attenuated reactive astrogliosis were crossed with a mouse model of AD-like pathology (transgenic mice expressing mutant human amyloid precursor protein and presenilin-1), the progression of neuropathological changes was facilitated, with increased plaque load and more abundant dystrophic neurites (FIGURE 9A) (125). Amyloid precursor protein expression and processing were both normal, which implies that reactive astrocytes affect plaque dynamics through interaction with amyloid plaques but not via synthesis or metabolism of amyloid protein. The processes of GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> astrocytes in the vicinity of amyloid plaques showed no signs of the characteristic hypertrophy of reactive astrocytes and almost no physical interactions with amyloid plaques (FIGURE 9B) (125). These results imply that reactive astrocytes inhibit the formation and growth of amyloid plaque in the brain by yet to be elucidated mechanisms involving physical interaction between astrocytes and amyloid plaques. Comparison between control AD and GFAP<sup>−/−</sup>/Vim<sup>−/−</sup> AD mouse brains showed decreased expression of matrix metalloproteinase-9 (MMP-9) mRNA in the latter. Thus it is possible that MMP-9 released by reactive astrocytes around plaques (14, 270) contributes to plaque degradation.

We do not know how reactive astrocytes affect the extent of neurite dystrophy in AD. Reactive astrocytes recycle neurotransmitters and control extracellular concentrations of many other molecules (170), and these neuroprotective functions are even more critical under tissue and cellular stress than
Some functions of astrocytes in the healthy and diseased CNS

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FIGURE 8. Reactive astrocytes show hypertrophy of their cellular processes but stay within their tiled domains. A: expression of GFAP, which forms bundles of intermediate filaments (green) in astrocytes in unchallenged mouse hippocampus (left) and in partially deafferented hippocampus (molecular layer of the dentate gyrus) 4 days after entorhinal cortex lesion that triggers astrocyte activation and reactive gliosis in the hippocampus. The processes of reactive astrocytes show distinct hypertrophy. [From Wilhelmsson et al. (260).] B: schematic drawing of astrocyte response to injury. The main cellular processes of reactive astrocytes get thicker (and thus visible over a greater distance; compare the circles); however, reactive astrocytes stay within their tiled domains. [From Wilhelmsson et al. (260).] C: examples of the functions of astrocytes in healthy CNS and at the early and late stages of neurological diseases.
In an unchallenged CNS (49, 173). Therefore, decreased presence of cellular processes of astrocytes that surround amyloid plaques, reduced glutamate transport (132), and imperfect physical barrier in AD mice with attenuated reactive astrogliosis compared with AD mice with fully reactive astrocytes (267), can all offer possible explanation for more prominent signs of neurodegeneration in a situation when astrocyte activation and reactive astrogliosis are attenuated.

In BD, known also as infantile neuronal ceroid lipofuscinosis (98, 99, 250), GFAP upregulation is a highly prominent first pathological sign (114, 144). When GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice were crossed with a BD mouse model, mice deficient in palmitoyl protein thioesterase 1 (PPT1) (85), the disease onset appeared earlier, neurodegeneration and immune cell infiltration were more prominent, and these mice died earlier than BD mice with normal response of astrocytes (143). These experiments show that attenuation of reactive astrogliosis by genetic ablation of the astrocyte IF system facilitates disease progression both in AD and BD, and suggests that reactive astrocytes are positive players in neurodegenerative diseases.

**VI. CROSSTALK BETWEEN REACTIVE ASTROCYTES AND ACTIVATED MICROGLIA: IMPLICATIONS FOR NEURODEGENERATION**

The activation of astrocytes and activation/recruitment of microglia and monocytes seem to show a bidirectional relationship, at least in the context of injury and neurodegeneration. For example, the infiltration with CD11b-positive microglia/monocytes after retinal detachment was blocked in GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice, suggesting that activated retinal glial cells are critical for the recruitment of microglia/mono-

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**FIGURE 9.** Alzheimer’s disease (AD) mice crossed with GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice with attenuated reactive astrogliosis develop more pronounced plaque deposits and have increased microglial infiltration around plaques. A: histological sections of the cerebral cortex and hippocampus of 4, 8, and 12-mo-old AD mice and AD GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice with amloid plaques visualized with antibodies against beta-amyloid. B: astrocytes (green fluorescent protein; green) in 12-mo-old mice were visualized by injection of an adenoviral vector and examined after 1 mo. Amyloid plaques (blue) were labeled with X-34. In AD mice, processes of reactive astrocytes are hypertrophic and astrocytes are in a direct contact with plaques. In contrast, in AD GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice, no hypertrophy of astrocyte processes and essentially no physical interaction between astrocytes and plaques is seen. Scale bar, 50 μm. C: the abundance of microglia (green), visualized by antibodies against Iba-1 around plaques (blue) of 8-mo-old mice, was higher and their infiltration of the plaques was more prominent in the brains of AD GFAP<sup>−/−</sup>Vim<sup>−/−</sup> mice compared with AD mice. The graphs show means ± SE (n = 3 mice/group). *P < 0.05, **P < 0.001. Scale bar = 20 μm. [Figure mount modified from Kraft et al. (125).]
cytes to the injured areas (156). The observation of reduced levels of monocyte chemoattractant protein-1 (MCP-1) in the \( \text{GFAP}^{-/-} \text{Vim}^{-/-} \) mice points to chemokines such as MCP-1 as mediators of such a recruitment (156). In contrast, the infiltration by CD11b-positive microglia/monocytes and lesion size was increased in mice in which astrocyte migration and the demarcation of the spinal cord traumatic lesion by astrocytes was inhibited by STAT3 ablation in reactive astrocytes. Notably, these mice showed also a more pronounced functional impairment (95, 163, 256). Similarly, selective ablation of the small RhoGTPase Cdc42 in astrocytes impaired their recruitment to the stab wound lesion and was accompanied by higher density of microglia in the lesion (192). Attenuation of reactive astrogliosis by constitutive GFAP and vimentin ablation was associated with increased numbers of microglial cells in plaque vicinity in a mouse model of AD (125) (FIGURE 9C). Furthermore, in the cortex of these mice, expression of microglia/monocyte markers CD11b and Iba-1 was increased (125), and the number of activated microglia/monocytes was larger in another mouse model of chronic neurodegeneration, namely, BD, when astrocyte activation was attenuated (143). As the neurodegenerative process was aggravated in both conditions, the microglial response in the brains of these mice may be the consequence of an unsuccessful compensation for the impaired ability of astrocytes to deal with the underlying cause (179). It is equally possible that the recruitment and activation of microglia is normally suppressed by activated astrocytes (125). Although the exact mechanism by which activated astrocytes affect the recruitment of blood-borne monocytes and migration, proliferation, and functional state of microglia are currently unknown, it is conceivable that the bidirectional astrocyte-microglia/monocyte cross-talk is at least partly dependent on the underlying cause of the neurodegenerative process. In support of this notion, tumor necrosis factor (TNF)-\( \alpha \) and interferon (IFN)-\( \gamma \), which are examples of candidate inflammatory cytokines and chemokines that could play a role in mediating such a cross-talk between astrocytes and microglia/monocytes, were strongly increased in BD but not AD brains with attenuated reactive astrogliosis (125, 143). Furthermore, the phenotype of reactive astrocytes depends to some degree on the type of insult as shown by the comparison of gene expression profiles of reactive astrocytes isolated from ischemic stroke and lipopolysaccharide (LPS)-induced neuroinflammation (271).

**VII. DOES GENDER AFFECT THE ASTROCYTE RESPONSE TO BRAIN INJURY?**

The morphology and function of astrocytes, at least in some regions of the developing and adult brain, differ between males and females (reviewed in Refs. 129, 208). Depending on whether they are derived from males or females, astrocytes show different sensitivity to hypoxia in vitro (195), and they also seem to respond differentially to an inflammatory challenge. Although the basal expression of interleukin (IL)-6, TNF-\( \alpha \), IL-1\( \beta \), and the LPS receptor (Toll-like receptor 4) in astrocytes from male and females are similar, the mRNA levels of IL-6, TNF-\( \alpha \), and IL-1\( \beta \) were manyfold higher in LPS-treated astrocytes derived from males or androgenized females than in astrocytes derived from control or vehicle-treated females (202). The mRNA levels of the chemokine IFN-inducible protein 10, on the other hand, were higher in LPS-treated astrocytes derived from control or vehicle-treated females than in those obtained from males or androgenized females (202). In addition, the effects of estrogen and progesterone on the function and viability of mitochondria in astrocytes are gender dependent (12). There is evidence to support the notion that perinatal exposure to testosterone predetermines the responses of astrocytes (202). Sexual dimorphism of astrocytes might at least partly explain the known gender differences in some neurodegenerative processes, such as higher susceptibility of women to multiple sclerosis and the tendency of men for a more progressive course of the disease (251). The prevalence and incidence of Parkinson’s disease, on the other hand, are higher in men compared with women, and sex-related differences have also been observed in the type of neuropsychiatric and cognitive changes as well as motor symptoms of Parkinson’s and Huntington’s disease (140, 219). Whether a specific modulation of astrocyte activation or function would show sexually dimorphic effects on the progression of chronic neurodegeneration or outcome after brain ischemia remains to be determined.

**VIII. NEGATIVE SIDES OF REACTIVE ASTROGLIOSIS**

Numerous undesired consequences of reactive astrogliosis have been demonstrated, in particular when astrocyte activation and reactive astrogliosis do not get resolved during the postacute stage or at the early chronic stage after the injury event (196).

A number of experimental models demonstrated the inhibitory effects of reactive astrogliosis, including glial scarring, on regeneration both in the brain and spinal cord with a host of molecules being implicated (4, 35, 54, 79). One such example is chondroitin sulfate proteoglycans expressed by oligodendrocyte precursor cells and astrocytes. Inhibition of chondroitin sulfate proteoglycans, the production of which is increased after CNS injury, is associated with improved axonal regeneration after trauma (27, 28, 130, 212, 254, 269). Ephrin-A5, expressed by astrocytes and upregulated after injury, is another such example as it limits functional recovery by blocking axonal sprouting (167). Attenuation of reactive astrogliosis achieved by genetic ablation of GFAP and vimentin also leads to positive outcome, although it is associated with more extensive tissue damage in the initial acute phase of CNS injury (132). \( \text{GFAP}^{-/-} \text{Vim}^{-/-} \) mice ex-
hhibit better regeneration of neuronal synapses after partial deafferentation of the hippocampus in the entorhinal cortex lesion model (262); better axonal regeneration after the optic nerve crush in the early postnatal period, which can be further enhanced by transgenic overexpression of Bcl-2 in neurons (42, 43); and improved regenerative response and functional recovery after spinal cord trauma (149a). We demonstrated increased basal and posttraumatic hippocampal neurogenesis in GFAP\(^{-/-}\)/Vim\(^{-/-}\) mice and proposed that astrocytes negatively regulate neurogenesis via the Notch pathway with the Notch signaling from astrocytes to NSC/NPSs depending on GFAP and vimentin and involving endocytosis (261). GFAP\(^{-/-}\)/Vim\(^{-/-}\) mice exposed to neonatal hypoxic-ischemic injury show increased number of newly born cortical neurons despite normal in-

<table>
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<th>Effects</th>
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<tr>
<td>Forms a barrier, confines the lesion, and prevents its spreading</td>
<td>71, 95, 163, 189, 249, 252</td>
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<tr>
<td>Reduces leukocyte infiltration, promotes blood-brain barrier repair</td>
<td>16, 36, 117</td>
</tr>
<tr>
<td>Limits neuronal loss in ischemic stroke and neurotrauma (e.g., by neuroprotection, counteracting hypoxic stress, detoxification of reactive oxygen species)</td>
<td>55, 58, 61, 71, 132, 154, 156, 216, 232, 235, 275</td>
</tr>
<tr>
<td>Reduces loss of neuronal synapses after deafferentation</td>
<td>262</td>
</tr>
<tr>
<td>Limits neurodegeneration, slows down progression of neurodegenerative diseases</td>
<td>125, 143, 242</td>
</tr>
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<tr>
<td>Limits regeneration of CNS axons</td>
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<tr>
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The negative, regeneration inhibiting side of prominent reactive astrogliosis and glial scarring has been appreciated for decades, and a host of molecules beyond IF proteins were implicated as potential targets (4, 29, 30, 35, 54, 72, 79, 221). Inhibition of chondroitin sulfate proteoglycans produced by oligodendrocyte progenitor cells and astrocytes (27, 28, 130, 212, 254, 269), downregulation of neurocan expression by astrocytes (213), or regional elimination of reactive astrocytes by \(\alpha\)-amino-butyrate administration (43) were all shown to improve axonal regeneration after injury. Similarly, genetic deficiency or pharmacological inhibition of EphA4 lead to attenuation of reactive astrogliosis and to improved axonal regeneration and functional recovery in a mouse model of spinal cord injury (79, 80). What we seem to understand and appreciate better now is the other side of astrocyte activation and reactive astrogliosis leading to beneficial effects and resulting in an improved handling of the acute cellular stress, in-

IX. WHAT NATURE Addressed Well and the Windows of Therapeutic Opportunity

Glial cells play a critical role in the normal brain development in *Drosophila* through axonal growth guidance and removal of apoptotic cells (97, 224). Whereas some astroglia-like cells are present in the expanded forebrain parenchyma in the cartilaginous fish such as rays and sharks, most bony fish lack parenchymal astrocytes in the gray and white matter of the brain and spinal cord (10, 103, 108). Instead, the so-called ependymoglia (radial glia) located at the ventricle of zebrafish have processes that span the entire brain and spinal cord (84, 193) and conceivably combine the function of mammalian ependymal cells and astroglia. In contrast to mammals, astrogliosis and glial scar formation around the injury are absent in the zebrafish brain, which is also permissivre for long-term neuronal survival and shows rapid wound closure (18). It is intriguing that during evolution, different successful strategies for wound healing and repair appeared (sometimes in parallel) and even disappeared to emerge again in more advanced organisms.

The negative, regeneration inhibiting side of prominent reactive astrogliosis and glial scarring has been appreciated for decades, and a host of molecules beyond IF proteins were implicated as potential targets (4, 29, 30, 35, 54, 72, 79, 221). Inhibition of chondroitin sulfate proteoglycans produced by oligodendrocyte progenitor cells and astrocytes (27, 28, 130, 212, 254, 269), downregulation of neurocan expression by astrocytes (213), or regional elimination of reactive astrocytes by \(\alpha\)-amino-butyrate administration (43) were all shown to improve axonal regeneration after injury. Similarly, genetic deficiency or pharmacological inhibition of EphA4 lead to attenuation of reactive astrogliosis and to improved axonal regeneration and functional recovery in a mouse model of spinal cord injury (79, 80). What we seem to understand and appreciate better now is the other side of astrocyte activation and reactive astrogliosis leading to beneficial effects and resulting in an improved handling of the acute cellular stress, in-

### Table 1. Examples of suggested positive and negative effects of astrocyte reactivity and reactive astrogliosis

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</tr>
<tr>
<td>Negatively affects the integration of neural grafts and neural stem/progenitor cells</td>
<td>116, 259</td>
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creased neuroprotection and, in some situations, in achieving relative isolation/sequestering of the toxic and potentially expanding lesion area from the rest of the CNS (71, 154, 249). The ability of astrocytes to mount antioxidative defenses allows them to quite impressively adapt to toxic environments, at least in some situations (235). In neurotrauma or ischemic stress, activated astrocytes limit oxidative damage to neurons by a glutathione-dependent mechanism (61). It is interesting that subthreshold ischemic insults activate stress-handling pathways that help to reduce damage during future ischemic episodes (59). This phenomenon is known as ischemic preconditioning or ischemic tolerance and offers an opportunity for designing novel treatment strategies (59).

As the relative number of astrocytes, expressed as a ratio to neuronal number, increased dramatically during phylogensis along with the increase in brain complexity (158), it is tempting to speculate that those acute responses of astrocytes allowing survival and some measure of repair, albeit at the price of a restricted regenerative capacity later, were also likely to be selected for during evolution. When not optimal in a particular disease context or within a particular time window, a given response might be amenable to therapeutic manipulation with the aim of enhancing regeneration and expanding the plasticity window, and consequently supporting, for example, a better recovery of the lost function, or more efficient “housekeeping” repair activities in the healthy aging CNS. These exciting opportunities for both therapy and prevention remain to be explored (159, 171).

It must not be forgotten that, somewhat contrary to a popular belief, we need to carefully ask in which situations increased regeneration is likely to be clinically beneficial and what types of regenerative responses would be desirable. This remains to be established in experimental models, in which the optimal regenerative responses that we seek through modulation of reactive astrocytes need to be defined. Too much regeneration, or maladaptive regenerative responses, might both pose a problem. To define the right therapeutic window for the acute or subacute stage of a particular pathology, while mitigating the negative effects of a given intervention, will pose another challenge. This issue will be even more important in polymorbidities, where multiple secondary prevention schemes are of key importance.

Viewed from an evolutionary perspective, it is likely that some of the stress-handling mechanisms connected to astrocyte activation in a pathological context, that evolved and bring benefit after a particular challenge (e.g., limited neurotrauma, ischemic or osmotic stress), are also triggered in pathological situations for which they were not selected, since all these situations share a number of pathophysiological features. Engaging such conserved “off-shelf” stress-handling mechanisms might benefit some situations, while in other situations, it might be detrimental. The latter can be the case, e.g., with a profound astrogial and inflammatory response with cytokine and chemokine production in retinal detachment-induced photoreceptor degeneration (211), where attenuated reactive response of astrocytes and Müller cells in GFAP−/−Vim−/− mice (116, 156) reduces Erk and c-fos activation, prevents the upregulation of MCP-1 and recruitment of monocytes to the injury site, and results in reduced photoreceptor degeneration (156). Therapeutic activation of specific stress-handling mechanisms that are not normally activated in a given disease context, or suppression of unwanted stress responses, can bring therapeutic benefits and be important both for treatment and prevention. A good conceptual example of such an approach might be protection against neurodegeneration in a mouse model of ALS achieved by the activation of the Nrf-2 stress response system (242).

**X. ASTROCYTES AS A TARGET: FROM EXPERIMENTAL DISEASE MODELS TO CLINICAL TRIALS**

Can we expect astrocytes to become the target for new therapeutic strategies that will become available in the coming decades for the treatment of diseases affecting CNS? The answer must be positive (17, 115, 150, 209). Extensive work in this area is currently ongoing in many laboratories, and a shift from assessing individually selected potential molecular targets to high throughput screening strategies, when molecular candidates can be evaluated in the context of the whole transcriptome or proteome, ensures that the tempo will increase and the work will become both more systematic and more comparable between individual laboratories, with the selection of potential target molecules less and less subjective (21, 39, 60, 93, 138).

We might even be surprised to learn that some of the already clinically used drugs act predominantly on astrocytes, or on the astrocyte network. It might be just a product of our largely neuron-centered view, which still prevails in neurology, that some of the clinically widely used drugs might predominantly target astrocytes with us being unaware of it. For example, astrocytes within epileptic foci both in human epilepsy and in animal models of epilepsy lose their characteristic domain organization and manifest a manfold increase in the process overlap between neighboring astrocytes (162). This loss of astrocyte domains might be a feature specific for epilepsy since neighboring reactive astrocytes in neurotrauma show only minimal interdigitation between their processes (260). Interestingly, the administration of valproic acid, a common antiepileptic drug, in an animal model of epilepsy both suppresses the seizures and largely reduces the process overlap between neighboring astrocytes (162). Thus it is possible that the loss of astrocyte domain organization is the primary event in at least some forms of epilepsy and the basis for recurrent excitation in the epileptic brain. It is also possible that reactive astrogliosis seen in the epileptic foci (182, 258) typi-
cally at very early stages of the disease (13, 41, 209, 234, 249) generates local synaptic perturbations (166) that might then further amplify the clinical symptoms. Such findings will further facilitate screening of drugs specifically targeting astrocytes.

In fact, the hunt for drugs targeting astrocytes is already ongoing. Here are two examples. Alexander disease is a rare and severe neurodegenerative disease with widespread reactive astrogliosis and the first known primary disorder of astrocytes (31). It is caused by a mutation in GFAP gene that acts in a gain-of-function fashion and leads on a cellular level to overexpression of a mutant GFAP and its toxic accumulation in Rosenthal fibers together with small stress proteins, such as HSP27 and αβ-crystallin (31, 150). The Messing laboratory successfully conducted a screen of FDA-approved compounds and identified clomipramine, a commonly used antidepressive drug, and few additional compounds as drug candidates for pharmacological reduction of the expression or accumulation of GFAP (44). Another screen of FDA-approved drugs, this time for their ability to stimulate the expression of glutamate transporter GLT1 (EAAT2) by astrocytes, helped to identify ceftriaxone, a representative of beta-lactam antibiotics, as a compound which was successfully applied to slow down progression of amyotrophic lateral sclerosis (ALS) in an animal model (199) and is currently being tested in a clinical trial (53). ALS, a neurodegenerative disease that predominantly affects motoneurons, nicely demonstrates the pitfalls of the neuron-centric view (249). For decades, neuron-autonomous pathogenesis of ALS remained unquestioned even though the mutations that cause some of the familiar ALS were known to be present in all cell types. More recent data suggest that at least in some forms of ALS, the neuronal damage is mediated by oligodendrocytes (111), microglia (25, 253), and astrocytes (57, 88, 155, 268), which thus emerge as a novel therapeutic target for this devastating disease.

Astrocytes are also considered a target for ischemic stroke (78, 198, 232, 275), a highly prevalent neurological condition with prominent astrocyte reactivity and reactive astrogliosis (176, 221, 223, 240). Apart from their neuroprotective effect on neurons and detoxification of various reactive oxygen species at the acute stage of ischemic stroke (96, 132, 216), reactive astrocytes profoundly affect the later postischemic stages, e.g., by secreting vascular endothelial growth factor (VEGF), which stimulates formation of new blood vessels as well as synaptogenesis (20, 274), or by secretion of synaptogenic thrombospondin (45). Thrombospondin 4 has recently been shown to control protective astrogensis in the adult subventricular zone in response to neurotrauma (22), and its expression was proposed to depend on astrocyte reactivity and reactive astrogliosis (7). VEGF secretion by reactive astrocytes can serve as a good example of a context-dependent response: the positive, stimulatory effect of VEGF on the formation of vessels and synapses in ischemic stroke (20, 263) contrasts with its induction of blood-brain barrier breakdown and lymphocyte infiltration in autoimmune CNS inflammation (8, 9). Treatment with glial cell line-derived neurotrophic factor (GDNF) enhances neuronal survival in the ischemic brain tissue (100, 119, 255). Although the expression of GDNF is low in an unchallenged brain, it is upregulated in reactive astrocytes in the peri-infarct region (117, 122, 257). Enhancement of GDNF expression by astrocyte targeting therapies thus appears as an attractive target in brain ischemia. Using a novel quantitative proteomics approach, Hauck et al. (91) showed that Müller cells in the retina produce a pool of neuroprotective factors such as osteoponin and connective tissue growth factor. Application of such screening strategies to astrocytes exposed to a variety of stimuli will enable better characterization of these cells and their responses under different conditions and will improve the understanding of the heterogeneity of astroglia.

Astrocytes also seem to play a role in the elimination of supernumerary neuronal synapses during development and in a pathological context. Recent work has shown that TGF-β secreted by immature astrocytes in the retina initiates synaptic elimination in the postnatal thalamus by upregulating the expression of the complement protein C1q in the retinal ganglion cells (24). C1q activates the classical complement pathway, which leads to the tagging of synapses with complement-activation derived C3b fragment, and the tagged synapses are subsequently eliminated by microglia (204, 228). Remarkably, in one of the most common neurodegenerative diseases, glaucoma, microglia upregulate C1q at an early stage of the disease, and C1q deficiency is protective against glaucoma in a mouse model (102). Although it is currently unknown which mechanisms regulate the expression of C1q in microglia in the context of chronic neurodegeneration or in the normal aging brain (227), the involvement of reactive astrocytes is not unlikely, not least in the light of increased expression of GFAP, a hallmark of astrocyte activation, that is found in brain tissue affected by neurodegenerative disorders as well as that obtained from normal aged individuals (19).

The complement system plays also a role in axotomy-induced elimination of synapses on spinal cord motoneurons, albeit in a C1q-independent manner (23). The role of astrocytes in this process and the implications of these findings for modulation of postischemic synaptic plasticity remain to be addressed. Recent work from the Barres laboratory provides evidence for an involvement of astrocytes in activity-dependent engulfment of both excitatory and inhibitory synapses in the developing as well as adult brain. In contrast to microglia, the direct synapse elimination by astrocytes does not require C1q (46). Interestingly, functional modulation of neuronal synapses by astrocytes may be a new therapeutic goal in stroke patients. Here is one example. A key negative regulator of neuronal activity is the inhibitory
neurotransmitter γ-aminobutyric acid (GABA) that exerts its fast effects through synaptic receptors. Through extrasynaptic receptors, GABA that spills over at active synapses negatively regulates also the background activity of neurons (this phenomenon is known as tonic inhibition). This GABA is normally removed from the extracellular space by neuronal and astrocyte transporters. The expression of GABA transporters on astrocytes is reduced in the ischemic penumbra, and tonic inhibition by excessive amounts of GABA limits both neural plasticity and functional recovery (48). In mouse models, pharmacological treatment that blocks GABA binding to its receptors, when provided at the right time after stroke, results in a faster recovery of function (48). However, when administered too early, such a treatment can increase the amount of brain tissue lost in the ischemic stroke. Thus the time of intervention is crucial. This is the case also with VEGF, the therapeutic use of which might be beneficial in the late poststroke period, but leads to increased capillary permeability and larger infarctions when administered in the early postischemic phase (239). As shown above, through not yet fully understood molecular mechanisms, astrocytes seem to control several aspects of synaptic plasticity. It is predominantly synaptic plasticity in the weeks and months after stroke which determines the degree of functional recovery and which can be enhanced through specific neurorehabilitation programs, noninvasive brain stimulation, and pharmacological modulation (40, 126, 159, 171). For example, ischemic stroke triggers ephrin-5A expression in reactive astrocytes, and this leads to the inhibition of axonal sprouting and motor recovery (167). Pharmacological blockade of ephrin-A5 combined with forced use of the affected limb was shown to promote new and widespread axonal projections within the entire cortical hemisphere at the side of experimentally induced ischemic stroke (167).

Another example of a drug that acts, at least partially, through modulating the functions of astrocytes is levodopa. Levodopa, L-DOPA (L-3,4-dihydroxyphenylalanine), is a dopamine precursor that is further converted to norepinephrine. When combined with physiotherapy, delayed treatment with levodopa improved functional motor recovery in ischemic stroke patients (205). Although its recovery-enhancing effects are largely ascribed to the increased levels of norepinephrine at the synapse (205, 229), there is also experimental evidence supporting the involvement of astrocytes in the positive actions of levodopa (201).

In addition to their involvement in the elimination of supernumerary synapses during development (204, 228), microglia also regulate synaptic activity in context-dependent manner. Microglia respond to concomitant exposure to hypoxia and inflammation (exemplified by LPS) by increased production of superoxide that in turn induces long-term synaptic depression in the neighboring neurons. The effect of LPS requires complement receptor 3, which in the brain is specifically expressed by microglia (272). In the healthy adult brain, microglia stimulate learning-dependent formation of neuronal synapses via brain-derived neurotrophic factor and neuronal tropomyosin-related kinase receptor B phosphorylation (169). Based on the above-mentioned cross-talk between microglia and astrocytes, as well as the fact that both cell types are in close contact with or even part of a synapse, it would not be surprising if astrocytes were also involved in this process, e.g., by coregulation of brain-derived neurotrophic factor release from microglia. Whether this is the case and how is this communication altered in the context of ischemia or other types of brain injury are important questions for future study. Despite the fact that they do not generate action potentials, NG2 cells receive glutamatergic synaptic contacts, and synaptic signal integration by NG2 cells has been proposed to be one of the crucial steps regulating activity-dependent myelination as well as remyelination of severed axons (145, 231). Thus astrocytes, microglia, as well as oligodendrocyte precursors are well equipped for directly sensing activity in individual neurons and neuronal circuits and play important roles in the regulation of adaptive neural plasticity.

Given the immense molecular complexity of the response of reactive astrocytes (and this most likely applies also to other cell types), it is unlikely that future therapeutic strategies will focus on a single molecular target or a single molecular pathway. More likely, the strategies used will be relatively gentle (136) and will be used to adjust multiple equilibria, often within defined therapeutic windows and in synergy between diverse regeneration-promoting modalities, both pharmacological (35) and nonpharmacological, such as targeted rehabilitation, multisensory stimulation physical exercise, and pharmacological modulations (159).

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DISCLOSURES

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REFERENCES


51. Cudiaowicz ME. Clinical Trial Ceftriaxone in Subjects With ALS. *ClinicalTrials.gov* # NCT00349622: 2012.


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