



Review

A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis

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Accepted 27 May 2004

Abstract

A strong glial reaction typically surrounds the affected upper and lower motor neurons and degenerating descending tracts of ALS patients. Reactive astrocytes in ALS contain protein inclusions, express inflammatory makers such as the inducible forms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), display nitrotyrosine immunoreactivity and downregulate the glutamate transporter EAAT2. In this review, we discuss the evidence sustaining an active role for astrocytes in the induction and propagation of motor neuron loss in ALS. Available evidence supports the view that glial activation could be initiated by proinflammatory mediators secreted by motor neuron in response to injury, axotomy or muscular pathology. In turn, reactive astrocytes produce nitric oxide and peroxynitrite, which cause mitochondrial damage in cultured neurons and trigger apoptosis in motor neurons. Astrocytes may also contribute to the excitotoxic damage of motor neurons by decreasing glutamate transport or actively releasing excitotoxic amino acid. In addition, reactive astrocytes secrete proapoptotic mediators, such as nerve growth factor (NGF) or Fas-ligand, a mechanism that may serve to eliminate vulnerable motor neurons. The comprehensive understanding of the interactions between motor neurons and glia in ALS may lead to a more accurate theory of the pathogenesis of the disease.

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Theme: Disorders of the nervous system

Topic: Neuromuscular diseases

Keywords: Amyotrophic lateral sclerosis; Astrocytes; Motor neurons; Nitric oxide; Peroxynitrite

Contents

1. Introduction	0
2. Astrocyte pathology in ALS	0
3. The origin of astrocytosis in ALS	0
3.1. Aging	0
3.2. Oxidative stress and peroxynitrite	0
3.3. Axotomy and neuronal damage	0
4. Neurotoxic potential of reactive astrocytes in ALS	0
4.1. Downregulation of astrocytic glutamate transporters	0
4.2. Cytokine production by astrocytes	0
4.3. Production of nitric oxide and peroxynitrite	0
4.4. Production of apoptotic factors	0

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44	5. Astrocytes and motor neuron death	0
45	6. Conclusions	0
46	7. Uncited references	0
47	Acknowledgements	0
48	References	0
49		

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51 **1. Introduction**

52 Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease originally described by Charcot in 1869, characterized by the selective degeneration of motor neurons from the cortex, brainstem and spinal cord that leads to progressive paralysis and muscle atrophy. Most hypotheses for this selective cell loss have primarily addressed early changes in motor neurons involving oxidative damage, defective cytoskeletal function, protein misfolding and aggregation and excitotoxicity from disruption of extracellular glutamate homeostasis. The degeneration of motor neurons is so blatant that it tends to obscure subtle changes in other cell types that may contribute to ALS. In this review, we will consider the origin of reactive astrogliosis in ALS and how reactive changes in astrocytes may contribute to the progressive nature of ALS.

57 About 10% of ALS cases show familial inheritance, 20% of which are caused by mutations in the gene encoding copper, zinc superoxide dismutase (SOD-1) [106]. An important clue to the pathogenesis of ALS was provided by the development of several strains of different transgenic animal models of the disease carrying the expression of high levels of mutated SOD-1 genes. Toxicity of mutant SOD-1 involves a dominant gain-of-function rather than simply diminished superoxide-scavenging activity [12,24,54]. Spinal motor neurons express high levels of mutant SOD-1 which might explain the selective vulnerability of these neurons. However, current evidence indicates that ALS-linked SOD-1 mutations must be expressed in both neuronal and non-neuronal cells to induce the disease [52,96]. These findings suggest that interactions between motor neurons and surrounding cells in the spinal cord, nerve or skeletal muscle are required for mutated SOD-1 to initiate neurodegeneration in ALS. Accordingly, a recent study by Clement et al. [23] using chimeric mice composed of mixtures of normal cells and cells expressing ALS mutant SOD-1 showed that motor neuron degeneration is not necessarily associated with the expression of SOD-1 mutations in the motor neuron per se but rather with its expression in a critical number of neighboring neuronal and non-neuronal cells.

92 Astrocytes represent the largest cell population in the central nervous system (CNS). They closely interact with neurons to provide structural, metabolic and trophic support and actively participate in modulating neuronal excitability and neurotransmission by controlling the extracellular levels of ions and neurotransmitters [13,40]. In vitro, astrocytes

98 exert potent trophic influences on motor neurons [4,32,94] 98
 99 through a variety of proteins and low molecular weight 99
 100 molecules [113,122], which can be modulated by neuro- 100
 101 protective drugs [94]. In response to injury, astrocytes and 101
 102 microglia display characteristic phenotypic changes charac- 102
 103 terized as astrocytosis or gliosis. Astrocytes respond to CNS 103
 104 damage by proliferating and adopting a reactive phenotype 104
 105 characterized morphologically by hypertrophic nuclei and 105
 106 cell bodies and elaboration of distinct long and thick 106
 107 processes with increased content of glial fibrillary acidic 107
 108 protein (GFAP). In addition, reactive astrocytes express a 108
 109 wide variety of markers such as cytoskeleton proteins, cell 109
 110 surface and matrix molecules, proteases, protease inhibitors 110
 111 and several growth factors and cytokines [31,104]. By 111
 112 secreting diffusible factors, damaged neurons or activated 112
 113 astrocytes interact in a complex manner with immune cells 113
 114 and with microglia. Activated microglia, in turn, secrete 114
 115 proinflammatory peptides, nitric oxide (NO) and excitotox- 115
 116 ins that induce astrocytosis or aggravate neuronal damage, 116
 117 therefore, perpetuating and amplifying a local pathogenic 117
 118 process [51]. However, subtler states of microglia activation 118
 119 may lead to downregulation of the neuroinflammatory 119
 120 process. Since gliosis also occurs in a variety of conditions 120
 121 such as cerebral ischemia, Alzheimer's disease, Parkinson's 121
 122 disease, frontotemporal dementia and Huntington's disease 122
 123 [120], it has long been suggested to be a non-specific 123
 124 response of glial cells to injury and often it is not considered 124
 125 as a primary pathogenic element in ALS. On the other hand, 125
 126 recent evidence indicates the existence of other molecular 126
 127 mechanisms by which activated astrocytes may contribute to 127
 128 either the death of neurons or to their survival in response to 128
 129 damage. Extensive reviews have been recently published 129
 130 about the pathogenesis of ALS [12,25,109], the role of 130
 131 microglia and inflammatory cells in ALS [83], and the 131
 132 immune function of astrocytes [31]. In this review, we 132
 133 examine the current evidence that supports an active role 133
 134 of astrocytes contributing both to the induction and to the 134
 135 propagation of motor neuron loss. Understanding of the 135
 136 interactions between neurons and glia in ALS may help to 136
 137 explain the progressive nature of ALS. 137

52 **2. Astrocyte pathology in ALS** 138

139 A strong glial reaction typically surrounds both upper 139
 140 and lower motor neurons in ALS patients [59,71,87,90,120]. 140
 141 Some degree of gliosis is also found in the lateral descend- 141
 142 ing corticospinal tracts and in the entering points of the 142

143 tracts into the gray matter [118], thus forming a continuum
 144 along the damaged regions. Microglia also proliferate and
 145 become activated in these regions, and invading T cells can
 146 be found around the capillaries [83]. Reactive astrocytes in
 147 ALS show increased immunoreactivity for GFAP and the
 148 calcium binding protein S100 β [85] and express inflamma-
 149 tory makers such as COX-2 [81], iNOS and neuronal NOS
 150 [5,115]. In addition, astrocyte pathology is accompanied by
 151 cytoplasmic hyaline inclusions [68] and markers of oxida-
 152 tive and nitrate stress [1,91,115].

153 A similar pattern of astrocytic changes has been de-
 154 scribed in the different animal models of ALS, including
 155 mice and rats carrying different SOD-1 mutations. In the
 156 case of mice expressing the G85R SOD-1 mutation,
 157 astrocytes display major morphological and functional
 158 changes characterized by the appearance of SOD1-contain-
 159 ing aggregates and decreased expression of glial glutamate
 160 transporter GLT-1 [18]. Typically, these changes become
 161 evident before the onset of motor symptoms and dramati-
 162 cally increase with the progression of the disease. How-
 163 ever, the selective expression of G85R mutation in
 164 astroglia, under the control of a GFAP promoter, failed
 165 to induce motor neuron loss and disease [52], indicating
 166 that glial pathology induced by mutant SOD-1 is not
 167 sufficient to initiate neurodegeneration. Although astro-
 168 cytes in these mice are larger and more globular compared
 169 to the stellate morphology typically found in wild type
 170 controls, the degree of morphological abnormalities is not
 171 as extensive as those observed in the G85R-SOD1 trans-
 172 genic mice at the end stage of the disease. Thus, the
 173 selective expression of SOD-1 mutations in glial cells
 174 cannot explain per se the striking inflammatory response
 175 during motor neuron disease.

176 Compared to G85R-SOD1 transgenic mice, G93A-SOD1
 177 mice develop astrocytosis later in the disease, after initial
 178 motor neuron changes and microglia invasion are observed
 179 [2,55,78]. However, reactive astrocytes in G93A mice also
 180 upregulate the expression of the calcium-binding protein
 181 S100A6 [63] as well as iNOS, and become immunoreactive
 182 for nitrotyrosine [3,116]. In addition, hypertrophic spinal
 183 cord astrocytes occurring in the late stages of the disease
 184 express the activated form of p38 mitogen-activated protein
 185 kinase [130], which is stimulated by nitric oxide and
 186 inflammatory mediators.

187 Transgenic rats expressing the G93A mutation also
 188 display earlier and more evident astrocytic alterations
 189 compared to the G93A SOD-1 mice. Rats with the highest
 190 copy numbers of the transgene and maximum expression
 191 of the mutant protein develop a paralytic disorder charac-
 192 terized by rapid motor neuron death accompanied by
 193 proliferation of microglia and hypertrophic astrocytes con-
 194 taining characteristic Lewy body-like hyaline inclusions
 195 [86]. Howland et al. [62] found that gliosis coincided with
 196 early vacuolization of the neuropil and with a striking
 197 focal loss of the GLT-1 glutamate transporter in the ventral
 198 horn.

199 Taken together, these studies about the pathology of the
 200 disease indicate that astrocytic activation occurs in ALS
 201 both in humans and animal models of the disease with some
 202 shared characteristics: (i) the extent of astrocyte activation
 203 correlates with the degree of neuronal degeneration. Early
 204 astrocytic reactivity without apparent motor neuron death
 205 may be observed in some models where astrocytes are
 206 located in the vicinity of motor neuron soma or dendrites,
 207 suggesting a neuronal-mediated induction of astrocytosis. In
 208 agreement, astrocyte reactivity dramatically increases in
 209 regions exhibiting overt motor neuron degeneration. (ii)
 210 When present, protein aggregates such as Lewy body-like
 211 hyaline inclusions containing SOD-1 are not restricted to
 212 motor neurons but are also abundant in surrounding astro-
 213 cytes. This suggests that defects in protein folding or
 214 processing are not cell type specific, but rather occur
 215 nonspecifically in cells involved in the degenerative pro-
 216 cess. (iii) The degree of astrocyte reactivity correlates with
 217 the expression of inflammatory mediators, increased pro-
 218 duction of reactive oxygen and nitrogen species and defec-
 219 tive glutamate homeostasis that may contribute to motor
 220 neuron degeneration.

221 3. The origin of astrocytosis in ALS

222 Symptomatic ALS mice develop a typical “isomorphic”
 223 gliosis characterized by proliferative, hypertrophic and
 224 globular astrocytes [104]. This type of gliosis has also
 225 been described after CNS trauma, epilepsy or axotomy and
 226 is mediated by complex signaling between neurons, micro-
 227 glia and astrocytes [82]. However, astrocytes do not
 228 respond in a stereotypic manner to all forms of cell or
 229 tissue damage. The combination of different mediators
 230 such as cytokines, chemokines, growth factors and adhe-
 231 sion molecules produced in the microenvironment will
 232 determine the final reactive phenotype [104]. Thus, it is
 233 difficult to define the underlying causes of astrocytosis
 234 occurring in ALS. However, astrocytic changes occurring
 235 in aging or following primary motor neuron damage might
 236 illustrate some of the mechanisms causing astrocyte reac-
 237 tivity in ALS.

238 3.1. Aging

239 ALS and related neurodegenerative diseases are closely
 240 associated with the aging process, including those cases
 241 with familial inheritance, suggesting that age-dependent
 242 changes occur in neuronal cells for the etiological defect
 243 to trigger neuronal degeneration. Aging in mouse brain is
 244 associated with astrocytic reactive changes resembling those
 245 occurring in neurodegenerative diseases [75]. Astrocytosis
 246 in the aged brain is evidenced by an increase in the relative
 247 number of astrocytes [95,111] and the upregulation of GFAP
 248 and calcium-binding protein S100 β expression [138]. In
 249 aging humans, the spinal cord also undergoes distinct
 250

cellular changes such as the appearance of spheroids (large eosinophilic bodies) in motor neurons and increased number of process-bearing astrocytes over-expressing GFAP in the anterior horn [28]. Moreover, astrocytes as well as microglia cultured from aged brains display some of the activated phenotypes of aging glia observed in vivo [111]. It is unknown whether these changes are due to primary neuronal damage or simply an attribute of aging glia. In any case, altered astrocytic function in the aging CNS may negatively affect synaptic activity and neuronal survival, and contribute to establish a local pro-inflammatory environment.

3.2. Oxidative stress and peroxynitrite

Oxidative stress caused by increased production of nitric oxide and peroxynitrite by damaged motor neurons may constitute a potential mechanism for astrocyte activation in ALS. Nitric oxide can diffuse through cell membranes and combine with superoxide by a diffusion-limited reaction to form the stronger oxidant peroxynitrite [65], which probably accounts for much of the cytotoxicity of nitric oxide in vivo. Both nitric oxide and superoxide are produced by motor neurons in response to trophic factor deprivation [35], Fas pathway activation [101] or loading with zinc-deficient SOD-1 [36]. In addition, peroxynitrite is a strong oxidant [11,97] and reacts with tyrosine residues to form nitrotyrosine, a stable post-translational modification that has been utilized as a footprint for the in vivo formation of peroxynitrite and other reactive nitrogen species [10]. Recently, we showed that cultured spinal cord astrocytes briefly exposed to peroxynitrite-induced reactive morphological changes characterized by the appearance of process-bearing cells displaying intense GFAP, iNOS and nitrotyrosine immunoreactivity [19,20]. A similar phenotype was induced by bacterial lipopolysaccharide (LPS), a well-known inflammatory stimulus to glial cells. LPS stimulated iNOS expression and nitrotyrosine formation, suggesting a role for peroxynitrite in mediating the astrocytic reactive phenotype.

Furthermore, oxidative stress and, in particular, peroxynitrite formed in reactive astrocytes could induce long-term effects in specific proteins such as connexins, glutamate transporters and enzymes that may dramatically affect the interactions between astrocytes and neurons. For example, astrocytes are extensively coupled by gap junctions of the Cx43 connexin subtype, which allows intercellular diffusion of ions and signaling molecules that regulate neuronal excitability and neurotransmission. In cultured astrocytes, the induction of NOS by pro-inflammatory stimulus or the exposure of astrocyte monolayers to peroxynitrite, leads to a long-term inhibition of gap junctional communications [15,112], thus preventing the normal regulatory role of astrocytes. Peroxynitrite also inhibits astrocytic glutamate transporters [131], causing an increase of neurotoxic influences on motor neurons through potentiating neuronal excitability and excitatory neurotransmission.

3.3. Axotomy and neuronal damage

Axotomy in adult motor neurons induces a series of structural and metabolic changes that affect neurons, astrocytes and other glial cells [26,50,53,102]. After facial nerve axotomy, astrocytes in the facial nucleus become hypertrophic and develop long processes. This is followed by a sustained increase in GFAP synthesis, lasting for several days [129]. Some inflammatory molecules such as fibroblast growth factor [64], major histocompatibility complex-encoded antigens [89], interferon gamma [70,89] and NOS [70] have been found elevated in motor neurons after nerve lesions or spinal cord injury and proposed to signal between motor neurons and glia. Another putative candidate for the motor neuron–astrocyte crosstalk is TGF- β , which was found expressed by motor neurons in the wobbler mice [66]. Cytokines such as interferon- α (IFN- α) are among the most potent inducers of class II MHC expression in astrocytes [31]. Aside from cytokines, neurotransmitters and neuropeptides are known to induce class II MHC, suggesting a scenario in which damaged motor neurons releasing different mediators can induce astrocytes to assume a phenotype with inflammatory features.

Further support for motor neurons mediating astrocyte activation is provided by a recent study showing that astrocyte and microglia activation around motor neurons in SOD-1 G93A mice occurs after the onset of distal axon degeneration [47]. Astrogliosis appears related to motor neuron stress due to denervation and reinnervation changes occurring in muscle rather than to motor neuron loss, suggesting a role for early neuronal dysfunction as a source of inflammatory mediators influencing glial cells. Further evidence for a role for motor neurons modulating astrocytes reactivity was provided in G93A SOD-1 mice expressing increased levels of insulin growth factor-1 (IGF-1) in spinal motor neurons and skeletal muscle [67]. Such treatment delayed the onset of motor deficits, sustained life in mice, decreased astrogliosis and delayed motor neuron pathology and neuropil vacuolization. It needs to be established whether these effects are due to an increased release of IGF-1 to the surrounding neuropil targeting glial cells or to a downregulation of the pro-inflammatory mediators secreted by motor neurons that induce astrogliosis.

Taken together, the available evidence suggests that functional stress in motor neurons without cell death is sufficient to elicit a secondary glial response resembling astrocytic changes in presymptomatic ALS mouse and rat models. In the axotomy model, these responses are reversible and possibly provide the means to select viable neurons and facilitate nerve growth. However, acute responses are essentially reversible, while chronic neurodegenerative conditions are progressive. Whereas acute injury elicits glial reaction followed by activation of mechanisms to dampen the inflammatory process to background levels, in neurodegenerative processes, glial cells may be subjected to a long

362 lasting activation. Alternatively, astrocytes may develop an
 363 atypical phenotype that allows them to avoid the anti-
 364 inflammatory signals. The final result would be the appear-
 365 ance of glial cells that exert neurotoxic influences on motor
 366 neurons, further perpetuating neuronal damage and glial
 367 activation in a toxic vicious loop. In this perspective, ALS
 368 might be caused in part by a defective crosstalk between
 369 motor neurons and surrounding astroglia.

370 4. Neurotoxic potential of reactive astrocytes in ALS

371 Recent studies have emphasized the involvement of
 372 astrocyte dysfunction in the pathogenesis of ALS through
 373 different synergistic mechanisms.

374 4.1. Downregulation of astrocytic glutamate transporters

375
 376 Many ALS patients have elevated glutamate levels in
 377 cerebrospinal fluid and a selective reduction of the astro-
 378 cytic glutamate transporter EAAT2 (GLT1), giving support
 379 to the excitotoxic hypothesis of motor neuron degeneration
 380 [107]. Excitatory amino acid transmission is dependent
 381 upon rapid clearance of released glutamate from the extra-
 382 cellular space by high affinity glutamate transporters located
 383 in the plasma membranes of presynaptic terminals and
 384 astrocytes. In patients with ALS, EAAT2 transporters are
 385 decreased or defective [108,114], which is coincident with
 386 the occurrence of astrocytosis. Significant loss of the
 387 EAAT2 transporters has also been documented in the spinal
 388 cord of SOD-1 G85R transgenic mice [18] and G93A
 389 transgenic rats [62]. In the rats, there is a focal loss of
 390 transporters around motor neurons in presymptomatic ani-
 391 mals and almost a complete loss at the end-stage of the
 392 disease. In contrast, neither EAAT1 nor EAAT2 transporters
 393 seem to be affected in presymptomatic or symptomatic mice
 394 carrying the G93A SOD1 mutation and are characterized by
 395 less pronounced, tardy astrocyte reactivity [117]. Functional
 396 abnormalities or defective processing of glutamate trans-
 397 porters may explain the significant reduction in the ability to
 398 transport glutamate in ALS. The presence of aberrant
 399 mRNA splice variants for EAAT2 in ALS has been hypoth-
 400 esized as a putative cause of EAAT2 loss [6,79]. Similarly,
 401 alterations in glutamate transporters have been found in
 402 Alzheimer's disease [74] and Lewy-body dementia [60],
 403 pathologies also characterized by the occurrence of astro-
 404 cytosis, suggesting that the loss of glutamate transporters in
 405 ALS may be secondary to astrocytic activation. Accord-
 406 ingly, recent evidence supports the view that reactive oxygen
 407 species produced by damaged motor neurons induce oxida-
 408 tion and disruption of glutamate uptake in neighboring
 409 astrocytes [98]. Oxidants and lipid peroxides inhibit the
 410 high affinity glutamate uptake in astrocytes and synapto-
 411 somes [14,137] by oxidation of critical sulfhydryl groups of
 412 the transporter protein. Similar effects are induced by
 413 peroxynitrite or hydrogen peroxide in reconstituted lipo-

414 somes containing glutamate transporters [131]. Alternative-
 415 ly, a neuroprotective effect could be explained by the
 416 inhibition of glutamate transporters by oxidants as an
 417 adaptative mechanism to prevent exaggerated glutamate
 418 release by reversal of the electrogenic glutamate uptake
 419 [128]. Other mechanism by which astrocytes may contribute
 420 to the excitotoxic damage of motor neurons is through the
 421 active release of glutamate. Inflammatory changes occurring
 422 in astrocytes can trigger the release of glutamate, which
 423 further exacerbates excitotoxic conditions for motor neu-
 424 rons. For example, reactive astrocytes in ALS express COX-
 425 2, an enzyme that catalyzes the synthesis of the inflamma-
 426 tory prostaglandin E2, which in turn stimulates glutamate
 427 release from astrocytes. Treatment of ALS mice with the
 428 COX-2 inhibitor Celecoxib delayed the onset of the disease
 429 and increased the survival rates [30], further suggesting a
 430 link between inflammation and excitotoxicity.

431 4.2. Cytokine production by astrocytes

432
 433 Much of the research on the pathology of neurodegen-
 434 erative diseases has been focused on neuroinflammatory
 435 mechanisms, proposed to exacerbate the progression of
 436 neurodegenerative diseases, including Alzheimer's [83]. In
 437 ALS, neuroinflammatory changes can be observed through
 438 the entire motor system. However, aside from glial activa-
 439 tion and its expression of some inflammatory mediators
 440 described in ALS, the pathophysiological role of relevant
 441 cytokines and chemokines has remained largely unexplored.
 442 For example, in the CNS, interleukin-1 β (IL-1 β) and IL-6
 443 exert a powerful regulation of glial cells [109]. Important
 444 functional interactions have been described between IL-1 β
 445 expression by glial cells and the occurrence of excitotoxic
 446 mechanisms and neuronal death in diverse forms of neuro-
 447 degeneration, which could be relevant in ALS pathophysiol-
 448 ogy. Proinflammatory IL-1 β and IL-6 are synthesized by
 449 neuroglia during epileptic activity [105], the response being
 450 greater when seizures are associated with neuronal damage,
 451 suggesting the release of neuronal mediators. The cellular
 452 responses to cytokines is intrinsically complex and involve a
 453 crosstalk with other inflammatory mediators. Interestingly,
 454 cytokine signaling can induce iNOS, COX-2 and NMDA
 455 receptor subunit phosphorylation, with different consequen-
 456 ces in glial and neuronal cells. Activation of iNOS in
 457 astrocytes by IL-1 β potentiates NMDA-mediated neurotox-
 458 icity in mixed cortical cultures [57]. Activation of murine
 459 astrocytes with tumour necrosis factor-alpha (TNF- α), IL-
 460 1 β and IFN γ induces IL-6, COX-2 and iNOS and makes the
 461 cells vulnerable to undergo apoptosis in response to Fas
 462 ligand (FasL) [39]. These results suggest a yet largely
 463 unexplored mechanism of massive astrocyte degeneration
 464 that has been recently reported in the spinal cord of
 465 chronically LPS-treated mutant SOD1 mice [88]. Activated
 466 astrocytes are potent producers of IL-6. While IL-6 can
 467 promote survival and protect neurons from degeneration, it
 468 can also promote astrocyte proliferation and activation [31].

469 Thus, these data are consistent with the hypothesis that
470 interleukines and other inflammatory cytokines and chemo-
471 kines play a role in initiating and modulating the inflam-
472 matory mechanisms in ALS, explaining relevant features of
473 the astrocyte pathology.

474

475 4.3. Production of nitric oxide and peroxynitrite

476 Free radical damage is a characteristic of pathologically
477 affected ALS tissues. Previous reports have shown that
478 reactive astrocytes in culture may contribute to free radical
479 formation and neuronal death. In particular, induction of
480 iNOS by LPS or cytokines seems to be required for
481 astrocytes to promote neuronal death [20,21,29,124]. Pro-
482 duction of nitric oxide or peroxynitrite by astrocytes induces
483 damage to mitochondrial complexes of co-cultured cortical
484 neurons [16,126] and enhances NMDA-induced excitotoxi-
485 city [57]. We have reported that production of nitric oxide
486 by reactive astrocytes is required for the induction of motor
487 neuron apoptosis in a co-culture model [20]. The co-cultures
488 were established by plating embryonic rat spinal motor
489 neurons onto monolayers of astrocytes that were first
490 exposed to peroxynitrite or LPS. Under control conditions,
491 astrocytes provided sufficient trophic support to allow motor
492 neurons to survive and extensively develop neurites without
493 addition of neurotrophic factors. In contrast, 30–40% of the
494 motor neurons plated on astrocytes pretreated with perox-
495 ynitrite or LPS undergo apoptosis over the next 24 h. Some
496 motor neurons plated on these reactive astrocytes were
497 smaller and displayed immunoreactivity for cleaved caspase
498 3, suggesting the activation of apoptotic mechanisms. Motor
499 neuron loss was not prevented by a cocktail of neurotrophic
500 factors that support motor neuron survival in the absence of
501 astrocytes, thus excluding a mechanism of peroxynitrite
502 inhibition of trophic factor production by astrocytes. Inhi-
503 bition of iNOS by nitro-L-arginine methyl ester and low
504 concentrations of aminoguanidine prevented motor neuron
505 loss, supporting the neurotoxic role of astrocytic nitric
506 oxide. In addition, damaged motor neurons were immuno-
507 reactive for nitrotyrosine. Nitric oxide itself cannot nitrate
508 tyrosine, which implies that it was transformed into perox-
509 ynitrite by reaction with superoxide [11]. Nitrotyrosine
510 staining has also been reported in cultured motor neurons
511 undergoing apoptosis [35,36], in mutant SOD-1 mice
512 [42,116] and in sporadic and familial cases of ALS
513 [1,8,43,115]. Further evidence to support a role for perox-
514 ynitrite in motor neuron death comes from the protection
515 provided by Mn-TBAP and uric acid. Mn-TBAP is a
516 membrane permeant SOD-mimetic and peroxynitrite scav-
517 enger [40]. On the other hand, uric acid does not directly
518 scavenge peroxynitrite [61] but is particularly effective at
519 inhibiting nitration by peroxynitrite. Thus, reactive astro-
520 cytes in ALS would not only impair neuronal excitability
521 and neurotransmission, but they would create the conditions
522 to actively induce motor neuron death by NO-dependent
523 mechanisms.

4.4. Production of apoptotic factors

526 Cytokines and trophic factors produced by activated
527 astrocytes such as FasL, TNF- α and NGF, are capable of
528 activating death receptors expressed in the diseased CNS.
529 Receptor-mediated apoptosis could play a role in motor
530 neuron loss observed in ALS without the direct involvement
531 of the immune system. These ligands show a dual function,
532 promoting cell survival or death depending on gene expres-
533 sion and activation state of the target cell [9]. Although FasL
534 is induced in reactive astrocytes as well as in microglia in
535 Alzheimer's and Huntington's disease [44,45], little is
536 known about its expression in ALS. Motor neurons co-
537 express Fas and FasL during the embryonic period of
538 naturally occurring cell death; however, no changes in
539 motoneuron survival were observed in mutant mice defi-
540 cient for Fas signaling [133]. In contrast, Fas signaling has
541 been implicated in motor neuron death induced by axotomy,
542 suggesting that this pathway may be activated in the
543 pathological degeneration of motor neurons [133]. Further-
544 more, motor neurons from transgenic mice overexpressing
545 ALS-linked SOD mutations G37R, G85R or G93A display
546 an increased susceptibility to Fas signaling [99,101]. The
547 apoptotic pathway activated by Fas seems to be specific to
548 motor neurons, requiring co-activation of caspase-8 and p38
549 as well as the production of nitric oxide by neuronal NOS
550 [101]. Another potential apoptotic candidate released by
551 astrocytes is nerve growth factor (NGF). Clearly, NGF is
552 critical for the differentiation and survival of specific
553 neuronal populations during development and for neural
554 plasticity in the mature nervous system [77,119,125]. While
555 NGF can signal through activation of the high affinity TrkA
556 receptor, it also can activate the non-selective neurotrophin
557 receptor p75^{NTR}, a member of the tumor necrosis factor
558 receptor superfamily. Motor neurons are generally thought
559 to be unresponsive to NGF because they lack the specific
560 TrkA receptor. However, they do respond to other members
561 of the neurotrophin family, including BDNF, NT-3 and NT-
562 4/5 [123].

563 Signaling through p75^{NTR}, in the absence of the
564 corresponding Trk receptor, has been shown to promote
565 apoptosis in specific neuronal types during normal CNS
566 development [7,48,49,136] and is probably used to eliminate
567 damaged neurons and oligodendrocytes in the mature ner-
568 vous system [84,100]. Motor neurons express p75^{NTR} during
569 the embryonic period of naturally occurring cell death when
570 over half of motor neurons die, but its expression gradually
571 ends after birth [22,34,134,141]. Although p75^{NTR} is not
572 present in mature motor neurons, the receptor can be re-
573 expressed following nerve injury [34,46,69,103,140] and has
574 been shown to mediate motor neuron loss after facial nerve
575 lesion in newborn [139] and adult mice [46]. Moreover,
576 p75^{NTR} is found in motor neurons of ALS patients [80,121],
577 suggesting that re-expression of the receptor might modulate
578 the death of neurons in damaged areas. p75^{NTR} expression
579 can be also observed on reactive astrocytes, microglia and

580 perivascular cells in chronic active MS lesions, suggesting an
581 additional role for NGF in regulating the immune response in
582 glial cells [135].

583 Astrogliosis is associated with increased expression and
584 release of several growth factors and cytokines, including
585 NGF [33]. Increased NGF levels have been shown in rodent
586 CNS following experimental lesions and in pathological
587 conditions characterized by prominent astrocytosis such as
588 Alzheimer’s disease [27,37]. Expression of NGF receptors
589 in active multiple sclerosis lesions suggests a role for NGF
590 in regulating the autoimmune response at both immune and
591 glial cell levels [17,135]. However, little is known about the
592 expression of NGF in ALS, although increased NGF levels
593 were reported in muscle of ALS patients [72,127]. Thus, it
594 is conceivable that NGF signaling between astrocytes and
595 p75^{NTR}-expressing motor neurons may contribute to the
596 induction of neuronal apoptosis in ALS. We have recently
597 found a prominent increase in NGF immunoreactivity in the
598 neuropil of the anterior horn in symptomatic mice carrying
599 the G93A mutation coincident with p75^{NTR} expression in
600 motor neurons [93], suggesting that increased NGF production
601 may parallel the development of astrocytosis in ALS
602 and contribute to motor neuron death. We also found that
603 reactive astrocytes secrete high molecular forms of NGF,
604 which could correspond to the precursors forms of NGF
605 (pro-NGF) [92]. It has been recently shown that the pro-
606 neurotrophin is enriched in the CNS [38] and could be
607 secreted by different cell types [56,58]. Some isoforms of

608 pro-NGF bind with high affinity to p75^{NTR} and thus induces
609 a specific apoptotic signal even in cells expressing both
610 p75^{NTR} and TrkA receptors [76].

611 Two recent reports further support a role for a NGF/
612 p75^{NTR} apoptotic pathway in ALS. Survival of SOD G93A
613 mice is significantly increased by systemic treatment with
614 an antisense peptide nucleic acid construct that targets the
615 p75 NTR gene and inhibits its expression [132]. In another
616 study, a modest but significant increase in survival was
617 reported in double transgenic mice expressing SOD1 G93A
618 but lacking p75^{NTR} [73]. Improved survival was only found
619 in female mice and was not correlated with increased motor
620 neuron survival but with less astrocyte activation in the
621 lumbar ventral spinal cord.

622 **5. Astrocytes and motor neuron death**

623 We hypothesize that oxidative stress, occurring in dam-
624 aged areas undergoing neurodegeneration, disrupts the inter-
625 actions between motor neuron and astrocytes (Fig. 1). In
626 response to damage, motor neurons can signal the surround-
627 ing astrocytes to become activated and upregulate critical
628 genes that may recapitulate the pattern found during develop-
629 ment. For example, the re-expression of p75^{NTR} and
630 neuronal NOS may help to determine which neurons survive
631 or undergo apoptosis. In turn, activated astrocytes are likely
632 to develop several phenotypes with distinct patterns of gene

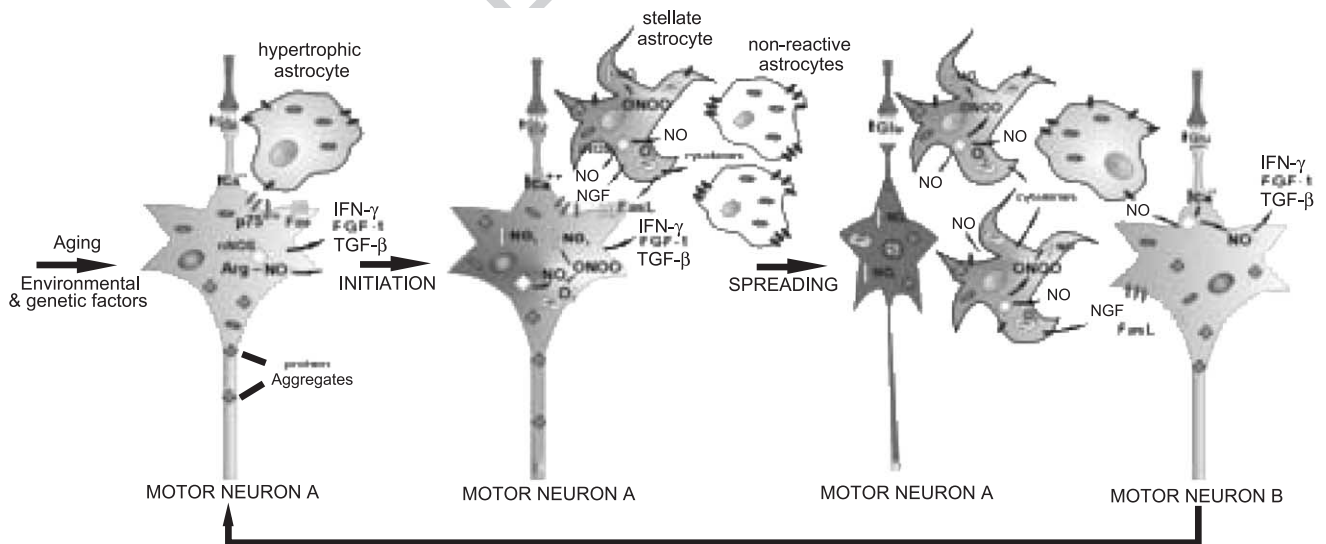


Fig. 1. Proposed pathogenic steps involving astrocyte to motor neuron signaling in ALS. INDUCTION. Aging or various genetic or environmental defects may induce early damage in *motor neuron A*, which upregulates the expression of nNOS, p75^{NTR}, Fas, cytokines and trophic factors. Neighboring astrocytes (and microglia—not shown) become activated in response to the mediators produced by motor neurons and adopt a hypertrophic morphology. INITIATION. The initial subtle pathological changes cause *motor neuron A* to become further damaged by a combination of increased excitatory inputs, defects in mitochondrial activity, slowed axonal transport and enhanced production of NO and peroxynitrite. Astrocytes become further activated, displaying a stellate morphology and decreased glutamate transporters and suffer increased oxidative stress mediated by NO and peroxynitrite. Astrocytes may amplify motor neuron damage by producing NO, cytokines and pro apoptotic mediators such as NGF or FasL. Astrocytes may also spread the damage to other glial cells that become activated. SPREADING. While *motor neuron A* shows regressive changes or may follow apoptosis, it becomes completely surrounded by reactive astrocytes. Both astrocytes and motor neurons exhibit extensive pathologic signs including accumulation of nitrotyrosine, protein aggregation and damaged mitochondria. Meanwhile, astrocyte activation has spread and may render *motor neuron B* more vulnerable to the primary etiologic factors and reinforce a vicious autotoxic loop.

633 expression and secretory capacity, which primarily serve to
 634 isolate the site where injury is occurring. In addition, reactive
 635 astrocytes may develop phenotypic features such as down-
 636 regulation of glutamate transporters and upregulation of
 637 inflammatory mediators and trophic factors that support the
 638 survival and growth of the “healthy” neurons still present in
 639 the injured regions while signaling the death of those already
 640 critically damaged. Increased levels of nitric oxide produced
 641 by astrocytes does not induce motor neuron apoptosis unless
 642 increased fluxes of superoxide are formed as a result of
 643 metabolic or mitochondrial dysfunction, perhaps stimulated
 644 by exaggerated excitatory transmission. Similarly, increased
 645 production of either FasL or NGF does not induce motor
 646 neuron apoptosis unless nitric oxide is produced or p75^{NTR} is
 647 expressed in those neurons. However, it would be the
 648 exception rather than the rule that the simple occurrence of
 649 reactive astrocytes in response to pathological conditions
 650 such as axotomy or trauma, triggers a progressive motor
 651 neuron disease with the characteristics of ALS. Changes in
 652 motor neurons and surrounding astrocytes should be revers-
 653 ible and strictly controlled to avoid propagation of the injury.
 654 We propose that these mechanisms are somehow critically
 655 and irreversibly disrupted in ALS.

656 6. Conclusions

657 The pathology of ALS is characterized by widespread
 658 signs of neuronal and astrocyte dysfunction which account
 659 for the appearance of protein aggregates, cytoskeletal ab-
 660 normalities and mitochondrial swelling. These changes not
 661 only affect motor neurons but also the surrounding astro-
 662 cytes and many interneurons. The origin of this pan-cellular
 663 pathology is intriguing and deserves further investigation.
 664 Because reactive astrocytes occurring in ALS may spread
 665 the phenotypic transformation to astrocytes located in adja-
 666 cent regions, they may greatly contribute to the elimination
 667 of vulnerable motor neurons and propagation of the disease.
 668 While some motor neurons display regressive changes and
 669 may undergo apoptosis, others exhibit compensatory axonal
 670 growth to re-innervate muscle fibers. This may increase the
 671 metabolic and functional burden on the remaining motor
 672 neurons to eventually promote further neuronal death. We
 673 speculate that motor neurons surrounded by reactive astro-
 674 cytes may not be appropriately supported with metabolic
 675 substrates or trophic factors and may become progressively
 676 vulnerable to inflammatory mediators and pro-apoptotic
 677 mediators. Deciphering the interactions between motor
 678 neurons and glia in ALS may reveal the basis for the
 679 progressive pathogenesis of the disease.

680 7. Uncited references

681 [41]
 682 [110]

Acknowledgements

This work was supported by the PEDECIBA program;
 the Linus Pauling Institute, the Environmental Health
 Sciences Center (ES0021) Oregon State University (USA)
 and grants from the National Institutes of Health R03
 TW006482; P01AT002034; R01 NS033291. We thank Dr.
 Mark Bevenssee for his insightful comments.

References

- [1] K. Abe, L.H. Pan, M. Watanabe, H. Konno, T. Kato, Y. Itoyama, 691
Upregulation of protein tyrosine nitration in the anterior horn cells of 692
amyotrophic lateral sclerosis, *Neurol. Res.* 19 (1997) 124–128. 693
- [2] M.E. Alexianu, M. Kozovska, S.H. Appel, Immune reactivity in a 694
mouse model of familial ALS correlates with disease progression, 695
Neurology 57 (2001) 1282–1289. 696
- [3] G. Almer, S. Vukosavie, N. Romero, S. Przedborski, Inducible 697
nitric oxide synthase up-regulation in a transgenic mouse model 698
of familial amyotrophic lateral sclerosis, *J. Neurochem.* 72 (1999) 699
2415–2425. 700
- [4] L.C. Ang, B. Bhaumick, B.H.J. Juurlink, Neurite promoting activity 701
of insulin, insulin-like growth factor I and nerve growth factor on 702
spinal motoneurons is astrocyte dependent, *Brain Res. Dev. Brain* 703
Res. 74 (1993) 83–88. 704
- [5] J.M. Anneser, M.R. Cookson, P.G. Ince, P.J. Shaw, G.D. Borasio, 705
Glial cells of the spinal cord and subcortical white matter up-regulate 706
neuronal nitric oxide synthase in sporadic amyotrophic lateral scler- 707
osis, *Exp. Neurol.* 171 (2001) 418–421. 708
- [6] G. Bai, S.A. Lipton, Aberrant RNA splicing in sporadic amyotrophic 709
lateral sclerosis, *Neuron* 20 (1998) 363–366. 710
- [7] G.L. Barrett, P.F. Bartlett, The p75 nerve growth factor receptor 711
mediates survival or death depending on the stage of sensory 712
neuron development, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 713
6501–6505. 714
- [8] M.F. Beal, L.J. Ferrante, S.E. Browne, R.T. Matthews, N.W. Kowall, 715
R.H. Brown Jr., Increased 3-nitrotyrosine in both sporadic and famil- 716
ial amyotrophic lateral sclerosis, *Ann. Neurol.* 42 (1997) 644–654. 717
- [9] B. Becher, P.A. Barker, T. Owens, J.P. Antel, CD95–CD95L: can 718
the brain learn from the immune system? *Trends Neurosci.* 21 719
(1998) 114–117. 720
- [10] J.S. Beckman, H. Ischiropoulos, L. Zhu, M. van der Woerd, C.D. 721
Smith, J. Chen, J. Harrison, J.C. Martin, M. Tsai, Kinetics of 722
superoxide dismutase- and iron-catalyzed nitration of phenolics 723
by peroxynitrite, *Arch. Biochem. Biophys.* 298 (1992) 438–445. 724
- [11] J.S. Beckman, J. Chen, H. Ischiropoulos, J.P. Crow, Oxidative chem- 725
istry of peroxynitrite, *Methods Enzymol.* 233 (1994) 229–240. 726
- [12] J.S. Beckman, A.G. Estévez, J.P. Crow, L. Barbeito, Superoxide 727
dismutase and the death of motoneurons in ALS, *Trends Neurosci.* 728
24 (2001) S15–S20. 729
- [13] P. Bezzi, A. Volterra, A neuron-glia signalling network in the active 730
brain, *Curr. Opin. Neurobiol.* 11 (2001) 387–394. 731
- [14] E.M. Blanc, J.N. Keller, S. Fernandez, M.P. Mattson, 4-hydroxy- 732
nonenal, a lipid peroxidation product, impairs glutamate transport in 733
cortical astrocytes, *Glia* 22 (1998) 149–160. 734
- [15] J.P. Bolaños, J.M. Medina, Induction of NOS inhibits gap junction 735
permeability in cultures rat astrocytes, *J. Neurochem.* 66 (1996) 736
2091–2099. 737
- [16] J.P. Bolaños, S.J.R. Heales, J.M. Land, J.B. Clark, Effect of peroxy- 738
nitrite on the mitochondrial respiratory chain: differential suscepti- 739
bility of neurons and astrocytes in primary cultures, *J. Neurochem.* 740
64 (1995) 1965–1972. 741
- [17] L. Bracci-Laudiero, L. Aloe, R. Levi-Montalcini, C. Buttinelli, D. 742
Schilter, S. Gillessen, U. Otten, Multiple sclerosis patients express 743

- 744 increased levels of nerve growth factor in cerebrospinal fluid, *Neurosci. Lett.* 147 (1992) 9–12.
- 745
- 746 [18] L.I. Buijn, M.W. Becher, M.K. Lee, K.L. Anderson, N.A. Jenkins, N.G. Copeland, S.S. Sisodia, J.D. Rothstein, D.R. Borchelt, D.L. Price, D.W. Cleveland, ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions, *Neuron* 18 (1997) 327–338.
- 749
- 750
- 751 [19] P. Cassina, H. Peluffo, L. Barbeito, Adaptive responses of spinal astrocytes to oxidative stress, in: B. Castellanos (Ed.), *Glial Cell Function*, Prog. Brain Res., vol. 132, Elsevier, Amsterdam, 2001, pp. 413–425.
- 752
- 753
- 754
- 755 [20] P. Cassina, H. Peluffo, M. Pehar, L. Martinez-Palma, A. Ressia, J.S. Beckman, A.G. Estévez, L. Barbeito, Peroxynitrite triggers a phenotypic transformation in spinal cord astrocytes that induces motor neuron apoptosis, *J. Neurosci. Res.* 67 (2002) 21–29.
- 756
- 757
- 758
- 759 [21] C.C. Chao, S. Hu, W.S. Sheng, D. Bu, M.I. Bukrinsky, P.K. Peterson, Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism, *Glia* 16 (1996) 276–284.
- 760
- 761
- 762 [22] A.Y. Chiu, E.W. Chen, S. Loera, A motor neuron-specific epitope and the low-affinity nerve growth factor receptor display reciprocal patterns of expression during development, axotomy, and regeneration, *J. Comp. Neurol.* 328 (1993) 351–363.
- 763
- 764
- 765
- 766 [23] A.M. Clement, M.D. Nguyen, E.A. Roberts, M.L. Garcia, S. Boille, M. Rule, A.P. McMahon, W. Doucette, D. Siwek, R.J. Ferrante, R.H. Brown, J.P. Julien, S.S. Goldstein, D.W. Cleveland, Wild-type non-neuronal cells extend survival of SOD1 mutant motor neurons in ALS mice, *Science* 302 (2003) 113–117.
- 767
- 768
- 769
- 770
- 771 [24] D.W. Cleveland, J. Liu, Oxidation versus aggregation—how do SOD1 mutants cause ALS? *Nat. Med.* 6 (2000) 1320–1321.
- 772
- 773
- 774 [25] D.W. Cleveland, J.D. Rothstein, From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS, *Nat. Rev., Neurosci.* 2 (2001) 806–819.
- 775
- 776
- 777 [26] J.L. Cova, H. Aldskogius, Effect of axotomy on perineuronal glial cells in the hypoglossal and dorsal motor vagal nuclei of the cat, *Exp. Neurol.* 93 (1986) 662–667.
- 778
- 779
- 780 [27] K.A. Crutcher, S.A. Scott, S. Liang, W.V. Everson, J. Weingartner, Detection of NGF like activity in human brain tissue: increased levels in Alzheimer's disease, *J. Neurosci.* 13 (1993) 2540–2550.
- 781
- 782
- 783 [28] F.F. Cruz-Sanchez, A. Moral, E. Tolosa, J. de Belleruche, M.L. Rossi, Evaluation of neuronal loss, astrocytosis and abnormalities of cytoskeletal components of large motor neurons in the human anterior horn in aging, *J. Neural Transm.* 105 (1998) 689–701.
- 784
- 785
- 786 [29] V.L. Dawson, H.P. Brahmabhatt, J.A. Mong, T.M. Dawson, Expression of inducible nitric oxide synthase causes delayed neurotoxicity in primary mixed neuronal glial cortical cultures, *Neuropharmacology* 33 (1994) 1425–1430.
- 787
- 788
- 789
- 790 [30] D.B. Drachman, K. Frank, M. Dykes-Hoberg, P. Teismann, G. Almer, S. Przedborski, J.D. Rothstein, Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS, *Ann. Neurol.* 52 (2002) 771–778.
- 791
- 792
- 793
- 794 [31] Y. Dong, E.N. Benveniste, Immune function of astrocytes, *Glia* 36 (2001) 180–190.
- 795
- 796
- 797 [32] K.L. Eagleson, T.R. Raju, M.R. Bennett, Motoneuron survival is induced by immature astrocytes from developing avian spinal cord, *Dev. Brain Res.* 17 (1985) 95–104.
- 798
- 799
- 800 [33] M. Eddleston, L. Mucke, Molecular profile of reactive astrocytes: implications for their role in neurologic disease, *Neuroscience* 54 (1993) 15–36.
- 801
- 802
- 803 [34] P. Ernfors, A. Henschen, L. Olson, H. Persson, Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons, *Neuron* 2 (1989) 1605–1613.
- 804
- 805
- 806 [35] A.G. Estévez, N. Spear, S.M. Manuel, R. Radi, C.E. Henderson, L. Barbeito, J.S. Beckman, Nitric oxide and superoxide contribute to motor neuron apoptosis induced by trophic factor deprivation, *J. Neurosci.* 18 (1998) 923–931.
- 807
- 808
- 809
- 810 [36] A.G. Estévez, J.P. Crow, J.B. Sampson, C. Reiter, Y. Zhuang, G.J. Richardson, M.M. Tarpey, L. Barbeito, J.S. Beckman, Induction of nitric oxide dependent apoptosis in motor neurons by zinc deficient superoxide dismutase, *Science* 286 (1999) 2498–2500.
- 811
- 812
- 813
- 814 [37] M. Fahnstock, S.A. Scott, N. Jetté, J.A. Weingartner, K.A. Crutcher, Nerve growth factor mRNA and protein levels measured in the same tissue from normal and Alzheimer's disease parietal cortex, *Mol. Brain Res.* 4 (1996) 175–178.
- 815
- 816
- 817
- 818 [38] M. Fahnstock, B. Michalski, B. Xu, M.D. Coughlin, The precursor pro nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease, *Mol. Cell. Neurosci.* 18 (2001) 210–220.
- 819
- 820
- 821
- 822 [39] J. Falsig, M. Latta, M. Leist, Defined inflammatory states in astrocyte cultures: correlation with susceptibility towards CD95 driven apoptosis, *J. Neurochem.* 88 (2004) 181–193.
- 823
- 824
- 825 [40] K.M. Faulkner, S.E. Liochev, I. Fridovich, Stable Mn(III) porphyrins mimic superoxide dismutase in vitro and substitute for it in vivo, *J. Biol. Chem.* 268 (1994) 23471–23476.
- 826
- 827
- 828 [41] S. Fedoroff, A. Vernadakis (Eds.), *Astrocytes: Biochemistry, Physiology, and Pharmacology of Astrocytes*, vol. 2, Academic Press, Orlando, 1986, 420 pp.
- 829
- 830
- 831 [42] R.J. Ferrante, L.A. Shinobu, J.B. Schulz, R.T. Mathews, C.E. Thomas, N.W. Kowall, M.E. Gurney, M.F. Beal, Increased 3 nitrotyrosine and oxidative damage in mice with a human copper/zinc superoxide dismutase mutation, *Ann. Neurol.* 42 (1997) 326–334.
- 832
- 833
- 834
- 835 [43] R.J. Ferrante, S.E. Browne, L.A. Shinobu, A.C. Bowling, M.J. Baik, U. MacGarvey, N.W. Kowall, R.H. Brown Jr., M.F. Beal, Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis, *J. Neurochem.* 69 (1997) 2064–2074.
- 836
- 837
- 838
- 839 [44] I. Ferrer, R. Blanco, B. Cutillas, S. Ambrosio, Fas and Fas-L expression in Huntington's disease and Parkinson's disease, *Neuropathol. Appl. Neurobiol.* 26 (2000) 424–433.
- 840
- 841
- 842 [45] I. Ferrer, B. Puig, J. Krupinski, M. Carmona, R. Blanco, Fas and Fas ligand expression in Alzheimer's disease, *Acta Neuropathol. (Berl.)* 102 (2001) 121–131.
- 843
- 844
- 845 [46] C.C. Ferri, F.A. Moore, M.A. Bisby, Effects of facial nerve injury on mouse motoneurons lacking the p75 low affinity neurotrophin receptor, *J. Neurobiol.* 34 (1998) 1–9.
- 846
- 847
- 848 [47] L.R. Fischer, D.G. Culver, P. Tennant, A.A. Davis, M. Wang, A. Castellano-Sanchez, J. Khan, M.A. Polak, J.D. Glassa, Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man, *Exp. Neurol.* 185 (2004) 232–240.
- 849
- 850
- 851
- 852 [48] J.M. Frade, Y.A. Barde, Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord, *Development* 126 (1999) 683–690.
- 853
- 854
- 855
- 856 [49] J.M. Frade, A. Rodriguez Tebar, Y.A. Barde, Induction of cell death by endogenous nerve growth factor through its p75 receptor, *Nature* 383 (1996) 166–168.
- 857
- 858
- 859 [50] S.A. Gilmore, T.J. Sims, J.E. Leiting, Astrocytic reactions in spinal grey matter following sciatic axotomy, *Glia* 3 (1990) 342–349.
- 860
- 861
- 862 [51] D. Giulian, T.J. Baker, Peptides released by amoeboid microglia regulate astroglial proliferation, *J. Cell Biol.* 101 (1985) 2411–2415.
- 863
- 864
- 865 [52] Y.H. Gong, A.S. Parsadanian, A. Andreeva, W.D. Snider, J.L. Elliott, Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration, *J. Neurosci.* 20 (2000) 660–665.
- 866
- 867
- 868 [53] M.B. Graeber, G.W. Kreutzberg, Delayed astrocyte reaction following facial nerve axotomy, *J. Neurocytol.* 17 (1988) 209–220.
- 869
- 870
- 871 [54] M.E. Gurney, H. Pu, A.Y. Chiu, et al., Motor neuron degeneration in mice that express a human cu, zn superoxide dismutase mutation, *Science* 264 (1994) 1772–1775.
- 872
- 873
- 874 [55] E.D. Hall, J.A. Oostveen, M.E. Gurney, Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS, *Glia* 23 (1998) 249–256.
- 875
- 876
- 877 [56] W. Hasan, T. Pedchenko, D. Krizan Agbas, L. Baum, P.G. Smith, Sympathetic neurons synthesize and secrete pro nerve growth factor protein, *J. Neurobiol.* 57 (2003) 38–53.

- 878 [57] S.J. Hewett, C.A. Csernansky, D.W. Choi, Selective potentiation of
879 NMDA induced neuronal injury following induction of astrocytic
880 iNOS, *Neuron* 13 (1994) 487–494.
- 881 [58] J.V. Heymach Jr., E.M. Shooter, The biosynthesis of neurotrophin
882 heterodimers by transfected mammalian cells, *J. Biol. Chem.* 270
883 (1995) 12297–12304.
- 884 [59] A. Hirano, Neuropathology of ALS: an overview, *Neurology* 47
885 (1996) S63–S66.
- 886 [60] L.S. Honig, D.D. Chambliss, E.H. Bigio, S.L. Carroll, J.L. Elliott,
887 Glutamate transporter EAAT2 splice variants occur not only in ALS,
888 but also in AD and controls, *Neurology* 55 (2000) 1082–1088.
- 889 [61] D.C. Hooper, S. Spitsin, R.B. Kean, J.M. Champion, G.M. Dickson,
890 I. Chaudhry, H. Koprowski, Uric acid, a natural scavenger of per-
891 oxynitrite, in experimental allergic encephalomyelitis and multiple
892 sclerosis, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 675–680.
- 893 [62] D.S. Howland, J. Liu, Y. She, B. Goad, N.J. Maragakis, B. Kim, J.
894 Erickson, J. Kulik, L. DeVito, G. Psaltis, L.J. DeGennaro, D.W.
895 Cleveland, J.D. Rothstein, Focal loss of the glutamate transporter
896 EAAT2 in a transgenic rat model of SOD1 mutant mediated amyotro-
897 phic lateral sclerosis (ALS), *Proc. Natl. Acad. Sci. U. S. A.* 99
898 (2002) 1604–1609.
- 899 [63] D. Hoyaux, J. Alao, J. Fuchs, R. Kiss, B. Keller, C.W. Heizmann, R.
900 Pochet, D. Freremann, S100A6, a calcium and zinc binding protein, is
901 overexpressed in SOD1 mutant mice, a model for amyotrophic lateral
902 sclerosis, *Biochim. Biophys. Acta* 1498 (2000) 264–272.
- 903 [64] K. Huber, C. Meisinger, C. Grothe, Expression of fibroblast growth
904 factor 2 in hypoglossal motoneurons is stimulated by peripheral
905 nerve injury, *J. Comp. Neurol.* 382 (1997) 189–198.
- 906 [65] R.E. Huie, S. Padmaja, The reaction of NO with superoxide, *Free
907 Radic. Res. Commun.* 18 (1993) 195–199.
- 908 [66] M.P. Junier, M. Culpier, N. Le Forestier, J. Cadusseau, F. Suzuki,
909 M. Peschanski, P.A. Dreyfus, Transforming growth factor alpha
910 (TGF alpha) expression in degenerating motoneurons of the murine
911 mutant wobbler: a neuronal signal for astrogliosis? *J. Neurosci.* 14
912 (1994) 4206–4216.
- 913 [67] B.K. Kaspar, J. Llado, N. Sherkat, J.D. Rothstein, F.H. Gage, Ret-
914 rograde viral delivery of IGF 1 prolongs survival in a mouse model
915 ALS model, *Science* 301 (2003) 839–842.
- 916 [68] S. Kato, H. Hayashi, K. Nakashima, E. Nanba, M. Kato, A. Hirano,
917 I. Nakano, K. Asayama, E. Ohama, Pathological characterization of
918 astrocytic hyaline inclusions in familial amyotrophic lateral sclero-
919 sis, *Am. J. Pathol.* 151 (1997) 611–620.
- 920 [69] V.E. Koliatsos, T.O. Crawford, D.L. Price, Axotomy induces nerve
921 growth factor receptor immunoreactivity in spinal motor neurons,
922 *Brain Res.* 549 (1991) 297–304.
- 923 [70] K. Kristensson, M. Aldskogius, Z.C. Peng, T. Olsson, H. Aldskogius,
924 M. Bentivoglio, Co induction of neuronal interferon gamma and
925 nitric oxide synthase in rat motor neurons after axotomy: a role in
926 nerve repair or death? *J. Neurocytol.* 23 (1994) 453–459.
- 927 [71] P.D. Kushner, D.J. Stephenson, S. Wright, Reactive astrogliosis is
928 widespread in the subcortical white matter of amyotrophic lateral
929 sclerosis brain, *J. Neuropathol. Exp. Neurol.* 50 (1991) 263–277.
- 930 [72] B.M. Kust, J.C. Copray, N. Brouwer, D. Troost, H.W. Boddeke,
931 Elevated levels of neurotrophins in human biceps brachii tissue of
932 amyotrophic lateral sclerosis, *Exp. Neurol.* 177 (2002) 419–427.
- 933 [73] B.M. Kust, N. Brouwer, I.J. Mantingh, H.W. Boddeke, J.C. Copray,
934 Reduced p75NTR expression delays disease onset only in female
935 mice of a transgenic model of familial amyotrophic lateral sclerosis,
936 *Amyotroph. Lateral Scler. Other Mot. Neuron Disord.* 4 (2003)
937 100–105.
- 938 [74] C.M. Lauderback, J.M. Hackett, F.F. Huang, J.N. Keller, L.I.
939 Szweda, W.R. Markesbery, D.A. Butterfield, The glial glutamate
940 transporter, GLT 1, is oxidatively modified by 4 hydroxy 2 nonenal
941 in the Alzheimer's disease brain: the role of Abeta1 42, *J. Neuro-
942 chem.* 78 (2001) 413–416.
- 943 [75] C.K. Lee, R. Weindruch, T.A. Prolla, Gene expression profile of the
944 ageing brain in mice, *Nat. Genet.* 25 (2000) 294–297.
- [76] R. Lee, P. Kermani, K.K. Teng, B.L. Hempstead, Regulation of
cell survival by secreted proneurotrophins, *Science* 294 (2001)
1945–1948.
- [77] R. Levi Montalcini, The nerve growth factor 35 years later, *Science*
237 (1987) 1154–1162.
- [78] J.B. Levine, J. Kong, M. Nadler, Z. Xu, Astrocytes interact intimately
with degenerating motor neurons in mouse amyotrophic lateral sclero-
sis (ALS), *Glia* 28 (1999) 215–224.
- [79] C.L. Lin, L.A. Bristol, L. Jin, M. Dykes Hoberg, T. Crawford, L.
Clawson, J.D. Rothstein, Aberrant RNA processing in a neurodegen-
erative disease: the cause for absent EAAT2, a glutamate transporter
in amyotrophic lateral sclerosis, *Neuron* 20 (1998) 589–602.
- [80] K.S. Lowry, S.S. Murray, C.A. McLean, P. Talman, S. Mathers, E.C.
Lopes, S.S. Cheema, A potential role for the p75 low affinity neuro-
trophin receptor in spinal motor neuron degeneration in murine and
human amyotrophic lateral sclerosis, *Amyotroph. Lateral Scler. Other
Mot. Neuron Disord.* 2 (2001) 127–134.
- [81] C. Maihofner, S. Probst-Cousin, M. Bergmann, W. Neuhuber, B.
Neundorfer, D. Heuss, Expression and localization of cyclooxy-
genase 1 and 2 in human sporadic amyotrophic lateral sclerosis,
Eur. J. Neurosci. 18 (2003) 1527–1534.
- [82] S.K. Malhotra, T.K. Shnitka, Diversity in reactive astrocytes, in: J.
De Vellis (Ed.), *Neuroglia in the Aging Brain*, Humana Press, New
Jersey, 2002, pp. 17–33.
- [83] P.L. McGeer, E.G. McGeer, Inflammatory processes in amyotrophic
lateral sclerosis, *Muscle Nerve* 26 (2002) 459–470.
- [84] J.E. Merrill, N.J. Scolding, Mechanisms of damage to myelin and
oligodendrocytes and their relevance to disease, *Neuropathol. Appl.
Neurobiol.* 25 (1999) 435–458.
- [85] A. Migheli, S. Cordera, C. Bendotti, C. Atzori, R. Piva, D. Schiffer,
S 100beta protein is upregulated in astrocytes and motor neurons in
the spinal cord of patients with amyotrophic lateral sclerosis, *Neuro-
sci. Lett.* 261 (1999) 25–28.
- [86] M. Nagai, M. Aoki, I. Miyoshi, M. Kato, P. Pasinelli, N. Kasai, R.H.
Brown Jr., Y. Itoyama, Rats expressing human cytosolic copper zinc
superoxide dismutase transgenes with amyotrophic lateral sclerosis:
associated mutations develop motor neuron disease, *J. Neurosci.* 21
(2001) 9246–9254.
- [87] D. Nagy, T. Kato, P.D. Kushner, Reactive astrocytes are widespread
in the cortical gray matter of amyotrophic lateral sclerosis, *J. Neuro-
sci. Res.* 38 (1994) 336–347.
- [88] M.D. Nguyen, T. D'Aigle, G. Gowing, J.P. Julien, S. Rivest, Exacer-
bation of motor neuron disease by chronic stimulation of innate
immunity in a mouse model of amyotrophic lateral sclerosis, *J. Neu-
rosci.* 24 (2004) 1340–1349.
- [89] T. Olsson, K. Kristensson, A. Ljungdahl, J. Maehlen, R. Holmdahl,
L. Klareskog, Gamma interferon like immunoreactivity in axotom-
ized rat motor neurons, *J. Neurosci.* 9 (1989) 3870–3875.
- [90] S.A. O'Really, J. Roedica, D. Nagy, R.A. Hallelwell, K. Alderson,
J.K. Marklund, P.D. Kushner, Motor neuron astrocyte interactions
and levels of Cu,Zn superoxide dismutase in sporadic amyotrophic
lateral sclerosis, *Exp. Neurol.* 131 (1995) 203–210.
- [91] W.A. Pedersen, W. Fu, J.N. Keller, W.R. Markesbery, S.A. Appel,
R.G. Smith, E. Kasarkis, M.P. Mattson, Protein modification by the
lipid peroxidation product 4 hydroxynonenal in the spinal cords of
amyotrophic lateral sclerosis patients, *Ann. Neurol.* 44 (1998)
819–824.
- [92] M.A. Pehar, M.P. Cassina, M.R. Vargas, R.M. Castellanos, L. Viera,
J.S. Beckman, A.G. Estevez, L. Barbeito, Astrocytic production of
proNGF and motor neuron apoptosis in ALS, *Program No. 96.18.*
(2003) Abstract. Society for Neuroscience, 2003, Online.
- [93] M.A. Pehar, M.P. Cassina, M.R. Vargas, R.M. Castellanos, L. Viera,
J.S. Beckman, A.G. Estevez, L. Barbeito, Astrocytic production of
nerve growth factor and motor neuron apoptosis: implications for
amyotrophic lateral sclerosis, *J. Neurochem.* (2004) (in press).
- [94] H. Peluffo, A. Estévez, L. Barbeito, J.M. Stutzmann, Riluzole pro-
motes survival of rat motoneurons in vitro by stimulating trophic

- activity produced by spinal astrocyte monolayers, *Neurosci. Lett.* 228 (1997) 207–211.
- [95] K. Pilegaard, O. Ladefoged, Total number of astrocytes in the molecular layer of the dentate gyrus of rats at different ages, *Anal. Quant. Cytol. Histol.* 18 (1996) 279–285.
- [96] A. Pramatarova, J. Laganier, J. Roussel, K. Brisebois, G.A. Rouleau, Neuron specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment, *J. Neurosci.* 21 (2001) 3369–3374.
- [97] R. Radi, J.S. Beckman, K.M. Bush, B.A. Freeman, Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide, *J. Biol. Chem.* 266 (1991) 4244–4250.
- [98] S.D. Rao, H.Z. Yin, J.H. Weiss, Disruption of glial glutamate transport by reactive oxygen species produced in motor neurons, *J. Neurosci.* 23 (2003) 2627–2633.
- [99] C. Raoul, C.E. Henderson, B. Pettmann, Programmed cell death of embryonic motoneurons triggered through the Fas death receptor, *J. Cell Biol.* 147 (1999) 1049–1062.
- [100] C. Raoul, B. Pettmann, C.E. Henderson, Active killing of neurons during development and following stress: a role for p75(NTR) and Fas? *Curr. Opin. Neurobiol.* 10 (2000) 111–117.
- [101] C. Raoul, A.G. Estevez, H. Nishimune, D.W. Cleveland, O. deLapeyriere, C.E. Henderson, G. Haase, B. Pettmann, Motoneuron death triggered by a specific pathway downstream of Fas. Potentiation by ALS linked SOD1 mutations, *Neuron* 35 (2002) 1067–1083.
- [102] I. Reisert, G. Wildemann, D. Grab, C. Pilgrim, The glial reaction in the course of axon regeneration: a stereological study of the rat hypoglossal nucleus, *J. Comp. Neurol.* 229 (1984) 121–128.
- [103] M. Rende, I. Giambanco, M. Buratta, P. Tonali, Axotomy induces a different modulation of both low affinity nerve growth factor receptor and choline acetyltransferase between adult rat spinal and brainstem motoneurons, *J. Comp. Neurol.* 363 (1995) 249–263.
- [104] J.L. Ridet, S.K. Malhotra, A. Privat, F.H. Gage, Reactive astrocytes: cellular and molecular cues to biological function, *Trends Neurosci.* 20 (1997) 570–577.
- [105] M. Rizzi, C. Perego, M. Aliprandi, C. Richichi, T. Ravizza, D. Colella, J. Veliskova, S.L. Moshe, M.G. De Simoni, A. Vezzani, Glia activation and cytokine increase in rat hippocampus by kainic acid induced status epilepticus during postnatal development, *Neurobiol. Dis.* 14 (2003) 494–503.
- [106] D.R. Rosen, T. Siddique, D. Patterson, et al., Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, *Nature* 362 (1993) 59–62.
- [107] J.D. Rothstein, Excitotoxicity hypothesis, *Neurology* 47 (1996) S19–S25.
- [108] J.D. Rothstein, M. Van Kammen, A.I. Levey, L.J. Martin, R.W. Kuncel, Selective loss of glial glutamate transporter GLT 1 in amyotrophic lateral sclerosis, *Ann. Neurol.* 38 (1995) 73–84.
- [109] N.J. Rothwell, S.J. Hopkins, Cytokines and the nervous system: II. Actions and mechanisms of action, *Trends Neurosci.* 18 (1995) 130–136.
- [110] L.P. Rowl, N.A. Shneider, Amyotrophic lateral sclerosis, *N. Engl. J. Med.* 344 (2001) 1688–1700.
- [111] I. Rozovsky, C.E. Finch, T.E. Morgan, Age related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down regulation, *Neurobiol. Aging* 19 (1998) 97–103.
- [112] J.C. Saez, D.C. Spray, E.L. Hertzberg, Gap Junctions: biochemical properties and functional regulation under physiological and toxicological conditions, *In Vitro Toxicol.* 3 (1990) 69–86.
- [113] J.I. Sagara, K. Miura, S. Bannai, Maintenance of neuronal glutathione by glial cells, *J. Neurochem.* 61 (1993) 1672–1676.
- [114] S. Sasaki, T. Komori, M. Iwata, Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis, *Acta Neuropathol. (Berl.)* 100 (2000) 138–144.
- [115] S. Sasaki, N. Shibata, T. Komori, M. Iwata, iNOS and nitrotyrosine immunoreactivity in amyotrophic lateral sclerosis, *Neurosci. Lett.* 291 (2000) 44–48.
- [116] S. Sasaki, H. Warita, K. Abe, M. Iwata, Inducible nitric oxide synthase (iNOS) and nitrotyrosine immunoreactivity in the spinal cords of transgenic mice with a G93A mutant SOD1 gene, *J. Neuropathol. Exp. Neurol.* 60 (2001) 839–846.
- [117] S. Sasaki, H. Warita, K. Abe, T. Komori, M. Iwata, EAAT1 and EAAT2 immunoreactivity in transgenic mice with a G93A mutant SOD1 gene, *NeuroReport* 12 (2001) 1359–1362.
- [118] D. Schiffer, S. Cordera, P. Cavalla, A. Migheli, Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis, *J. Neurol. Sci.* 139 (1996) 27–33.
- [119] A.F. Schinder, M. Poo, The neurotrophin hypothesis for synaptic plasticity, *Trends Neurosci.* 23 (2000) 639–645.
- [120] H.M. Schipper, Astrocytes, brain aging, and neurodegeneration, *Neurobiol. Aging* 17 (1996) 467–480.
- [121] J.L. Seeburger, S. Tarras, H. Natter, J.E. Springer, Spinal cord motoneurons express p75^{NTR} and p145^{trkB} mRNA in amyotrophic lateral sclerosis, *Brain Res.* 621 (1993) 111–115.
- [122] I. Selak, S.D. Skaper, S. Varon, Pyruvate participation in the low molecular weight trophic activity for central nervous system neurons and glia, *J. Neurochem.* 5 (1985) 23–28.
- [123] M. Sendtner, G. Pei, M. Beck, U. Schweizer, S. Wiese, Developmental motoneuron cell death and neurotrophic factors, *Cell Tissue Res.* 301 (2000) 71–84.
- [124] S.D. Skaper, L. Facci, A. Leon, Inflammatory mediator stimulation of astrocytes and meningeal fibroblasts induces neuronal degeneration via the nitridergic pathway, *J. Neurochem.* 64 (1995) 266–276.
- [125] W.D. Snider, Functions of the neurotrophins during nervous system development: what the knockouts are teaching us, *Cell* 77 (1994) 627–638.
- [126] V.C. Stewart, M.A. Sharpe, J.B. Clark, S.J.R. Heales, Astrocyte derived nitric oxide causes both reversible and irreversible damage to the neuronal mitochondrial respiratory chain, *J. Neurochem.* 75 (2000) 694–700.
- [127] H.J. Stuenkel, K. Kunze, Tissue nerve growth factor concentrations in neuromuscular diseases, *Eur. J. Neurol.* 5 (1998) 487–490.
- [128] M. Szatkowski, B. Barbour, D. Attwell, Non vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake, *Nature* 348 (1999) 443–446.
- [129] W. Tetzlaff, M.B. Graeber, M.A. Bisby, G.W. Kreutzberg, Increased glial fibrillary acidic protein synthesis in astrocytes during retrograde reaction of the rat facial nucleus, *Glia* 1 (1988) 90–95.
- [130] M. Tortorolo, P. Veglianesi, N. Calvaresi, A. Botturi, C. Rossi, A. Giorgini, A. Migheli, C. Bendotti, Persistent activation of p38 mitogen activated protein kinase in a mouse model of familial amyotrophic lateral sclerosis correlates with disease progression, *Mol. Cell. Neurosci.* 23 (2003) 180–192.
- [131] D. Trotti, D. Rossi, O. Gjesdal, L.M. Levy, G. Racagni, N.C. Danbolt, A. Volterra, Peroxynitrite inhibits glutamate transporter subtypes, *J. Biol. Chem.* 271 (1996) 5976–5979.
- [132] B.J. Turner, I.K. Cheah, K.J. Macfarlane, E.C. Lopes, S. Petratos, S.J. Langford, S.S. Cheema, Antisense peptide nucleic acid mediated knockdown of the p75 neurotrophin receptor delays motor neuron disease in mutant SOD1 transgenic mice, *J. Neurochem.* 87 (2003) 752–763.
- [133] G. Ugolini, C. Raoul, A. Ferri, C. Haenggeli, Y. Yamamoto, D. Salaun, C.E. Henderson, A.C. Kato, B. Pettmann, A.O. Hueber, Fas/tumor necrosis factor receptor death signaling is required for axotomy induced death of motoneurons in vivo, *J. Neurosci.* 23 (2003) 8526–8531.
- [134] B.A. Urschel, C.E. Hulsebosch, Distribution and relative density of p75 nerve growth factor receptors in the rat spinal cord as a function of age and treatment with antibodies to nerve growth factor, *Brain Res. Dev. Brain Res.* 69 (1992) 261–270.
- [135] P. Valdo, C. Stegagno, S. Mazzucco, E. Zuliani, G. Zanusso, G. Moretto, C.S. Raine, B. Bonetti, Enhanced expression of NGF

- 1146 receptors in multiple sclerosis lesions, *J. Neuropathol. Exp. Neu-*
1147 *rol.* 61 (2002) 91–98.
- 1148 [136] C.E. Van der Zee, T. Hagg, p75NGFR mediates death of cholinergic
1149 neurons during postnatal development of the neostriatum in mice,
1150 *J. Chem. Neuroanat.* 14 (1998) 129–140.
- 1151 [137] A. Volterra, D. Trotti, C. Tromba, S. Floridi, G. Racagni, Glutamate
1152 uptake inhibition by oxygen free radicals in rat cortical astrocytes,
1153 *J. Neurosci.* 14 (1994) 2924–2932.
- 1154 [138] A.P. Wagner, G. Reck, D. Platt, Evidence that V+ fibronectin, GFAP
1155 and S100 beta mRNAs are increased in the hippocampus of aged
1156 rats, *Exp. Gerontol.* 28 (1993) 135–143.
- [139] S. Wiese, F. Metzger, B. Holtmann, M. Sendtner, The role of 1157
p75NTR in modulating neurotrophin survival effects in developing 1158
motoneurons, *Eur. J. Neurosci.* 11 (1999) 1668–1676. 1159
- [140] W. Wu, Potential roles of gene expression change in adult rat spinal 1160
motoneurons following axonal injury: a comparison among c-jun, 1161
off-affinity nerve growth factor receptor (LNGFR), and nitric oxide 1162
synthase (NOS), *Exp. Neurol.* 141 (1996) 190–200. 1163
- [141] Q. Yan, E.M. Johnson Jr., An immunohistochemical study of the 1164
nerve growth factor receptor in developing rats, *J. Neurosci.* 8 (1988) 1165
3481–3498. 1166

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