

Preface

The study of the neuroscience and neurobiology of aging continues to be an intensely active area of research. However, most of the research has been conducted on neurons. *Neuroglia in the Aging Brain* reviews the current knowledge of the supporting structure of the nervous system, the neuroglia, in aging.

Neuroglia in the Aging Brain discusses the role of glial cells in normal aging and in pathological aging, i.e., neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, because a great deal of knowledge can be gleaned from the study of these more severe forms of aging. The book is divided into six sections: cellular and molecular changes of aged and reactive astrocytes; neuron–glia intercommunication; neurotrophins, growth factors, and neurohormones in aging and regeneration; metabolic changes; astrocytes and the blood–brain barrier in aging; and astrocytes in neurodegenerative diseases.

It is my hope that all of those scientists engaged in this interesting area of research will find this book to be useful. Many thanks to all of the authors for their fine contributions.

Jean S. de Vellis

Diversity in Reactive Astrocytes

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1. INTRODUCTION

Normal astrocytes in the adult undergo hypertrophy and proliferation and transform into reactive astrocytes following many types of central nervous system (CNS) injury (1–5). This process is termed astrogliosis and may result in the formation of a glial scar.

Morphological studies on astrogliosis by neuroanatomists and pathologists at the beginning of this century focused on the most florid examples encountered in the immediate vicinity of destructive lesions of the CNS, such as lacerations, infarcts, abscesses, and multiple sclerosis plaques (6). Accordingly, the criteria which became standard for defining the reactive state were astrocytic hypertrophy and mild to moderate proliferation, an elaboration of long, thick cytoplasmic processes and an increase in glial filaments composed of glial fibrillary acidic protein (GFAP) (7).

Duchesne et al. (8,9) were the first to draw attention to biochemical diversity in reactive astrocytes in four different models of CNS parenchymal injury. After a hiatus of a decade, there is now growing awareness that reactive astrocytes in different categories of CNS lesions are biochemically heterogeneous, largely as a result of detailed studies of the astroglial reaction in different experimental models of CNS injury (1), combined with the use of a panel of methods to detect GFAP and a number of other conventional and novel “astrocyte-specific” and companion biochemical markers (2,10). From these investigations it is evident that astrocytes do not respond in a stereotypic fashion to all forms of CNS insult, but rather are capable of a variety of types of response, as defined by qualitative, quantitative, temporal and spatial differences in the patterns of molecules which they elaborate in different types of parenchymal injury. This biochemical diversity in reactive astrocytes appears to largely depend on the nature of the CNS injury and the microenvironment of the injury site. Thus, in cellular and molecular terms it is no longer appropriate to hold to a single general definition of astrogliosis.

In this chapter, we consider the likely combinations of “damage signals” and factors in different CNS pathologies that act to induce subtypes of reactive astrocytes. Finally, attention is directed to the future use for mechanistic studies of new and evolving cell culture models of reactive astrogliosis.

2. SUBTYPES OF REACTIVE ASTROCYTES

2.1. Proximal and Distal Reactive Astrocytes in Trauma Models

Proximal reactive astrocytes develop in the immediate vicinity of a destructive lesion of the adult CNS in which there is cellular necrosis and disruption of the blood brain barrier. Distal reactive astrocytes develop at a distance from such a lesion, in a much less disturbed microenvironment (9). The “local astrocytic response” (11) and the “remote astrocytic response” (12) are alternative terms used to describe the foregoing two topographical and intensity-graded patterns of astroglial reactivity.

Proximal reactive astrocytes are the subtype which has been studied in the greatest detail (2–4). Stab wounds of the rat cerebrum provide a convenient and reproducible model system for their induction (1,13). The center of the lesion is necrotic and hemorrhagic. Astrocytes become reactive and undergo hypertrophy within 3 h postinjury in a 1 to 2 mm surrounding zone. The astroglial response spreads through the ipsilateral cortex and into subcortical white matter, reaching a peak between 3 and 7 d. The contralateral cerebral hemisphere also may be remotely affected. The margins of the wound are infiltrated by inflammatory mononuclear cells (i.e., lymphocytes, blood-derived macrophages, and intrinsic brain microglial cells). Between 6 and 12 h postinjury, there is a rapid increase in GFAP mRNA in proximal reactive astrocytes which is followed 2 d later by an increase in their GFAP content (11,14,15). Both GFAP mRNA and GFAP decline to near normal levels by 21 d. Between 3 and 6 d postinjury, approx 13% of the proximal reactive astrocytes are the progeny of cells which have divided (16). Thus, the majority of proximal reactive astrocytes arise from normal astrocytes or their precursors in the region. Over a period of several weeks proximal reactive astrocytes participate in the formation of a persistent astroglial scar (anisomorphic gliosis) whereas distal reactive astrocytes revert to normal (isomorphic gliosis) (6,17). Allowing for differences in the design and execution of experiments, comparable findings to those obtained with cerebral stab wounds have been observed following a cryogenic injury, focal X-irradiation, laser-irradiation, and focal ischemia (2).

Spinal cord transection in adult rats produces an astroglial reaction which is maximal at 14 d. Concurrently, there is both rostral and caudal spread of the reaction (18). In neonatal rats, however, astrogliosis remains limited to the site of spinal cord injury. Barrett et al., (19) suggested that the remote astrocytic reaction is due to degeneration of the long ascending and descending fiber tracts which are myelinated in the adult but not in the neonate. Gliosis is more severe in the lacerated spinal cord than in the lacerated cerebrum, probably because of the greater amount of initial tissue necrosis at the former site. An immunohistochemical study of lacerated adult rat spinal cord by Predy et al., (20), employing a monoclonal antibody (Mab J1–31) raised against multiple sclerosis plaque tissue by means of hybridoma technology (21), revealed that J1–31 antigen is a more intense marker for proximal reactive astrocytes than GFAP, but is a less intense marker for distal reactive astrocytes than GFAP in the rat spinal cord (22) (Figs. 1 and 2).

Many antigens are either unregulated or are expressed *de novo* in proximal reactive astrocytes. GFAP, an intermediate filament protein found only in astrocytes, has become the classical marker for reactive astrocytes in general, and for proximal reactive astrocytes in particular (7). Other molecules which are nonspecifically increased in

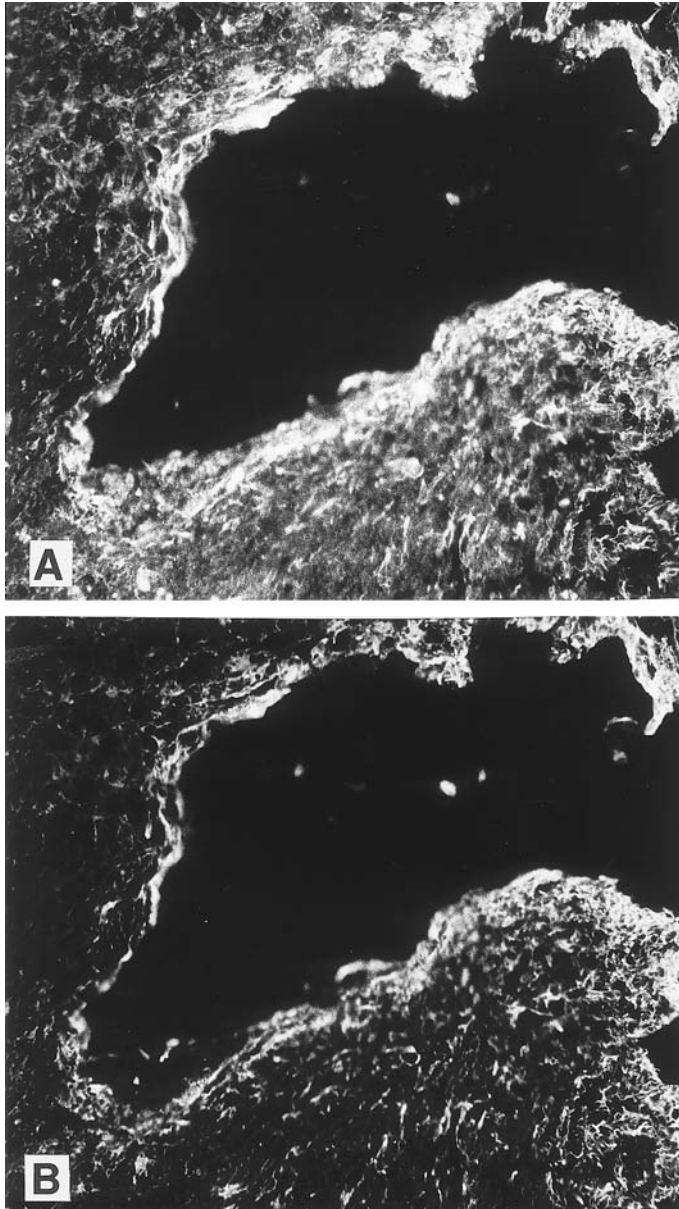


Fig. 1. Sectioned lacerated rat spinal cord showing more intense staining of proximal reactive astrocytes with Mab J1-31 (**A**) than with anti-GFAP (**B**) in the vicinity of the wound. Details of protocols for spinal cord laceration and immunofluorescence staining are given in Predy et al., (20) $\times 106$. (Reproduced with permission from (2).)

proximal reactive astrocytes include S-100 β protein, vimentin; NSE, GS and GAP-43 protein (2,4,5,23).

In recent years, hybridoma technology has fostered the production of some new monoclonal antibodies which recognize specific markers for proximal reactive astrocytes. These include J1-31 antigen (21,22), 6.17 antigen and M22 antigen (5), and

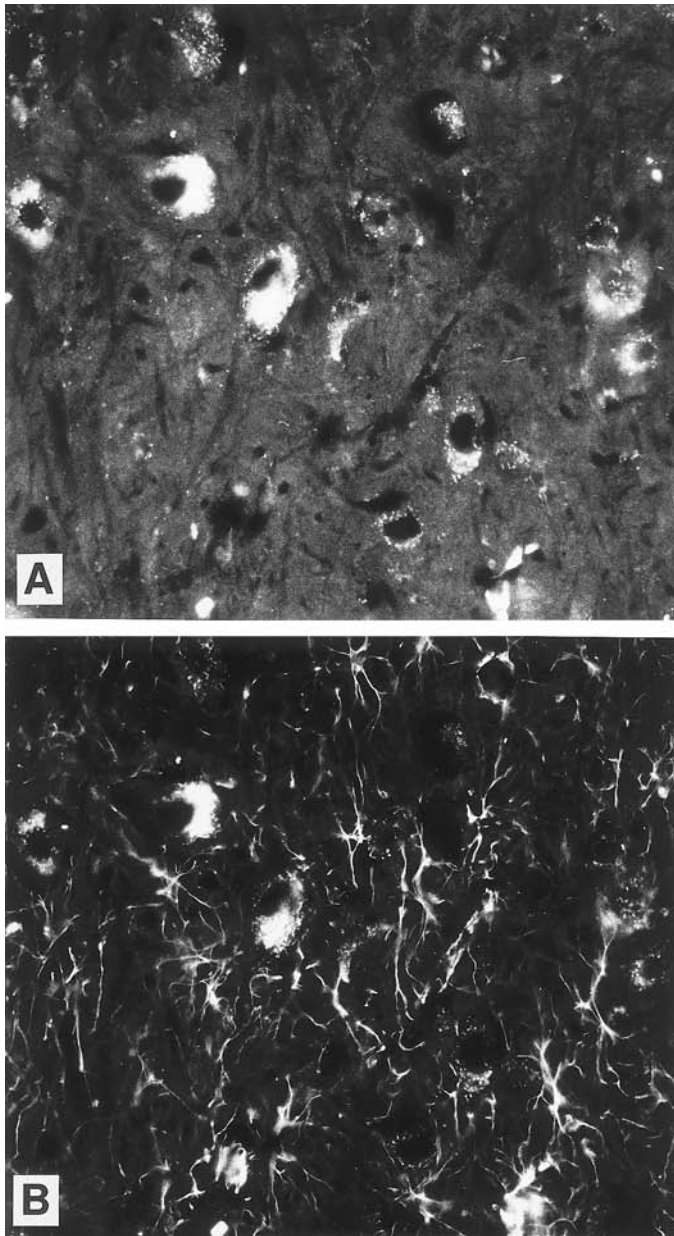


Fig. 2. Sectioned lacerated rat spinal cord in a region at a distance from the wound, showing a lack of astroglial staining with Mab J1-31 (**A**) in contrast to intense staining with anti-GFAP (**B**), in a double-labeled preparation. Autofluorescence is apparent in the soma of neurons in both photographs (arrows). Details of protocols for spinal cord laceration and immunofluorescence staining are given in Predy et al. (20). (Reproduced with permission from (2).)

13All and 01E4 epitopes (24,25). The foregoing require further characterization, however. Table 1 indicates that although proximal and distal reactive astrocytes share some biochemical characteristics in common, they also differ significantly with regard to other characteristics. To date, distal reactive astrocytes have been less thoroughly stud-

Table 1
Comparison of the biochemical characteristics of normal astrocytes, proximal reactive astrocytes and distal reactive astrocytes².

Biochemical characteristics Category ¹	Normal Astrocytes	Proximal Reactive Astrocytes (PRA's)	Distal Reactive Astrocytes (DRA's)
Intermediate filaments	GFAP ↑ GFAP content ↑ Vimentin-neg	GFAP immunoreactivity ↑↑↑ GFAP content ↑↑ Vimentin ↑ (10% of PRA's)	GFAP immunoreactivity ↑↑ GFAP content ↑ Vimentin ±
Gene expression	GFAP mRNA±	GFAP mRNA ↑↑	GFAP mRNA ↑↑
Enzymes	Oxidoreductive enzyme activities: succinate, glucose-6-phosphate, lactate and glutamate dehydrogenases are at normal levels	Oxidoreductive enzyme activities: succinate, glucose-6-phosphate and glutamate dehydrogenases ↑ Enzymes of glycolysis ↑↑↑ Enzymes of the hexose monophosphate shunt (LDH) ↑↑↑	Oxidoreductive enzyme activities: succinate, glucose-6-phosphate, lactate and glutamate dehydrogenases, remain at normal levels
Growth factors	βFGF-neg	βFGF ↑↑	bFGF-neg
Other biologically active proteins	S-100β protein ↑	S-100β protein ↑↑	S-100β protein ↑
Gangliosides	GD ₃ -neg	GD ₃ ↑	GD ₃ -neg
Other marker epitopes	J1-31-neg 6.17-neg M22-neg	J1-31 ↑ 6.17 ↑↑ M22 ↑↑	J1-31-neg 6.17 ↑↑ M22 ↑

¹ The selection of marker molecules shown above is based on the availability of published comparative biochemical data for normal astrocytes and sub-types of reactive astrocytes under consideration.

² For sources of data see reviews by Eddleston and Mucke (21); Malhotra and Shmitka (2); Ridet, et al., (3); Ridet and Privat (4).

ied than proximal reactive astrocytes. Hence the imbalance in available data concerning these two sub-types of reactive astrocytes (4,23).

2.2. Mechanisms of Activation of Proximal Reactive Astrocytes

The induction of proximal reactive astrocytes in the immediate vicinity of a traumatic lesion of the CNS is a highly complex process involving a multiplicity of “damage signals” and factors and severe alterations in local microenvironmental conditions. The details are far from clear, and remain to be fully elucidated. From pathological studies, it is obvious that mechanical trauma produces local destruction of neural and glial cells and their connections, disruption of blood vessels, and an escape of the cellular and fluid components of blood into the injury site. This complex environment contains serum proteins, blood platelets, eicosanoids, biologically active peptides, myelin breakdown products, fibrin split products and purine nucleosides and nucleotides, and so on, which can activate astrocytes and/or function as trophic factors for mononuclear inflammatory cell (2,4,26). Soon after injury, activated astrocytes and mononuclear inflammatory cells (i.e., lymphocytes, blood-derived macrophages and intrinsic brain microglial cells) and activated endothelial cells elaborate many cytokines (such as ciliary neurotrophic factor [CNTF], nerve growth factor [NGF], basic fibroblast growth factor [bFGF], and insulin growth factor-1 [IGF-1]), which act over short distances in an autocrine or paracrine fashion, to produce a broad range of synergistic or antagonistic effects in different cell types (5,27).

Although the intact normal brain is regarded as an immunoprivileged organ, it does contain resident astrocytes and microglia, which when activated produce a number of potent immune molecules, e.g., TNF- α , TGF β , IL-1, IL-6 and IFN- γ (5,27). TNF- α , IFN- γ and IL-6 have multiple effects in the CNS in controlling glial and neuronal activation, proliferation and survival, thus influencing both degenerative and repair processes. Some of the beneficial and detrimental actions induced by TNF- α and IFN- γ are largely mediated by nitric oxide synthase (NOS)-derived NO production (5). Indeed, NO is involved not only in cytotoxic reactions, but also in the survival and differentiation of neurons. In general, high concentrations are neuroprotective. TNF- α and IFN- γ also upregulate the expression of ICAM-VCAM surface adhesion molecules by astrocytes and brain endothelial cells, thereby favoring astroglial migration, adhesion and anchoring, and neuronal differentiation, during the repair of CNS damage and commencing astrogliosis (27). From the foregoing, it is clear that bidirectional communication exists between resident parenchymal cells (neurons and astrocytes), and resident and infiltrating cells of the immune system in the CNS. It logically follows that *in vitro* studies require *in vivo* confirmation, because indirect synergistic effects predominate *in vivo*, rather than single direct effects (1). The challenge for the future will be to investigate in detail how different factors interact and transform normal astrocytes into proximal reactive astrocytes *in vivo*. The transcriptional mechanisms that are involved in the activation of astrocytes are still a matter of conjecture. There is abundant evidence from *in vitro* studies to indicate that glial cells receive numerous signals which employ cAMP as the second messenger (28,29). Also, signaling through the protein kinase C pathway may play a major role in mediating the developmental proliferation of astrocytes and their activation in astrogliosis in the adult brain (30).

2.3. Mechanisms of Activation of Distal Reactive Astrocytes

The origin of distal reactive astrocytes in both the ipsilateral and contralateral cerebral hemispheres following a cerebral laceration has been the subject of speculation and experimentation (1,13,31,32). Several possible mechanisms have been suggested to explain the phenomenon:

1. The release and widespread diffusion from the site of injury of cytokines and growth factors induces astrocyte reactivity at a distance (31).
2. Neuronal degeneration at the site of CNS injury causes anterograde and retrograde fibre degeneration and includes remote astroglial response in the corresponding projection territories (12).
3. Proximal reactive astrocytes migrate away from the site of injury to remote regions (33).
4. Injury results in decreased gap junctional activity which may be propagated through the astroglial syncytial network (34).

To specifically address the origin of contralateral (remote) astrogliosis, Moundjian et al., (31) performed a callosotomy in rats which also had received a cerebral stab wound, in order to prevent the migration of astrocytes via the corpus callosum. Cerebral callosotomy also served to transect all associative fibers, and thereby enhance neuronal degeneration in both hemispheres. The results of this spatiotemporal study strongly implicated diffusible substances in the induction of the remote astroglial response, rather than the other possibilities listed above. The lack of major local cell destruction, integrity of the blood-brain barrier, absence of blood components, and the pattern of the microglial/macrophage response in the remote astroglial reaction may be additional determinants of its special characteristics.

2.4. Reactive Astrocytes in Axotomy Models

Traumatic injury to a peripheral motor or sensory nerve or the sectioning of a CNS fiber tract (axotomy), induces in the corresponding projection territory, a series of structural and metabolic changes in neuronal cell bodies, dendrites and presynaptic terminals (axon reaction), which are rapidly followed by an astrocytic response (isomorphic gliosis) in the immediate vicinity (35). Details are provided in published descriptions of the axotomy reaction after transection of various motor nerves in the rat (36–41).

After facial nerve axotomy, protoplasmic astrocytes in the facial nucleus undergo hypertrophy and transform into fibrous astrocytes. Increased GFAP synthesis is detectable at 24 h and peaks by 3 d (40). In these motor nerve axotomy models, reactive astrocytes do not proliferate (39), or express vimentin (40), or immunostain with Mab J1–31, (22, see Table 2 and Fig. 3). In the latter context, they resemble the distal reactive astrocytes observed in lacerated rat spinal cord (22).

Until recently, the widely held view has been that “damage signals” emanating from axotomized neurons are responsible for the reactive changes that are observed in neighboring astrocytes. Candidates for the intercellular signaling process have included neurotransmitters (for which astroglial cells possess receptors), or ions particularly K^+ , which is released by active neurons and is taken up by astrocytes (42). Excitatory amino acids (particularly glutamate) may also play a role, because excitotoxicity has been implicated in a number of acute and chronic neurological diseases (43). Also,

Table 2
Comparison of proximal reactive astrocytes induced by cerebral laceration vs reactive astrocytes induced by axotomy

Subtype of reactive astrocyte	Proximal reactive astrocyte	Axotomy-induced reactive astrocyte	References
Model system	Cerebral laceration	Axotomy	
Pattern of glial scar	Anisomorphic gliosis	Isomorphic gliosis	(17,35)
Blood brain barrier	Disrupted	Intact	(71)
Hyaluronate binding protein	Negative	Positive	(72)
Axonal growth	Permissive	Nonpermissive	(72)
Microglial proliferation	Immediate	Delayed	(17)
Astroglial proliferation	Yes	No	(38)
Vimentin	In about 10% of reactive astrocytes	Negative	(16,40)
J1–31 antigen	Postive	Negative	(22)

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reactive changes in astroglia may be related to the synaptic reorganization that follows neuronal injury (42).

However, evidence presented by Svensson et al., (44), strongly suggests that the activation of astrocytes following hypoglossal nerve transection is mediated by factors (such as IL-1) released by reactive microglial cells. Their experimental approach was to block the usual axotomy-induced proliferation of indigenous microglial cells in rat brain by the intraventricular infusion of cytosine-arabinoiside (ARA-c) (44). Subsequently, astrocytes in the projection territory of the axotomized hypoglossal nerve failed to show expected increases in GFAP and GFAP mRNA, leading to the conclusion that axotomy-induced astrogliosis is mediated indirectly via the microglial reaction.

2.5. Reactive Astrocytes in Cortical Epileptogenic Foci

Temporal lobe seizures are the most common type of active epilepsy in adults, comprising about 40% of all cases (45). A broad range of pathological lesions have been identified in the temporal lobe in association with intractable complex partial seizures, including mild to severe gliosis (Ammon's horn sclerosis), malformation (mild cortical dysplasia, microdysgenesis, tuberous sclerosis, or angiomatous malformations), neoplasms (gliomas or mixed tumors of the CNS), and inflammatory scars from infection or infarcts (46–48).

Ammon's horn sclerosis (AHS) is the most common of the foregoing lesions among patients treated surgically for intractable temporal lobe epilepsy. Pathologically, AHS is characterized by atrophy of the hippocampal formation, loss of neurons and gliosis in CA1 and CA4 and in the dentate nucleus (47). Several theories have been proposed to explain the pathogenesis of AHS, including abnormal circuitry in the hippocampal formation due to a developmental error, hypoxic-ischemic damage (due to birth injury or to post-natal trauma), or that epileptic seizures can cause cortical gliosis (47,49). The currently favored view is that seizures provoke the death of neurons as a result of

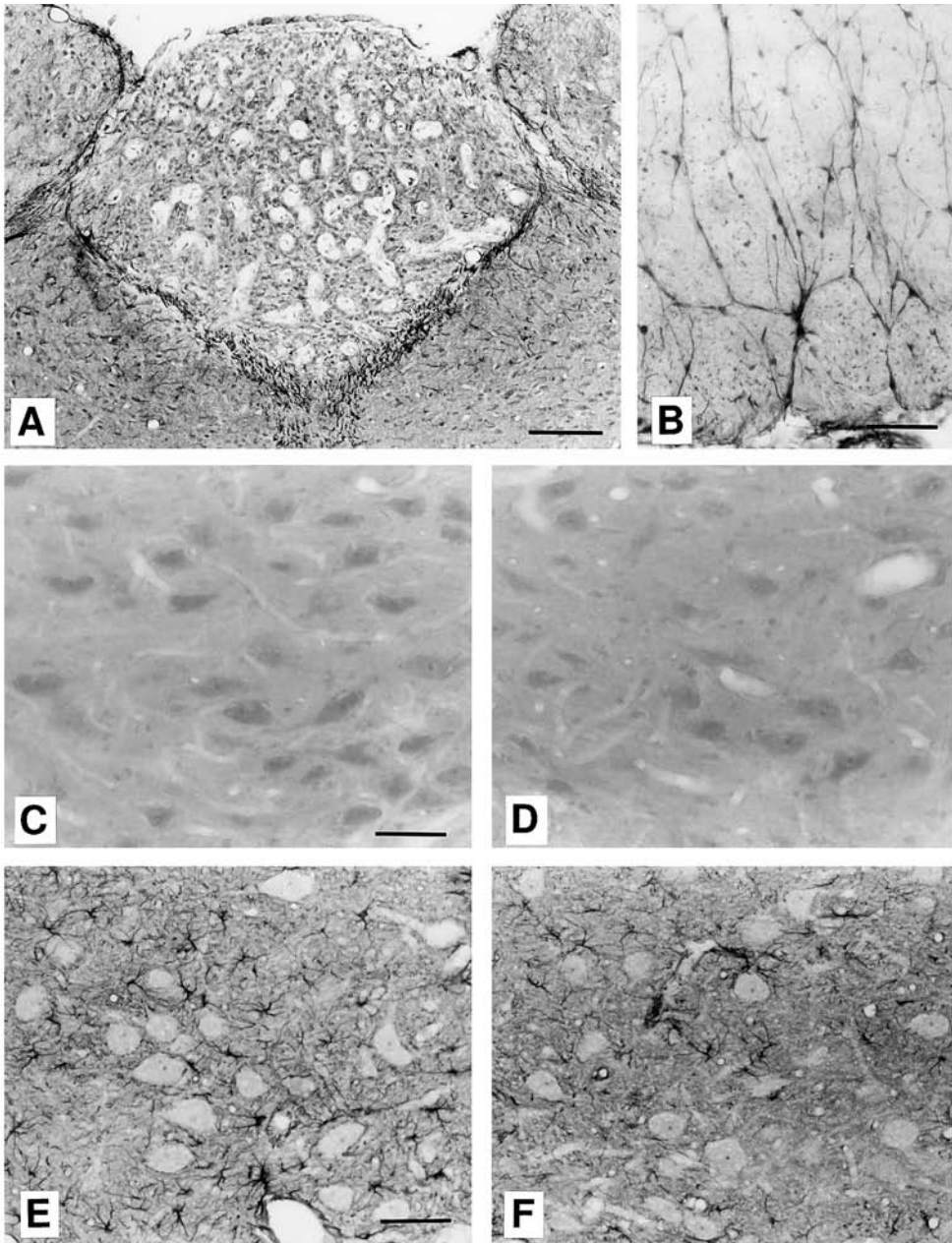


Fig. 3. (A-D) J1-31 immunolabeling of rat brain stem, 1 wk after transection of the right hypoglossal nerve. Immunoreactive processes surround the area postrema and extend toward the central canal (A). Subpial astrocytes and their processes are likewise positive for J1-31 (B). However, no specific immunoreactivity is observed in the right hypoglossal nucleus (C,D). Bar 100 μ m (A), 50 μ m (B-D). (E-F) GFAP immunolabeling in the right hypoglossal nucleus 1 week after transection of the right hypoglossal nerve. Note the increased intensity of immunoreactivity on the side of the operation (E,F). Bar 50 μ m. (Reproduced with permission from (22).)

excitotoxicity. Over-stimulation of neurons occurs when there is excessive activation of glutamate receptors (45). According to this hypothesis, gliosis is a secondary response to the death of neurons. Also, neuronal cell death may be followed by the formation of new synaptic connections with abnormal hyperexcitability.

Steward et al. (42) have shown experimentally that when mild electrical stimulation of the rat hippocampus was employed to elicit neuronal activity and acute-onset seizures, GFAP and mRNA levels increased rapidly and dramatically at the sites of stimulation, as well as in areas that were synaptically activated by the seizures. These early-stage increases in GFAP mRNA did not appear to be related to the presence of necrotic neurons. Candidates for signaling mechanisms included an upregulation of several “immediate early genes” (such as c-fos and c-jun), and an increased release by active neurons of glutamate and K^+ (50).

Experimental models of chronic focal epilepsy have involved the intracortical or topical cortical administration of alumina (51), iron (52) or cobalt (8,53). In relation to the topic of biochemical diversity among reactive astrocytes, Brotchi et al. (54) applied the term “activated astrocytes” to a subset of reactive astrocytes in human epileptogenic cortex and in cobalt-treated rat cortex, which display elevated levels of glutamate dehydrogenase (GS), glucose-6-phosphate dehydrogenase, and lactate dehydrogenase. Glucose 6-phosphate dehydrogenase was the first enzyme to rise and the last to decline to normal (9).

Hamberger et al. (48) have monitored the levels of neuron specific enolase (NSE), two glial cell specific proteins (S-100 β protein and GFAP), and CNAM neural cell adhesion molecule) in surgically excised specimens of human epileptogenic cortex. Gliosis varied from mild to severe. However, no correlation was found between the GFAP content and the duration of focal epilepsy. There was only a 30–40% increase above normal of S-100 β protein. The NSE values were close to normal, which correlated with the observation that the concentration of neurons in the cortex of most patients remains relatively unaffected, even after years of seizures.

2.6. Regional Differences in Resident Normal Astrocytes also May Influence the Development of Sub-Types of Reactive Astrocytes

Controversy exists as to whether the region-specific properties of normal astroglial cells are the result of intrinsic coded information within the glial cell, or whether neurons in different brain regions induce uncommitted astroglial cells to express specific proteins in support of a particular type of neuron-glial interaction (55,56).

In different regions of the intact rat brain, not all normal astrocytes are GFAP-positive (57); also there are large quantitative differences in glutamate dehydrogenase (GS) activity, and S100 β protein in astrocytes in different brain regions (58,59).

Reviews by Hatten et al. (56) and Norton et al. (1) give credence to the suggestion that in certain instances phenotypic diversity in reactive astrocytes may in part be related to the diversity of normal astrocytes in the region. We previously collected together some reports of experiments by others which could fit this pattern of reactivity (2). Two examples suffice here. Alonso and Privat (60) studied the fine structural organization and immunostaining characteristics of reactive astrocytes in glial scars produced in adult rats by two surgical stab wounds in two close-by locations of the same

brain area. With a lesion of the ventral hypothalamus, axonal regeneration occurred and was associated with GFAP-, vimentin-, laminin-, and PSA-NCAM-positive reactive astrocytes. On the other hand, in the dorsal hypothalamus, the same type of lesion resulted in a nonpermissive scar, which exhibited only slight PSA-NCAM and laminin staining. Electron microscopy disclosed that the nonpermissive scar in the dorsal hypothalamus contained significantly more gap junctions than the permissive gliotic scar in the ventral hypothalamus (4).

Another example of regional diversity in reactive astrocytes is described by Fernaud-Espinosa, et al. (17) in a study on the differential activation of microglia and astrocytes in aniso- and isomorphic glial scars. Microglial and macroglial responses were compared after two different types of brain damage in two distinct regions in rat brain (i.e., the cerebral cortex and the hippocampus). Each site was subjected to trauma resulting in anisomorphic gliosis. The microglial response seemed to be only linked to the type of lesion inflicted on the CNS, whereas the astrocytic response also appeared to depend on the region of the brain that was damaged. Reactive astrocytes in the hippocampus expressed β -amyloid precursor protein (β -APP) immunoreactivity after both aniso- and isomorphic gliosis, but this marker protein was not evident in reactive astrocytes in the cerebral cortex, under the same experimental conditions.

2.7. Models of Reactive Astroglial In Vitro

The complexity of the cellular and molecular events occurring in animal models of brain injury has prompted efforts to develop convenient, reliable model systems using astroglial cell cultures, for controlled experiments on astroglial gliosis *in vitro*. Wu and Schwartz (25) have reviewed the current range of cell culture models which are available to biochemically characterize reactive astrocytes, i.e., primary cultures of neonatal astrocytes, co-cultures of astrocytes with either neurons or microglia, and organ cultures. Each of the foregoing addresses a different set of questions. The *in vitro* models if employed as a panel, may help to identify the various patterns of “damage signals” and factors responsible for the biochemical diversity observed in reactive astrocytes *in vivo* in different categories of CNS lesions.

Yu et al. (61,62) have described a “mechanical injury model of reactive gliosis *in vitro*” evoked by scratching with a plastic pipet tip, confluent primary cultures of astrocytes prepared from the cerebral cortex of 7- to 10-d-old rat pups. Injured astrocytes along the scratch track started to swell within 6 h of injury, and between 1 and 3 d displayed stellation, an increased content of GFAP, and larger and more organized assemblies of cytoplasmic filaments.

Eng et al. (63) quantitated the changes in gene expression which occurred after a scratch wound of cultured rat astrocytes; *c-fos* mRNA increased 30-fold and heat shock protein (Hsp) mRNA increased four-fold within 60 min. Both returned to low levels by 12 h postinjury. The early genes which are activated may influence cell division, protect against further injury, and induce later genes (such as metallothionein) through the induction of transcription factors.

Two key nuclear signal transduction mechanisms in astroglia appear to be the basis for a flexible genomic switch which allows extracellular signals to change genetic programs and protein expression patterns:

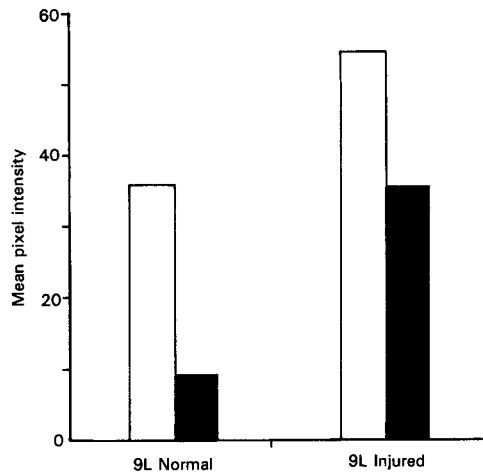


Fig. 4. Astrogliosis in vitro in 9L rat glioma cells induced by mechanical injury (“scratch wound” model). Histogram comparing the relative intensities of the fluorescent signals from double-labeled images for GFAP (solid columns) and J1-31 antigen (open columns) of normal and mechanically injured 9L cells. (Reproduced with permission from (65).)

1. Specific transmitter receptor stimulation by extracellular signals can produce a highly integrated nuclear third messenger (NTM) response with coordinated assembly at the level of NTM transcription and translation;
2. Multiple signals can dynamically change the expression of specific target genes via negative or positive cooperative interactions among various classes of NTMs at the genomic level (64).

In 1995, Malhotra et al. (65) showed that 9L rat glioma cells grown on coverslips, like primary cultures of astrocytes, undergo astrogliosis when subjected to mechanical trauma from a “scratch wound.” Cells along the scratch track display mild hypertrophy and increases in GFAP and J1-31 antigen immunoreactivities, from trace to moderate levels in the case of GFAP, and from moderate to high levels in the case of the J1-31 antigen (Fig. 4). Reaction to mechanical injury by the 9L cells occurred without interactions with microglia, neurons or oligodendroglia. In the same report, we reviewed the literature on the origin and characteristics of the 9L rat glioma cell line and listed the advantages of using cultures of 9L cells, rather than primary cultures of astrocytes, for studies on astrogliosis in vitro. Space limitations preclude the presentation of full details here.

9L rat glioma cells (66), and primary cultures of astrocytes from neonatal rat brain (67) also undergo astrogliosis after exposure to low levels of cadmium chloride (a prototype neurotoxicant [68]). Moreover, when cultures of 9L cells were subjected to mechanical injury and then exposed to cadmium chloride, there was a marked increase in the expression of the astrocytic marker, J1-31 antigen. This was due to a summation of stimulatory effects from these two injurious agents. A moderate coordinated response was detected for the expression of the classical marker, GFAP, and cell hypertrophy was only slightly increased over that produced by either injurious agent alone (Fig. 5). The foregoing findings suggest that more than one transcription mechanism

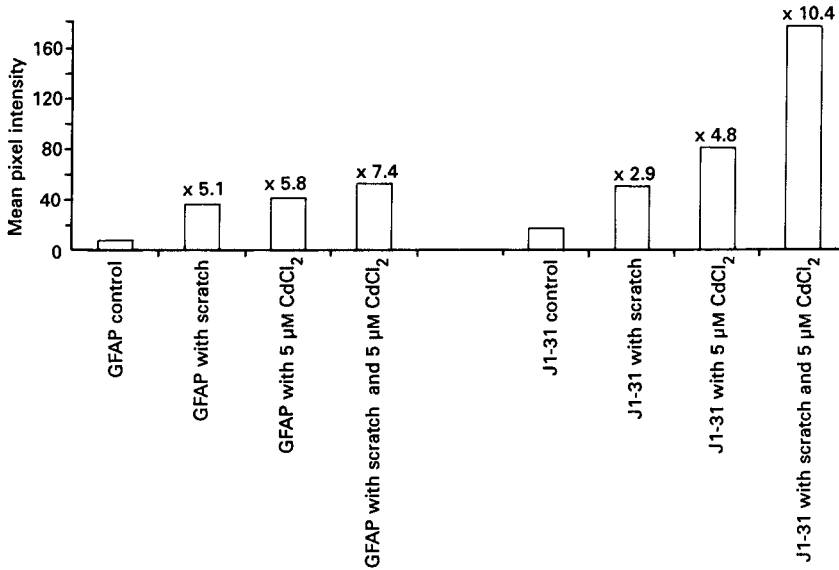


Fig. 5. Coordinated mechanical and chemical injuries upregulate astrogliosis in vitro in 9L rat glioma cells. Histogram comparing the relative intensities of the fluorescence signals from immunolabeled images from 9L cells for GFAP and J1–31 antigen respectively, in normal cells, mechanically injured cells, CdCl₂-chemically injured cells, and in cells with coordinated injuries from both agents. Increases shown above columns. Controls, without scratch or CdCl₂. (Reproduced with permission from (66).)

is involved in the activation of astroglia, and that mechanical and CdCl₂-induced injuries probably respectively affect different receptors and second- and third-messenger pathways. Thus, in a relatively simple in vitro model system, biochemical diversity in reactive astrocytes could be demonstrated by altering the conditions of astroglial cell injury.

3. CONCLUDING REMARKS

Recent reports on a wide range of experimental animal models of CNS injury, combined with the application of immunostaining methods for a considerably expanded panel of conventional and novel astrocyte-specific markers, indicate that reactive astrocytes are capable of graded levels and different types or responses, as defined by the molecular they elaborate in different categories of local pathology (1,2,4). Biochemical heterogeneity and functional diversity are also prominent among other activated types of specialized cells such as microglia (69) and fibroblasts (70). This adaptive plasticity of reactive astrocytes largely appears to be a modulated response to the different micro-environmental conditions, particularly the combinations of immune molecules, cytokines, eicosinoids, serum factors, peptides, nucleotides, and adhesion molecules that exist in different categories of CNS lesions. Regional biochemical differences in the normal astrocytes from which reactive astrocytes arise, as well as local nonglial influences, may be contributory factors in certain brain regions.

Future research, no doubt, will expand on the lines of investigation already established. New and evolving cell culture models of reactive astrogliosis should facilitate

mechanistic studies. The challenge then will be to determine both the common and the diverse signaling mechanism which underlie the conversion of normal astrocytes into their respective reactive subtypes. The foregoing should lead to a better understanding of the special roles played by the biochemical subtypes of reactive astrocytes which are present in different categories of CNS pathology.

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REFERENCES

1. Norton, W.T., Aquino, D.A., Hozumi, I., Chiu, F-C., and Brosnan, C.F. (1992) Quantitative aspects of reactive gliosis: a review. *Neurochem. Res.* **17**, 877–885.
2. Malhotra, S.K. and Shnitka, T.K. (1994) Adaptive plasticity and diversity among reactive astrocytes in central nervous system lesions. *Biomedical Letters* **49**, 273–302.
3. Montgomery, D.L. (1994) Astrocytes: form, functions, and roles in disease. *Vet. Path.* **31**, 145–167.
4. Ridet, J.L., Malhotra, S.K., Privat, A., and Gage, F.H. (1997) Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci.* **20**, 570–577.
5. Ridet, J.-L. and Privat, A. (1999) Reactive astrocytes, their roles in CNS injury and repair mechanisms, in *Advances in Structural Biology* (Malhotra, S.K. ed.) Vol. 6 (in press). Jai Press Inc. Stamford, CT, USA.
6. Greenfield, J.G. (1958) General pathology of nerve cell and neuroglia, in *Neuropathology* (Greenfield, J.G., Blackwood, W., Meyer, A., McMenemey, W.H. and Norman, R.M., eds.) Edward Arnold Ltd. London, pp. 1–66.
7. Eng, L.F. and Ghirnikar, R.S. (1994) GFAP and astrogliosis. *Brain Pathol.* **4**, 229–237.
8. Duchense, P.Y., Ghuens, J., Brotchi, J., and Gerebtzoff, M.A. (1979) Normal and reactive astrocytes: a comparative study by immunohistochemistry and by a classical histological technique. *Cell Molec. Biol.* **24**, 237–239.
9. Duchesne, P.Y., Gerebtzoff, M.A., and Brotchi, J. (1981) Four types of reactive astrocytes. *Bibliothca Anat.* **19**, 313–316.
10. Malhotra, S.K., Shnitka, T.K., and Elbrink, J. (1990) Reactive astrocytes – a review. *Cytobios* **61**, 133–160.
11. Condorelli, D.F., Dell’Albani, P., Kaczmarek, L., Messina, L., Spampinato, G., Avola, R., Messina, A., and Giuffrida Stella, A.M. (1990) Glial fibrillary acidic protein messenger RNA and glutamine synthetase activity after nervous system injury. *J. Neurosci. Res.* **26**, 251–257.
12. Hajós, F., Kálmán, M., Zilles, K., Schleicher, A., and Sotonyi, P. (1990) Remote astrocytic response as demonstrated by glial fibrillary acidic protein immunohistochemistry in the visual cortex of dorsal lateral geniculate nucleus lesioned rats. *Glia* **3**, 301–310.
13. Mathewson, A.J. and Berry, M. (1985) Observations on the astrocyte response to a cerebral stab wound in adult rats. *Brain Res.* **327**, 61–69.
14. Hozumi, I., Chiu, F-C., and Norton, W.T. (1990a) Biochemical and immunocytochemical changes in glial fibrillary acidic protein after stab wounds. *Brain Res.* **524**, 64–71.
15. Hozumi, I., Aquino, D.A., and Norton, W.T. (1990b) GFAP mRNA levels following stab wounds in rat brain. *Brain Res.* **534**, 291–294.

16. Miyake, T., Hattori, T., Masaru, F., Kitamura, T., and Fujita, S. (1988) Quantitative studies on proliferative changes of reactive astrocytes in mouse cerebral cortex. *Brain Res.* **451**, 133–138.
17. Fernaud-Espinosa, I., Nieto-Sampedro, M., and Bovolenta, P. (1993) Differential activation of microglia and astrocytes in aniso- and isomorphic gliotic tissue. *Glia* **8**, 277–291.
18. Barrett, C.P., Guth, L., Donati, E.J., and Krikorian, J.G. (1981) Astroglial reaction in the gray matter of lumbar segments after midthoracic transection in the adult rat spinal cord. *Expl. Neurol.* **73**, 365–375.
19. Barrett, C.P., Donati, E.J., and Guth, L. (1984) Differences between adult and neonatal rats in their astroglial response to spinal injury. *Expl. Neurol.* **84**, 374–385.
20. Predy, R., Malhotra, S.K., and Das, G.D. (1988) Enhanced expression of a protein antigen (J1–31 antigen, 30 kilodaltons) by reactive astrocytes in lacerated spinal cord. *J. Neurosci. Res.* **19**, 397–404.
21. Malhotra, S.K., Wong, F., Cumming, P., Ross, S.D., Shnitka, T.K., Manickavel, V., Warren, K.G., and Jeffrey, V. (1984) A monoclonal antibody for cytoskeletal antigenic determinant(s) distinguishable from glial fibrillary acidic protein in astrocytes. *Microbios Letters* **26**, 151–157.
22. Malhotra, S.K., Svensson, M., Aldskogius, H., Bhatnagar, R., Das, G.D., and Shnitka, T.K. (1993) Diversity among reactive astrocytes: proximal reactive astrocytes in lacerated spinal cord preferentially react with monoclonal antibody J1–31. *Brain Res. Bull.* **30**, 395–404.
23. Eddleston, M. and Mucke, L. (1993) Molecular profile of reactive astrocytes – implications for their role in neurologic disease. *Neuroscience* **54**, 15–36.
24. Welter, E., Bolesta, M.J., and Landis, D.M.D. (1993) Monoclonal antibodies which bind to reactive astrocytes. *Soc. Neurosci. Abstr.* **19**, 60.
25. Wu, V.W. and Schwartz, J.P. (1998) Cell culture models for reactive gliosis: New perspectives. *J. Neurosci. Res.* **51**, 675–681.
26. McMillian, M.K., Thai, L., Hong, J-S., O’Callaghan, J.P., and Pennypacker, K.R. (1994) Brain injury in a dish: a model for reactive gliosis. *Trends Neurosci.* **17**, 138–142.
27. Muñoz-Fernández, M.A. and Fresno, M. (1998) The role of tumor necrosis factor, interleukin 6, interferon- γ and inducible nitric oxide synthase in the development and pathology of the nervous system. *Prog. Neurobiol.* **56**, 307–340.
28. Hansson, E. (1989) Co-existence between receptors, carriers, and second messengers on astrocytes grown in primary cultures. *Neurochem. Res.* **14**, 811–819.
29. Melcangi, R.C., Celotti, F., Castano, P., and Martini, L. (1992) Intracellular signalling systems controlling the 5 α -reductase in glial cell cultures. *Brain Res.* **585**, 411–415.
30. Yong, V.W. (1992) Proliferation of human and mouse astrocytes *in vitro*: signalling through the protein kinase C pathway. *J. Neurol. Sci.* **111**, 92–103.
31. Moudjian, R.A., Antel, J.P., and Yong, V.W. (1991) Origin of contralateral reactive gliosis in surgically injured rat cerebral cortex. *Brain Res.* **547**, 223–228.
32. Takamiya, Y., Kohsaka, S., Toya, S., Otani, M., and Tsukada, Y. (1988) Immunohistochemical studies on the proliferation of reactive astrocytes and the expression of cytoskeletal proteins following brain injury in rats. *Rev. Brain Res.* **38**, 201–210.
33. Janeczko, K. (1989) Spatiotemporal patterns of the astroglial proliferation in rat brain injured at the postmitotic stage of postnatal development: a combined immunocytochemical and autoradiographic study. *Brain Res.* **485**, 236–243.
34. Anders, J.J., Niedermair, S., Ellis, E., and Salopek, M. (1990) Response of rat cerebral cortical astrocytes to free- or cobalt induced injury: an immunocytochemical and Gap-FRAP study. *Glia* **3**, 476–486.
35. Aldskogius, H. and Svensson, M.A. (1993) Neuronal and glial cell responses to axon injury, in *Advances in Structural Biology, Vol. 2*, (Malhotra, S.K., ed) pp. 191–223. Jai Press.
36. Reisert, I., Wildermann, G., Grab, D., and Pilgrim, C.H. (1984) The glial reaction in the course of axon regeneration: a stereological study of the rat hypoglossal nucleus. *J. Comp. Neurol.* **229**, 121–128.

37. Cova, J.L. and Aldskogius, H. (1985) A morphological study of glial cells in the hypoglossal nucleus of the cat during nerve regeneration. *J. Comp. Neuro.* **233**, 421–428.
38. Cova, J.L. and Aldskogius, H. (1986) Effect of axotomy on perineuronal glial cells in the hypoglossal and dorsal motor vagal nuclei of the cat. *Expl. Neuro.* **93**, 662–667.
39. Graeber, M.B. and Kreutzberg, G.W. (1988) Delayed astrocyte reaction following facial nerve axotomy. *J. Neurocytol.* **17**, 209–220.
40. Tetzlaff, W., Graeber, M.B., Bisby, M.A., and Kreutzberg, G.W. (1988) Increased glial fibrillary acidic protein synthesis in astrocytes during retrograde reaction of the rat facial nucleus. *Glia*, **1**, 90–95.
41. Gilmore, S.A., Sims, T.J., and Leiting, J.E. (1990) Astrocytic reactions in spinal gray matter following sciatic axotomy. *Glia* **3**, 342–349.
42. Steward, O., Torre, E.R., Tomasulo, R., and Lothman, E. (1991) Neuronal activity up-regulates astroglial gene expression. *Proc. Natn. Acad. Sci. USA* **88**, 6819–6823.
43. Whetsell, W.O. Jr. and Shapira, N.A. (1993) Biology of disease. Neuroexcitation, excitotoxicity and human neurological disease. *Lab. Invest.* **68**, 372–387.
44. Svensson, M. and Aldskogius, H. (1993) Evidence for activation of astrocytes via reactive microglial cells following hypoglossal nerve transection. *J. Neurosci. Res.* **35**, 373–381.
45. McNamara, J.O. (1992) The neurobiological basis of epilepsy. *Trends Neurosci.* **15**, 357–359.
46. Paul, L.W. and Scheibel, A.B. (1986) Structural substrates of epilepsy. *Adv. Neurol* **44**, 775–786.
47. Armstrong, D.D. (1993) The neuropathology of temporal lobe epilepsy. *J. Neuropath. Exp. Neurol.* **52**, 433–443.
48. Hamberger, A., Bock, E., Nordborg, C., et al. (1993) Biochemical correlates to cortical dysplasia, gliosis, and astrocytoma infiltration in human epileptogenic cortex. *Neurochem. Res.* **18**, 511–518.
49. Mathieson, G. (1975) Pathological aspects of epilepsy with special reference to the surgical pathology of focal seizures. *Adv. Neurol.* **8**, 108–137.
50. Torre, E.R., Lothman, E., and Steward, O. (1993) Glial response to neuronal activity: GFAP-mRNA and protein levels are transiently increased in the hippocampus after seizures. *Brain Res.* **631**, 256–264.
51. Harris, A.B. (1975) Cortical neuroglia in experimental epilepsy. *Expl. Neurol.* **49**, 691–715.
52. Hammond, E.J., Ramsey, R.E., Villareal, H.J., and Wilder, G.J. (1980) Effects of intracortical injection of blood and blood components on the electrocortigram. *Epilepsia* **21**, 3–14.
53. Fisher, J., Holubar, J., and Malik, V. (1968) Neurohistochemical study of the development of experimental epileptogenic cortical cobalt-gelatine foci in rats and their correlation with the onset of epileptogenic electrical activity. *Acta Neuropathol.* **11**, 45–54.
54. Brotchi, J., Tanaka, T., and Leviel, V. (1978) Lack of activated astrocytes in the kindling phenomena. *Expl. Neurol.* **58**, 119–125.
55. Wilkin, G.P., Marriott, D.R., and Cholewinski, A.J. (1990) Astrocyte heterogeneity. *Trends Neurosci.* **13**, 43–46.
56. Hatten, M.E., Liem, R.K.H., Shelanski, M.L., and Mason, C.A. (1991) Astroglia in CNS injury. *Glia* **4**, 233–243.
57. Hajós, F. and Kálmán, M. (1989) Distribution of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the rat brain. II. Mesencephalon, rhombencephalon and spinal cord. *Expl. Brain Res.* **78**, 164–173.
58. Patel, A.J., Weir, M.D., Hunt, A., Tahourdin, C.S.M., and Thomas, M.G.T. (1985) Distribution of glutamine synthetase and glial fibrillary acidic protein and correlation of glutamine synthetase with glutamate decarboxylase in different regions of the rat central nervous system. *Brain Res.* **331**, 1–9.
59. Didier, M., Harandi, M., Aquera, M., et al. (1986) Differential immunocytochemical staining for glial fibrillary acidic (GFA) protein, S-100 protein and glutamine synthesis in the rat subcom-

- misural organ, nonspecialized ventricular ependyma and adjacent neuropil. *Cell Tissue Res.* **245**, 343–351.
60. Alonso, G. and Privat, A. (1993) Reactive astrocytes involved in the formation of lesional scars differ in the mediobasal hypothalamus and in other forebrain regions. *J. Neurosci. Res.* **34**, 523–538.
 61. Yu, A.C.H., Kwan, H.H., Lee, Y.L., and Eng, L.F. (1993a) Morphologic changes in mechanically damaged astrocytes. *Trans. Am. Soc. Neurochem.* **24**, 242.
 62. Yu, A.C.H., Lee, Y.L., and Eng, L.F. (1993b) Astroglialosis in culture. 1. The model and the effect of antisense oligonucleotides on glial fibrillary acidic protein synthesis. *J. Neurosci. Res.* **34**, 295–303.
 63. Eng, L.F., Lee, L.Y., Murphy, G.M., and Yu, A.C.H. (1995) A RT-PCR study of gene expression in a mechanical injury model. *Prog. Brain Res.* **105**, 219–229.
 64. Szekely, A.M., Grayson, D., and Costa, E. (1993) Nuclear signal transduction via immediate early genes in neurons and glia. *Biochem. Soc. Trans.* **21**, 61–65.
 65. Malhotra, S.K., Bhatnagar, R., Shnitka, T.K., Herrera, J.J., Koke, J.R., and Singh, M.V. (1995) Rat glioma cell line as a model for astroglialosis. *Cytobios.* **82**, 39–51.
 66. Malhotra, S.K., Luong, L.T., Bhatnagar, R., and Shnitka, T.K. (1997) Up-regulation of reactive astroglialosis in the rat glioma 9L cell line by combined mechanical and chemical injuries. *Cytobios.* **89**, 115–134.
 67. Rising, L., Vitarella, D., Kimelberg, H.K., and Aschner, M. (1995) Cadmium chloride (CdCl₂)-induced metallothionein (MT) expression in neonatal rat primary astrocyte cultures. *Brain Res.* **678**, 91–98.
 68. O'Callaghan, J.P. (1993) Quantitative features of reactive gliosis following toxicant-induced damage of the CNS. *Ann. N.Y. Acad. Sci.* **679**, 195–210.
 69. Flaris, N.A., Densmore, T.L., Molleston, M.C., and Hickey, W.F. (1993) Characterization of microglia and macrophages in the central nervous system of rats: definition of the differential expression of molecules using standard and novel monoclonal antibodies in normal CNS and in four models of parenchymal reaction. *Glia* **7**, 34–40.
 70. Sappino, A.P., Schurch, W., and Gabbiani, G. (1990) Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab. Invest.* **63**, 144–161.

