Aging and Neurodegenerative Diseases: Models and Assessment of the Impact and Responses to ROS / RNS

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Overview

Discussion of Alzheimer's disease
and amyotrophic lateral sclerosis (ALS)

Methods for studying oxidative stress with examples
From recent human and animal studies

What the animal models are beginning to tell us
about the relationship of oxidative stress
to neuroinflammation

Oxidative Damage in Neurological Disease

Implicated in:	rodent models:
Alzheimer's disease (AD)	Several genetic models *e.g.* Tg2576, APP/PS1 mice
Amyotrophic lateral sclerosis (ALS, Lou Gherig's disease)	G93A-SOD1, G85R SOD1, other SOD1 mice; ALS2 mouse peripherin mouse
Huntington's disease	R6/2 mouse, 3-nitropropionate
Parkinson's disease	MPTP induced lesions; LPS-induction models
Stroke	Gerbil, rat models for carotid and MCAO occlusion

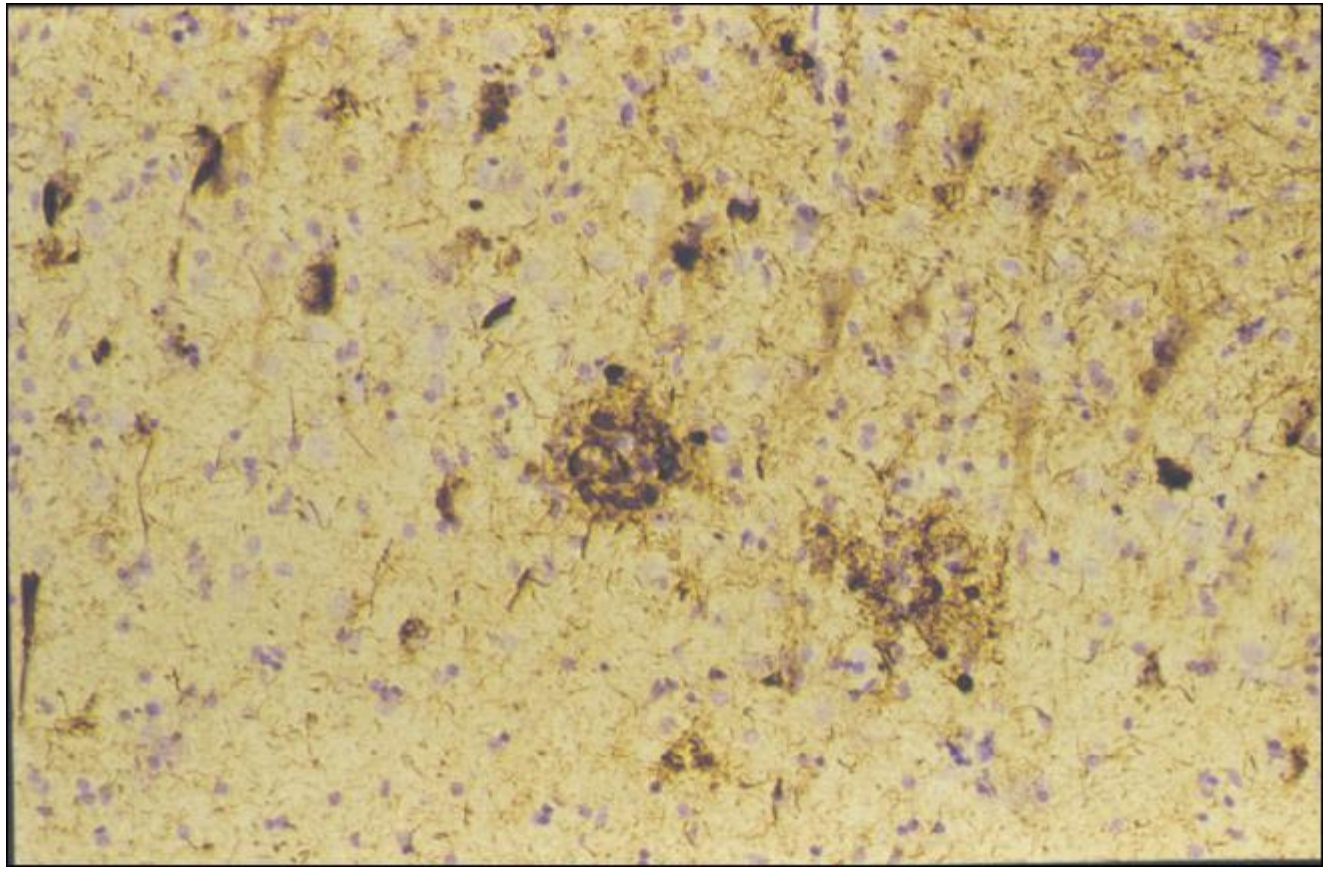
Alzheimer's Disease

Characterized by amyloid protein deposition in plaques and by intraneuronal inclusions of various proteins (*eg.* hyperphosphorylated tau)

Glial activation around plaques, and associated neuron damage / death

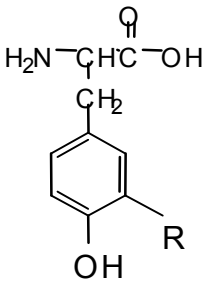
Region-specific accumulation of oxidative damage that correlates with histopathology

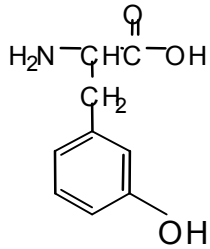
Histopathology of AD: Plaques and Tangles

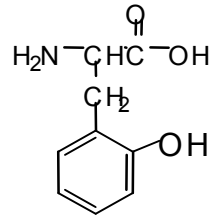


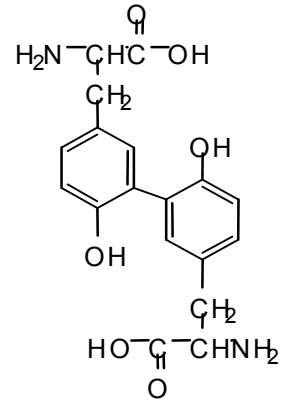
Histochemistry: anti-phospho-p38 / cresyl violet

Oxidative damage to brain can be measured (sometimes) using HPLC with electrochemical detection







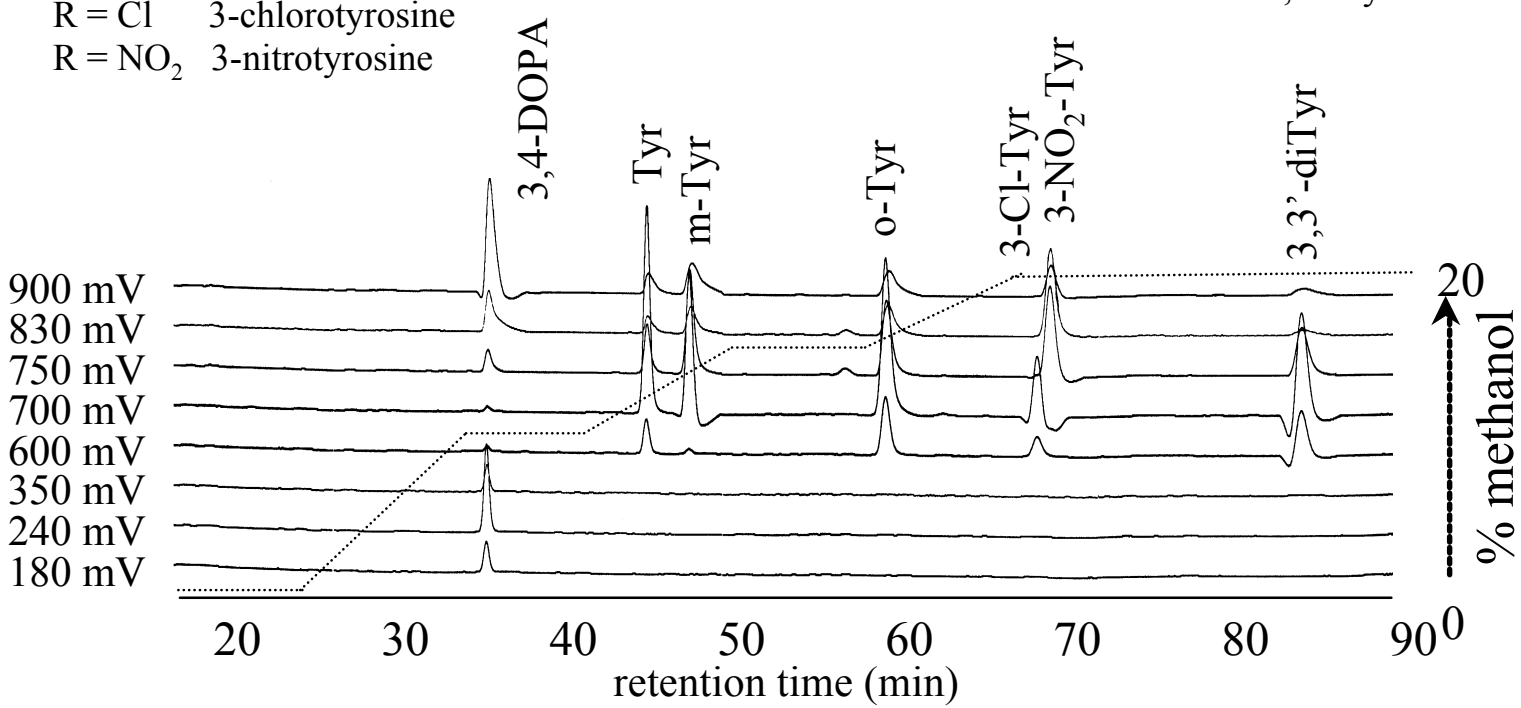


R = H tyrosine
R = OH 3,4-DOPA
R = Cl 3-chlorotyrosine
R = NO_2 3-nitrotyrosine

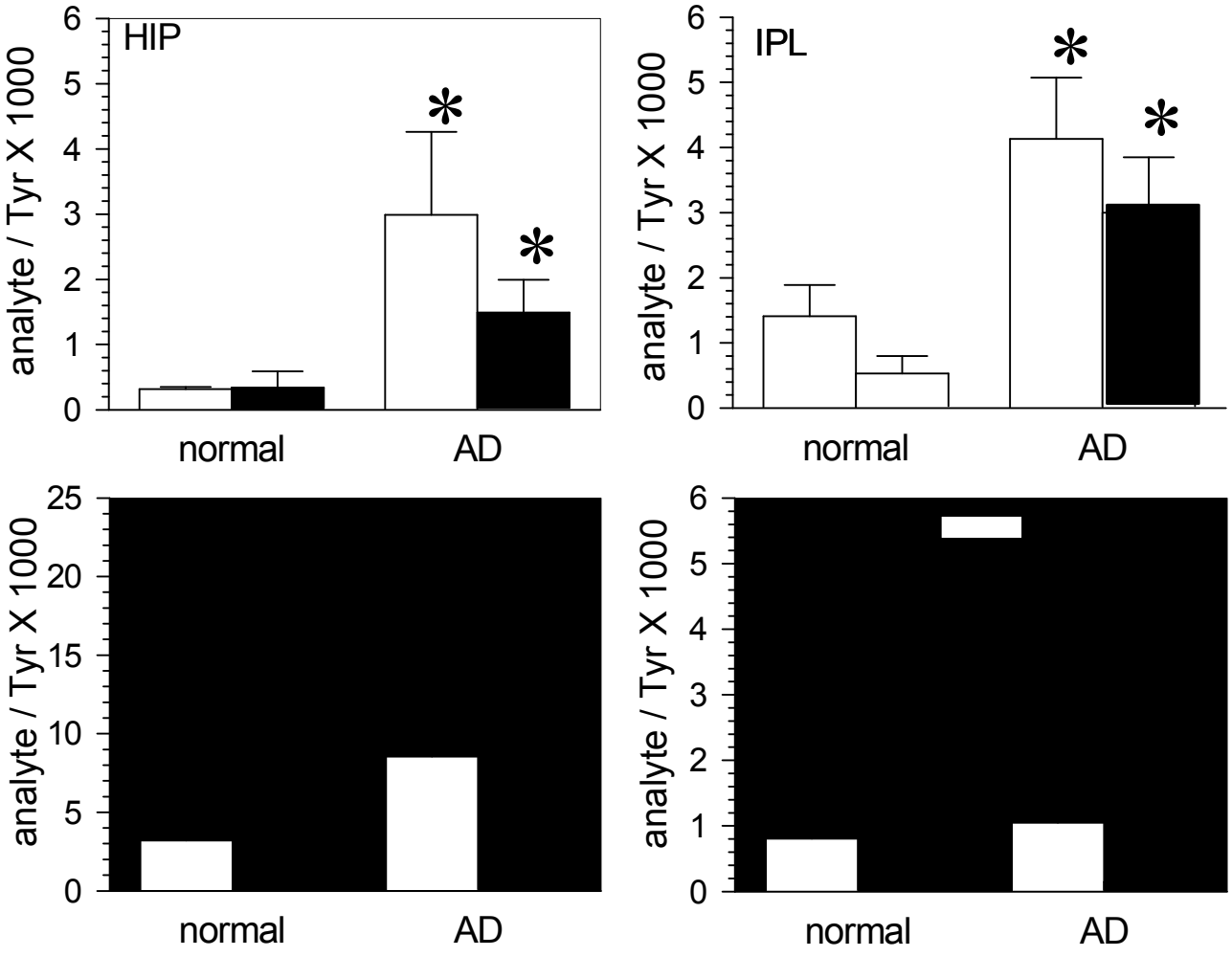
m-tyrosine

o-tyrosine

3,3-dityrosine



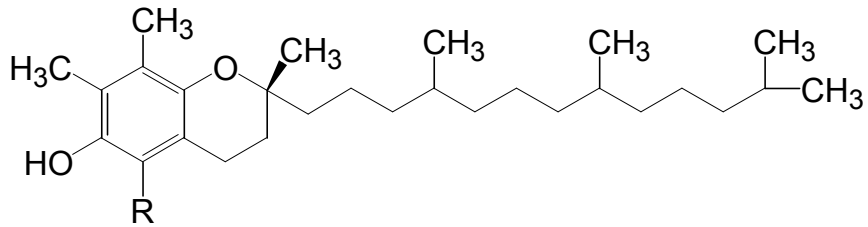
Protein 3-NO_2-Tyr is Elevated Region-Selectively in Human AD Brain

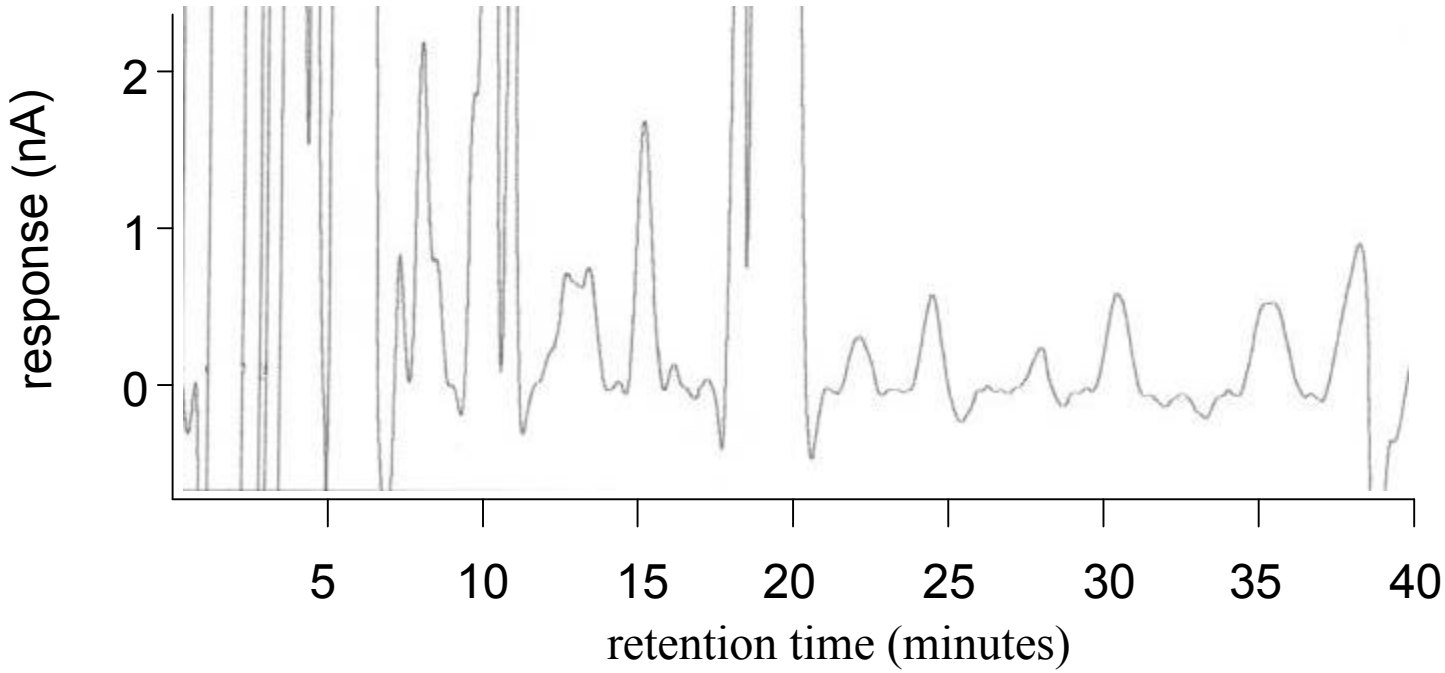


Hensley et al., J. Neurosci. **18**: 8126-8132 (1998)

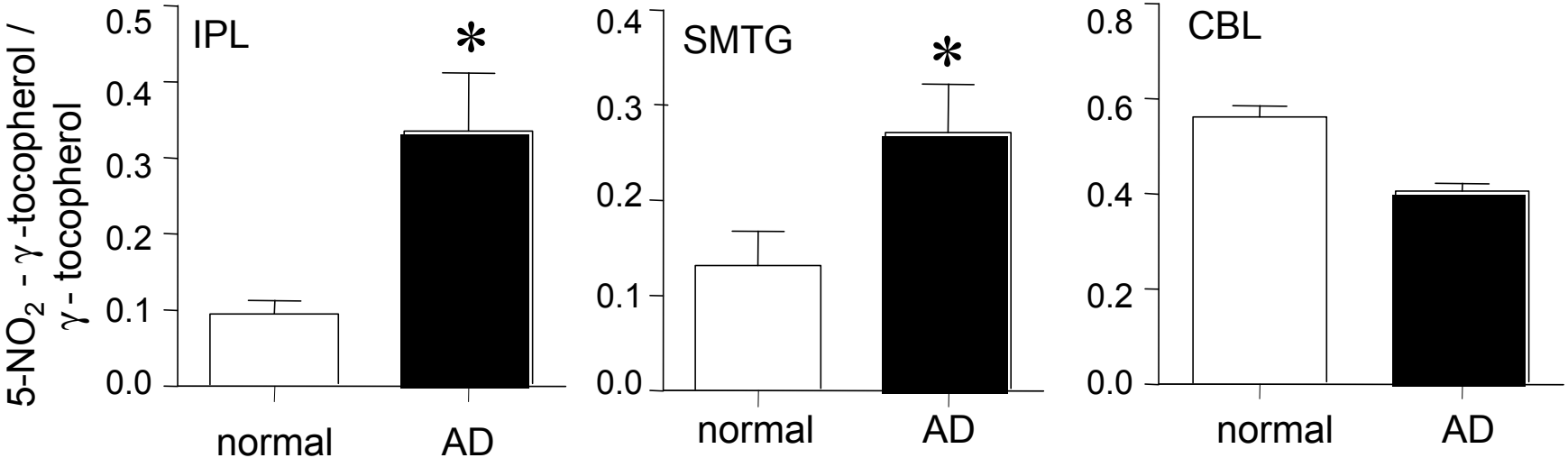
Lipid phase nitration: 5-NO_2-g-tocopherol can be detected in AD brain tissue using HPLC-ECD

R = CH_3 α-tocopherol
R = H γ-tocopherol
R = NO_2 5-NO_2-γ-tocopherol



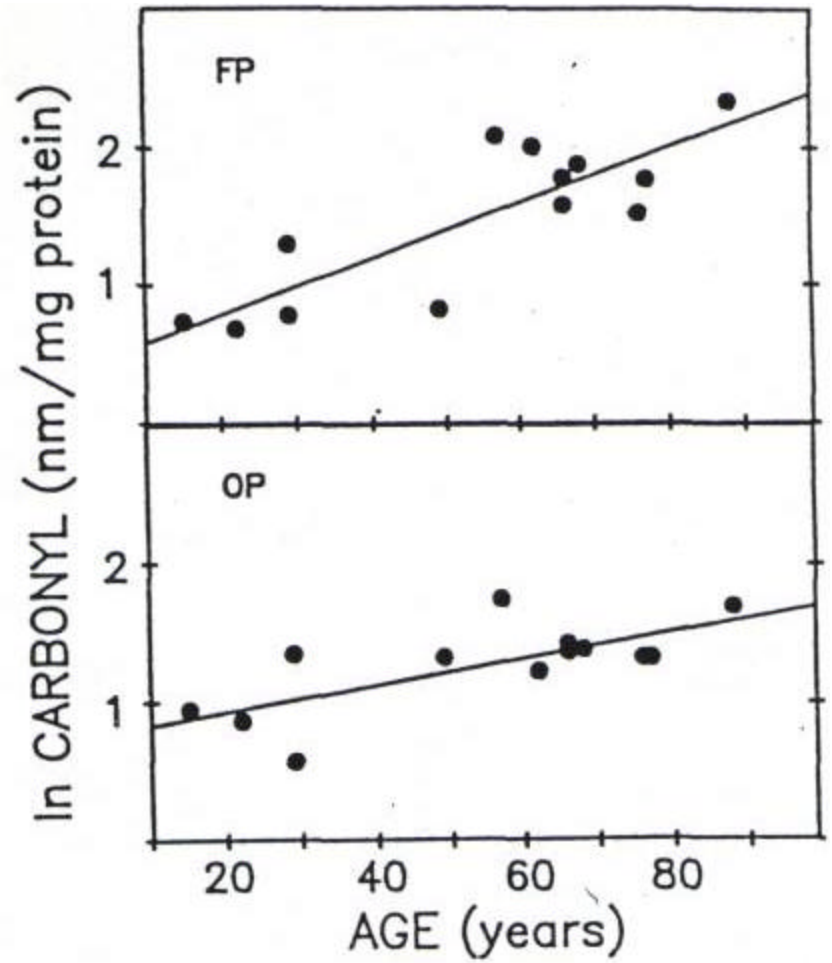


5-NO_2-g-tocopherol is elevated in the AD brain in a region-selective manner that correlates with 3-NO_2-Tyr elevations



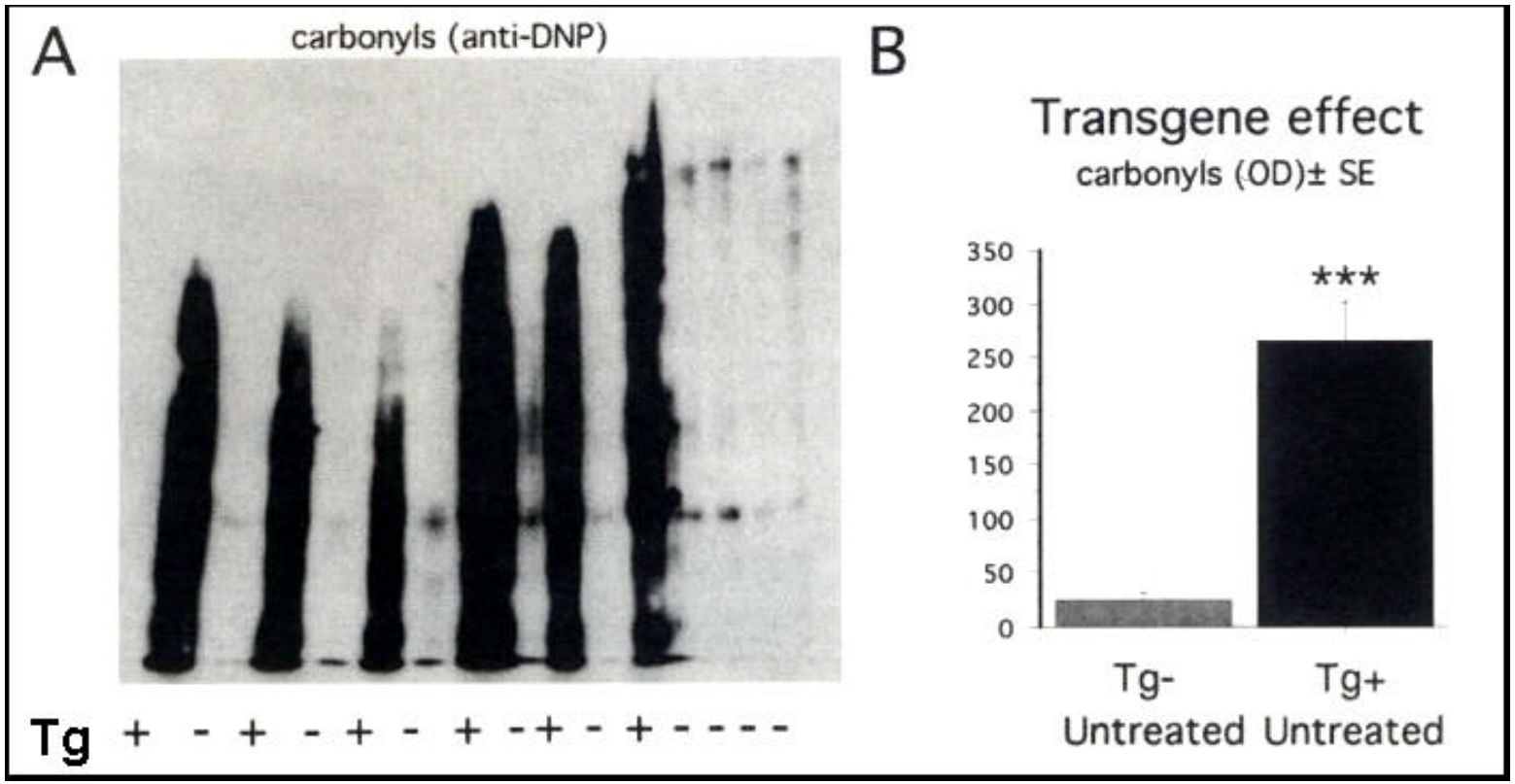
Williamson *et al., Nitric Oxide: Biol Chem*, **6**: 221-227 (2002)

Brain protein carbonyl load increases with age and further increases in Alzheimer's diseased cortex



Smith et al., Proc. Natl. Acad. Sci. USA **88**: 10540-10543 (1991)

Mouse models of AD partially reproduce the oxidative damage aspect of the disease



Lim *et al., J. Neurosci*. **21**: 8370-8377 (2001)

Oxidative damage in humans and animal models of ALS

ALS is a fatal motor neuron disease causing death of neurons in the spinal cord, brainstem and motor cortex.

It is essentially untreatable (+6 month life extension with the NMDA receptor antagonist riluzole).

Prognosis: Progressive paralysis followed by death in 3-5 years. Death is usually by pneumonia and near complete paralysis.

ALS may be sporadic or familial

About 20 % of all ALS cases are heritable

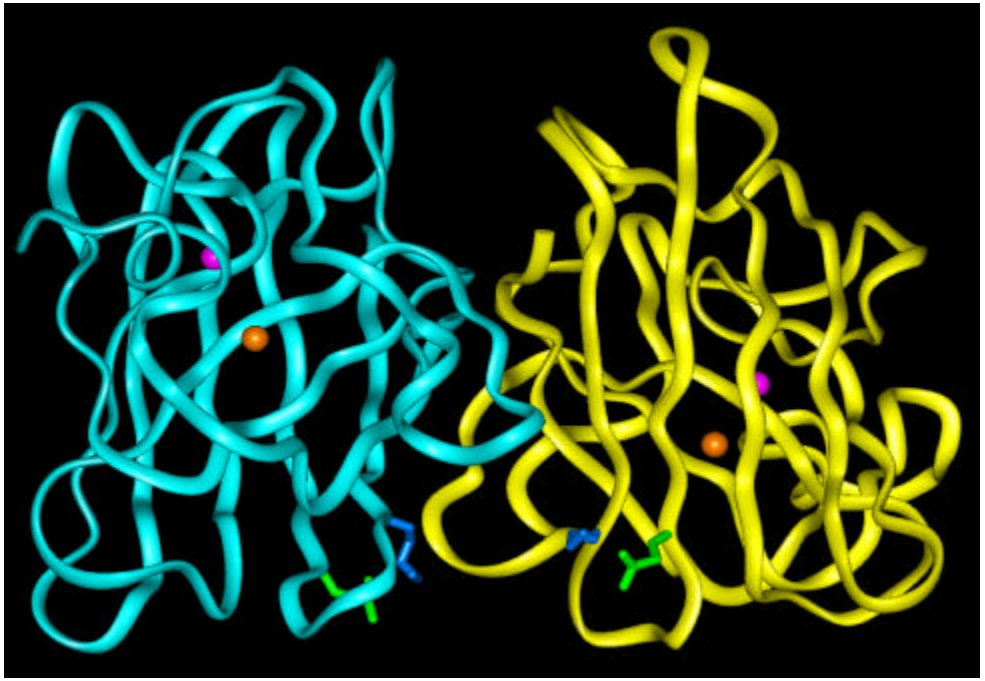
Of these, 20-30% are caused by gain-of-toxic Function mutations in Cu,Zn-SOD (SOD1)

[Deng *et al. Science* **20**: 1047-1051 (1993)]

$$2O_2^{\bullet -} + 2H^+ \xrightarrow[\text{SOD1}]{} H_2O_2 + O_2$$

SOD1 normally detoxifies ROS; it is unclear what is the toxic gain-of-function associated with mutant SOD1.

Cu,Zn-Superoxide Dismutase (SOD1)



SOD1 knockout mice are viable; no CNS disease.
Human wild-type SOD1 over-expressing mice are healthy

Mutant SOD1 causes ALS-like disease in mice when ubiquitously expressed but not when expression is specifically targeted to neurons (Pramatarova *et al.* 2001; Lino *et al.* 2002)

Why do mutant SOD1 enzymes cause motor neuron disease?

SOD1 mutants have increased peroxidase activity and convert H_2O_2 to $^{\bullet}OH$ (Valentine and Bredesen 1996; Yim *et al.* 1997)

Mutant SOD1 lose metals easily. Metal deficient enzymes promote protein nitration and render neurons susceptible to apoptosis (Crow, Beckman *et al.* 1997; Estevez *et al.* 1999)

SOD1 mutants aggregate inside neurons, contribute to toxicity (Bruijn *et al.* 1998)

Alternative Explanations

Maybe SOD1 mutants exert their pathogenic effects through non-neuronal cells.

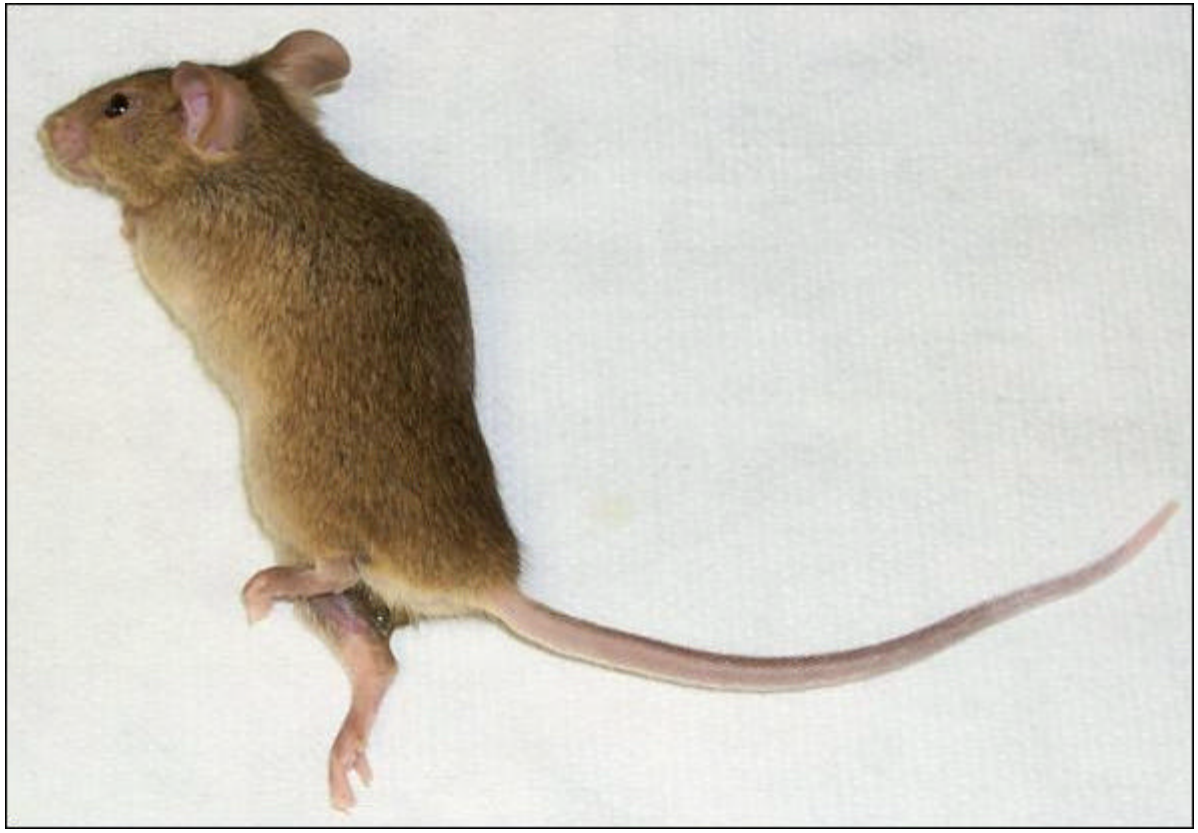
astrocytes

microglia

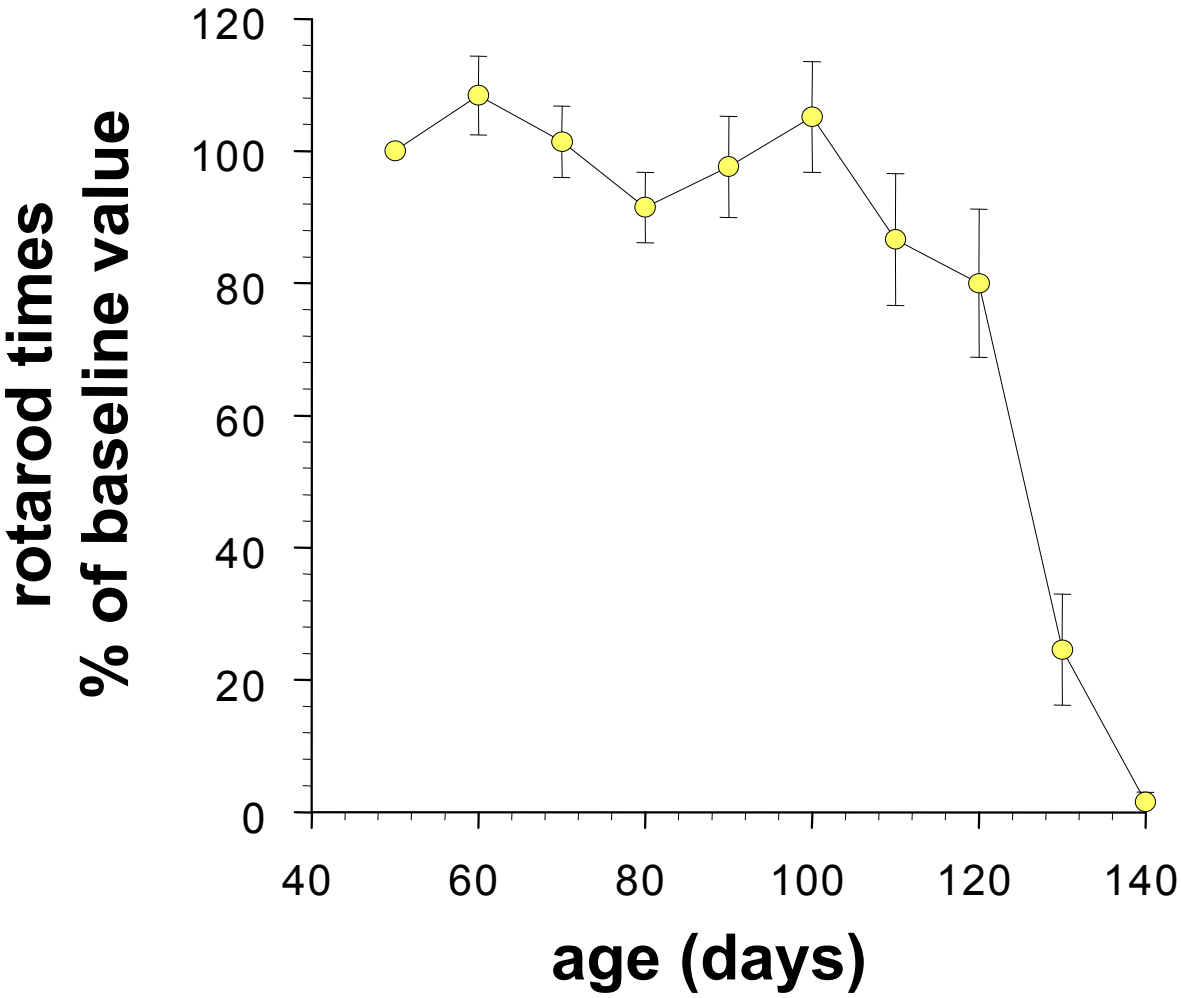
→ **Neuroinflammation:**

- **ROS, RNS**
- **cytokines**
- **apoptosis initiators**
- **limited involvement of lymphocytes**

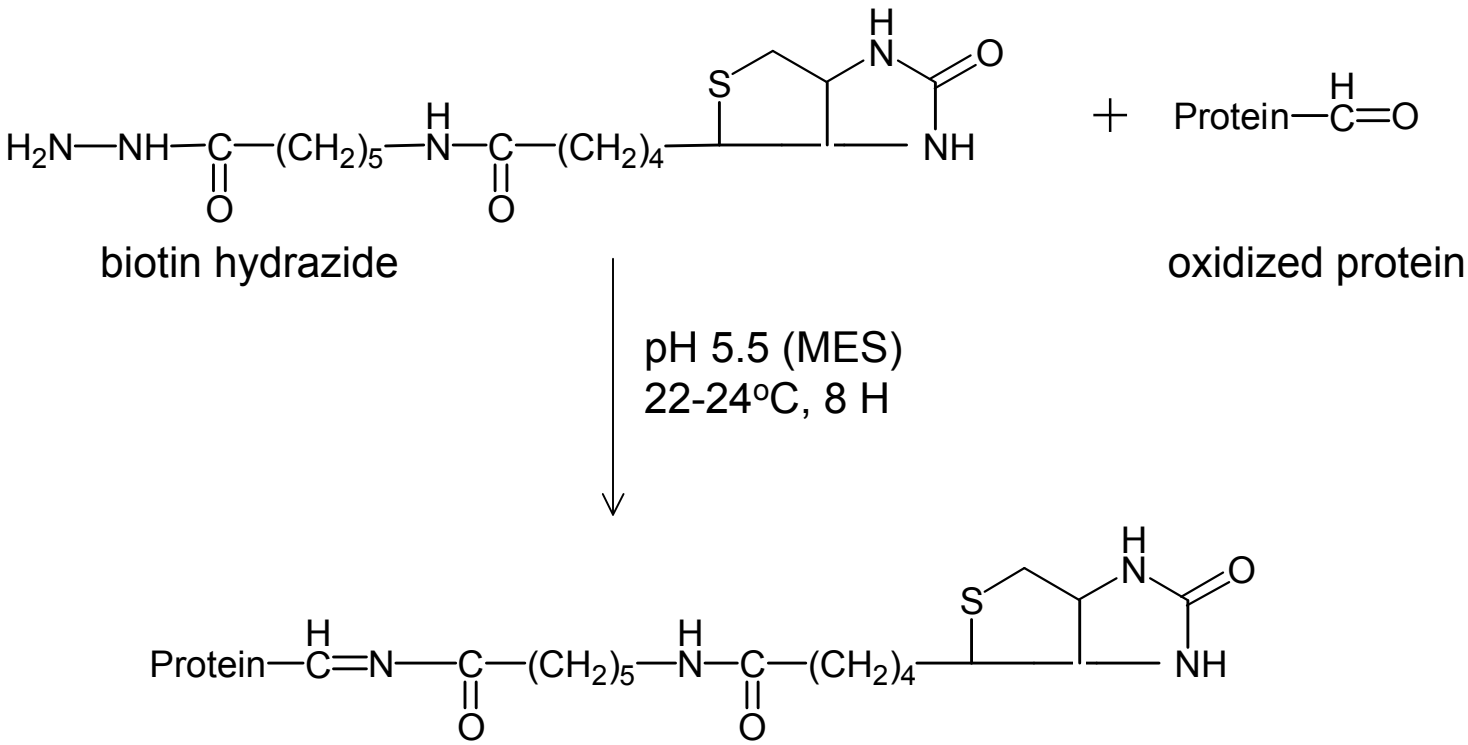
Transgenic mice expressing the G93A-SOD1 mutation develop ALS-like disease



G93A-SOD1 mutant mice experience progressive decline in motor function



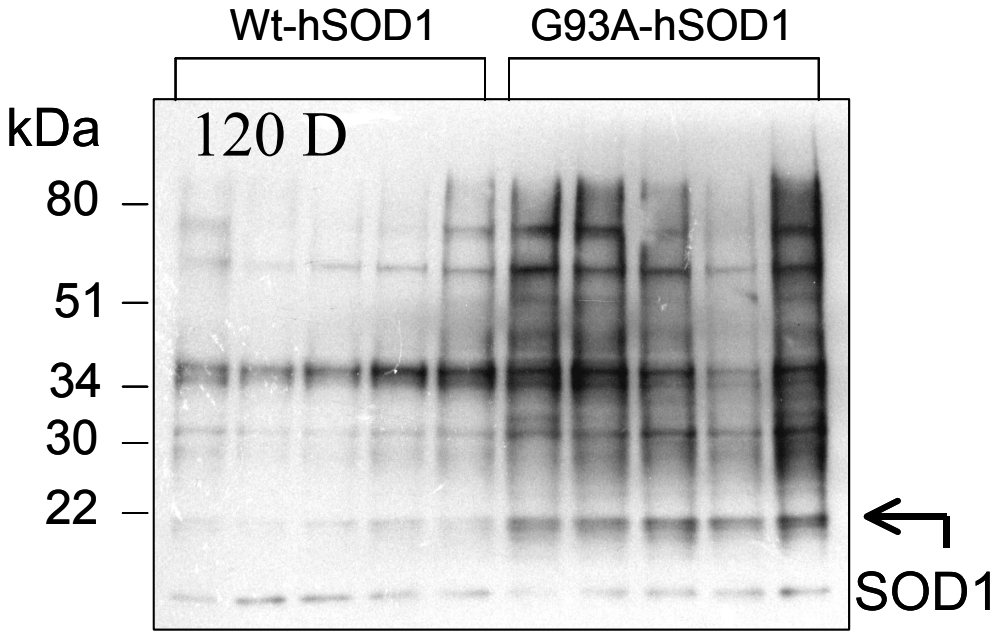
Protein carbonyl assessment with biotin hydrazide as developed to study protein oxidation in ALS

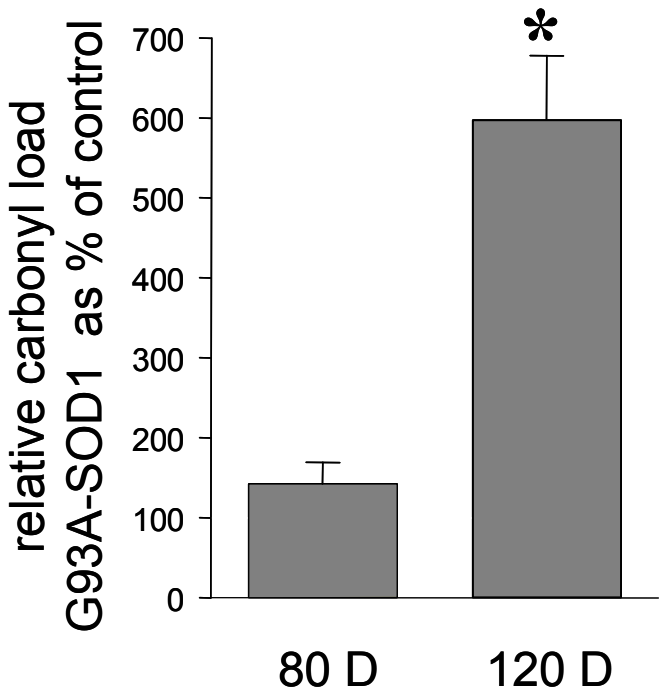


stable hydrazone derivative, detectable with streptavidin-HRP and standard chemiluminescence reagents

Hensley *et al., J. Neurochem*. **82**: 365-374 (2002)

Protein carbonyl levels are increased in the G93A-SOD1 mouse spinal cord at 120 D





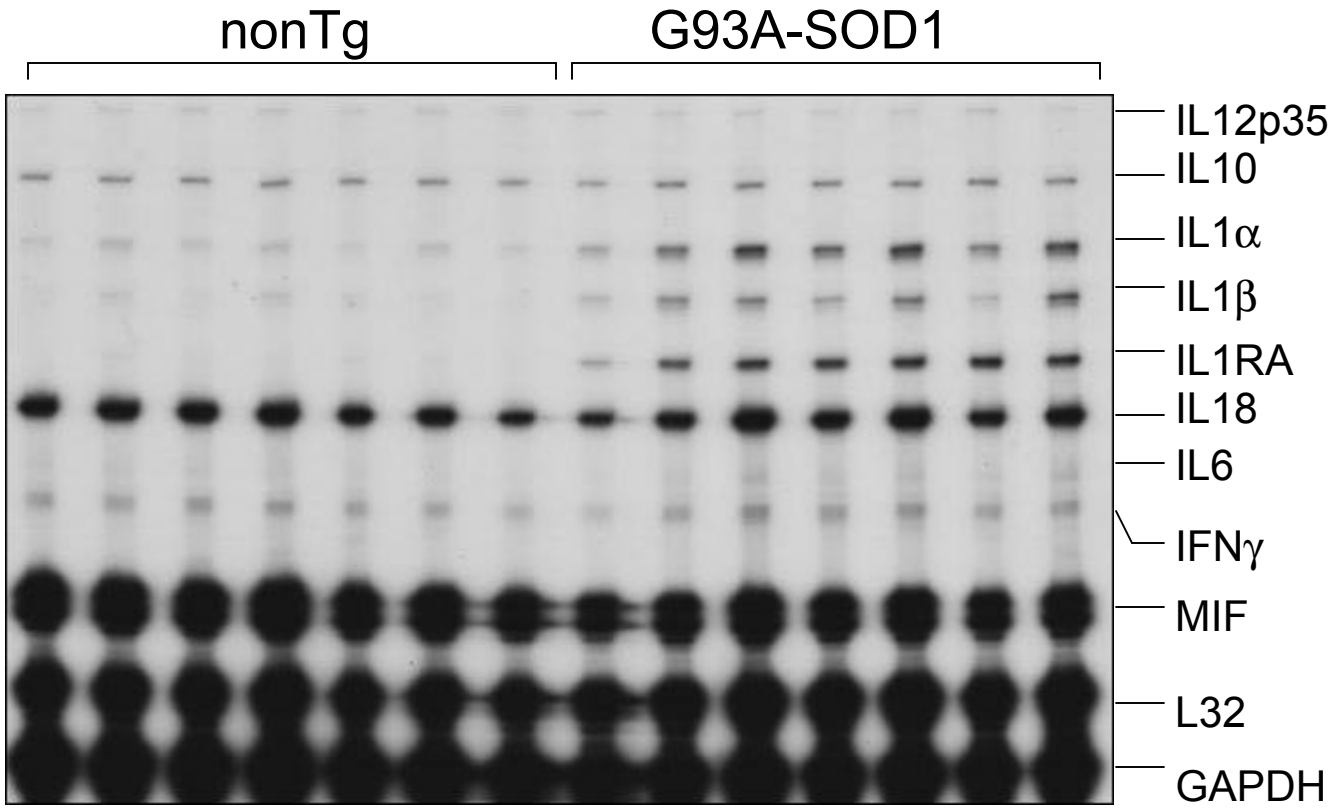
Hensley *et al., J. Neurochem*. **82**: 365-374 (2002)
Andrus, Fleck and Gurney *J. Neurochem*. **71:** 2041-2048 (1998)

Where are these ROS coming from?

Hypothesis:

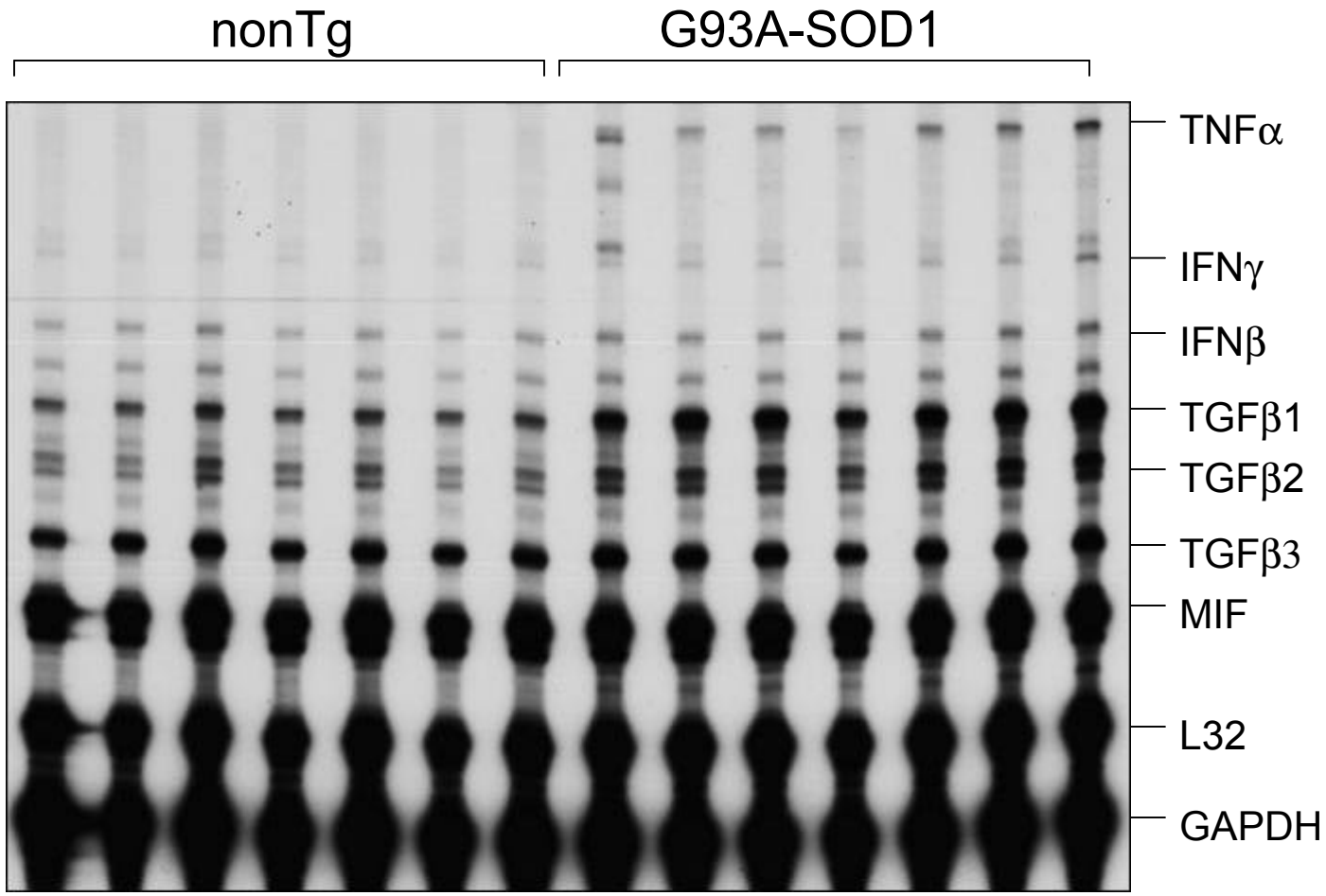
Activated microglia, dysregulated cytokine networks and the neuroinflammatory process

RPA analysis indicates broad-spectrum elevations of cytokine messages at 120D in G93A-SOD1 mice



Hensley *et al., J. Neurochem*. 82: 365-374 (2002)

TNFa and TGFb1/2 are also upregulated at 120 D



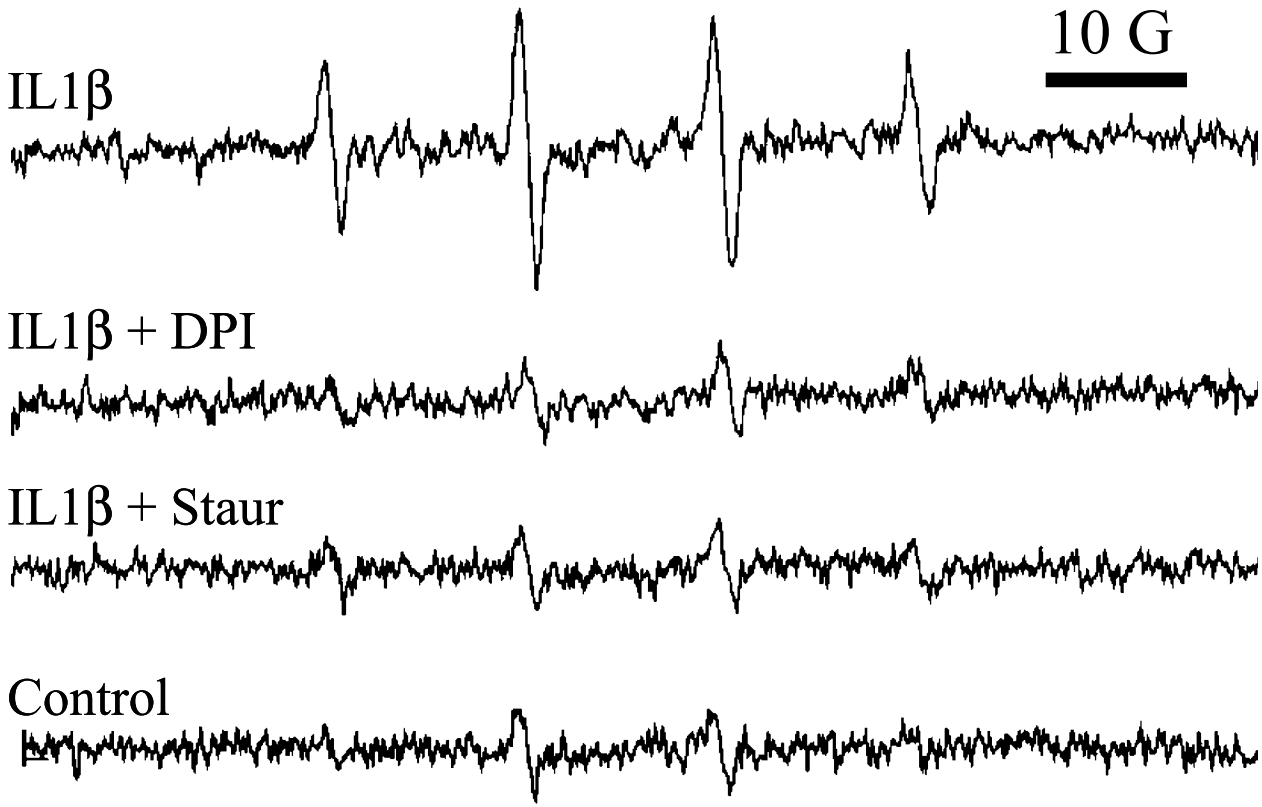
Glia-activating cytokines are increased in G93A-SOD1 mice in an age-dependent fashion

	difference at 80 D	difference at 120 D	
	% of nonTg	% of wt-hSOD	% of nonTg
TNFα	152*		717*
TNFRI	164*		333*I
IL1α	217*	397*	294*
IL1β	178*	760*	183*
IL1RA	355*	2085*	415*
IFNγ			147*
IL6	ND	155*	ND
IL10	133	117	127
IL12-p35	235*	135*	131
IL18	158*	99	107
MIF	116*	73*	89
IL2	ND		90
IL3	ND		99
IL4	ND		123

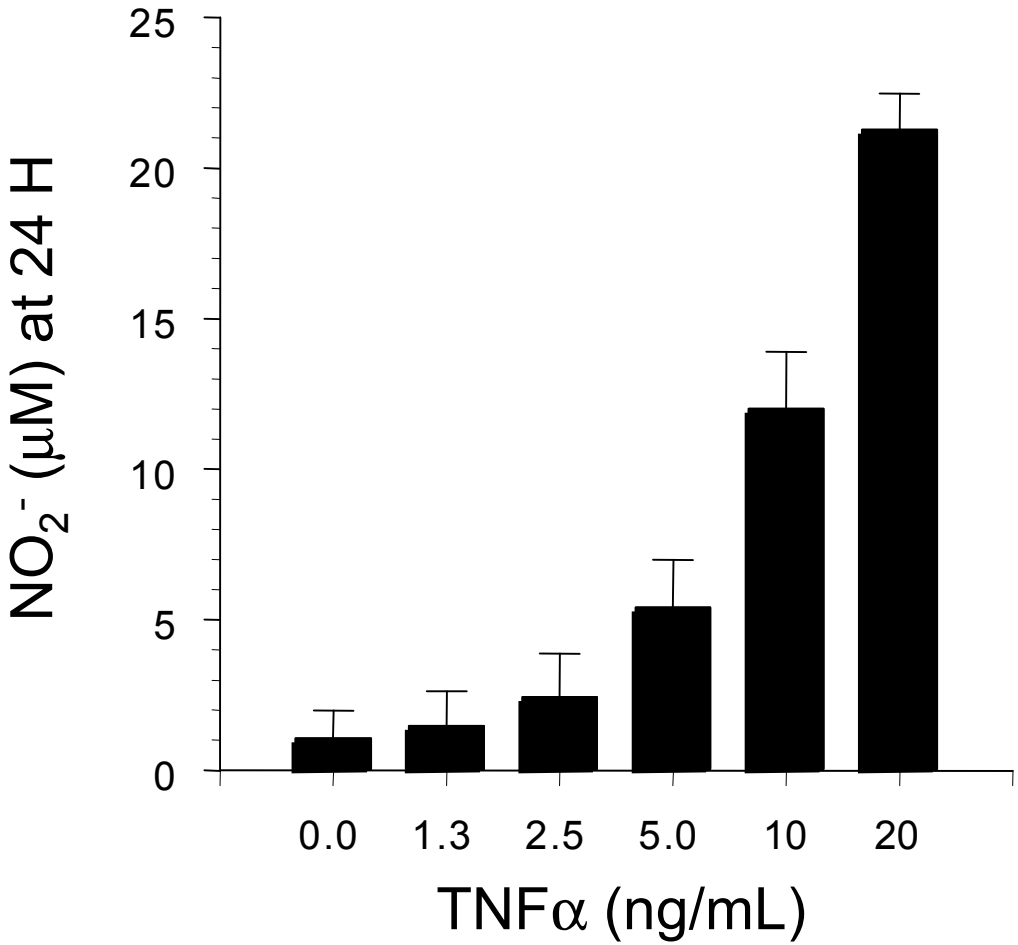
Cytokines and chemokines are elevated at the protein level in G93A-SOD1 mouse spinal cord

	pg / mg protein		
Analyte	NonTg	G93A-SOD1	% increase
TNFα	42 ± 7	65 ± 3*	55
IFNγ	1063 ± 96	1500 ± 91*	41
IL6	488 ± 64	740 ± 115*	52
IL1α	0.54 ± 0.10	1.0 ± 0.10*	85
IL1β	120 ± 18	164 ± 26*	37
IL2	456 ± 30	744 ± 38*	63
IL3	6.2 ± 1.2	8.9 ± 1.1*	44
IL4	1.9 ± 0.2	2.3 ± 0.1*	21
IL5	438 ± 92	595 ± 26*	36
IL10	515 ± 71	640 ± 35*	24
IL12p40	4.6 ± 0.5	5.9 ± 0.8*	28
IL12p70	9.4 ± 1.9	13.0 ± 1.6*	38
IL17	2.9 ± 0.22	3.1 ± 0.38	7
KC	5.8 ± 1.1	8.9 ± 1.5*	53
MIP-1α	248 ± 43	323 ± 41*	30
RANTES	17 ± 3	34 ± 6*	100
GM-CSF	1055 ± 54	1113 ± 57	5

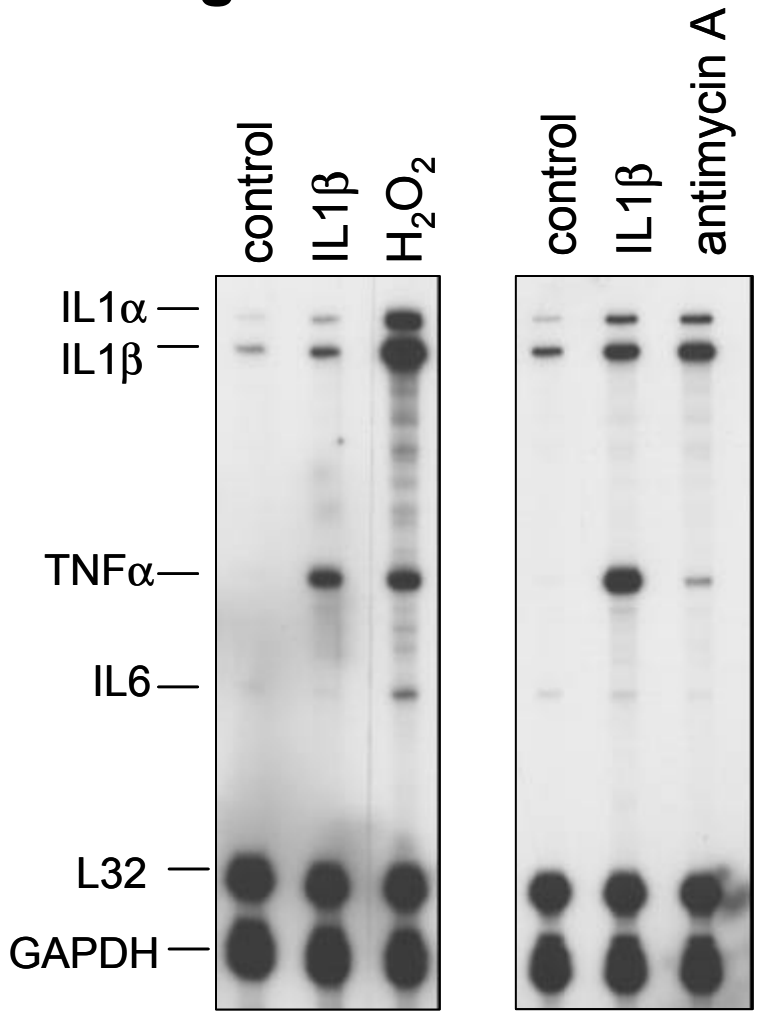
Cytokines induce ROS (I):
IL1b Treatment of mixed glial cell culture



Cytokines induce ROS (II): TNFa treatment of Walker EOC-20 microglia

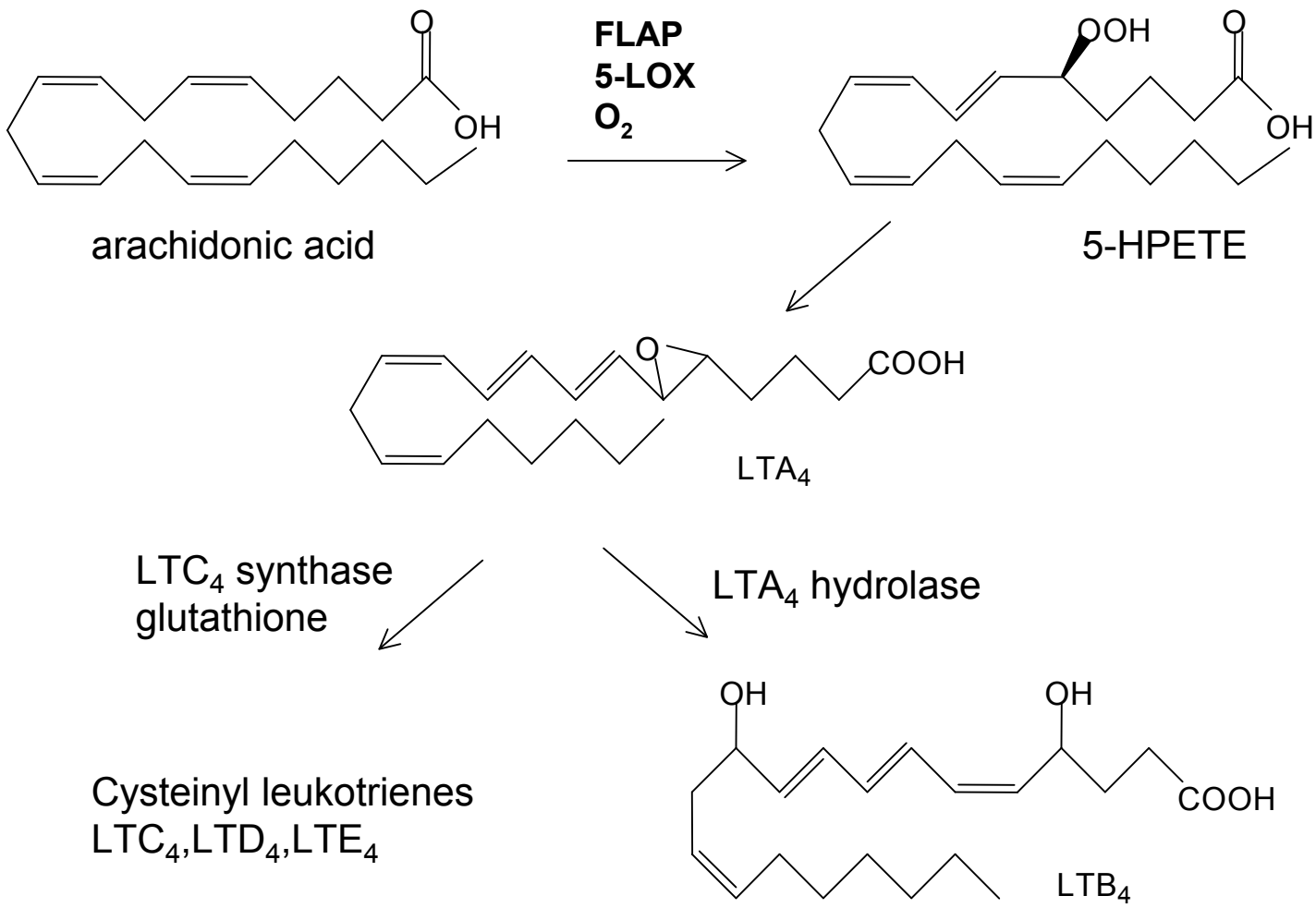


Conversely: ROS can induce cytokine transcription in glial cell cultures

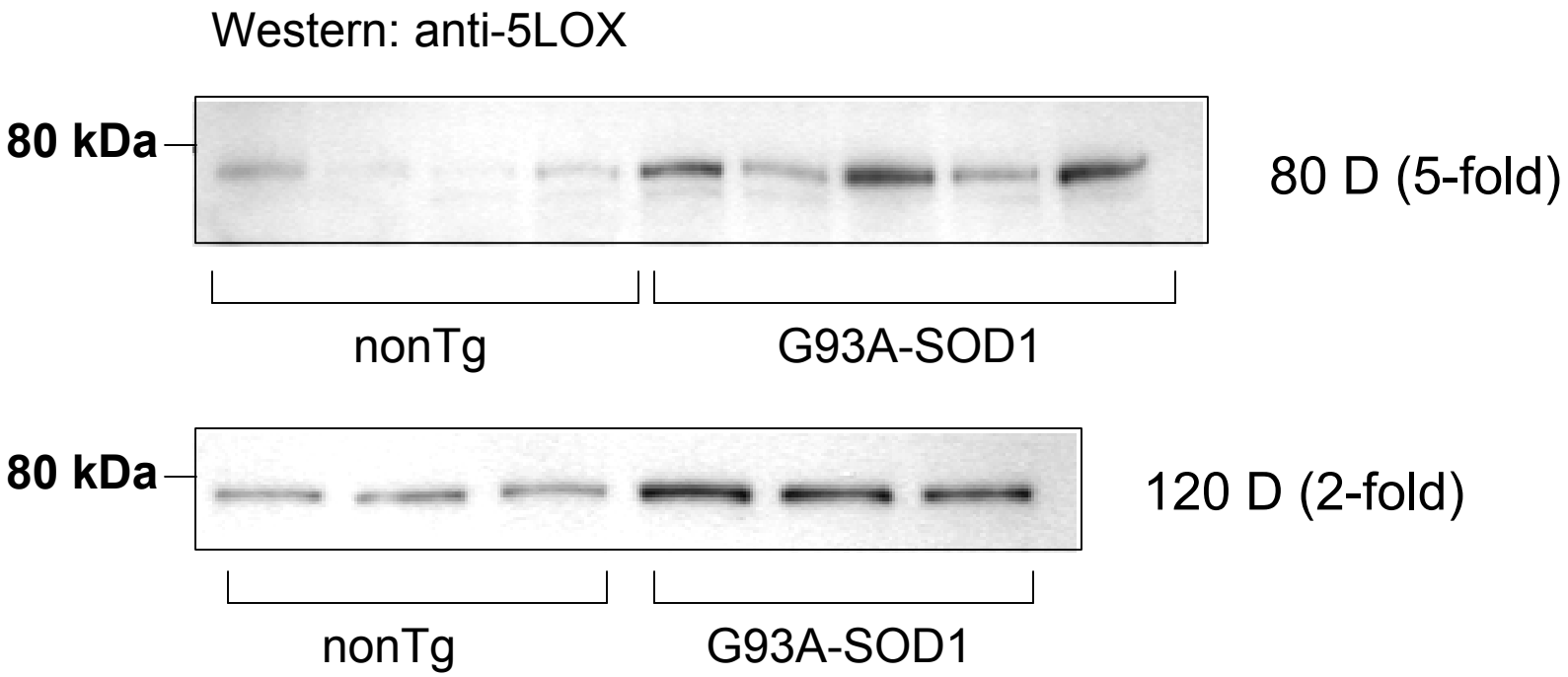


Gabbita *et al., Arch. Biochem. Biophys.* **376***:* 1-13 (2000)

Other sources of ROS in ALS: Arachidonic acid metabolism



5-LOX is upregulated in G93A-SOD1 mouse spinal cord



Summary

Oxidative stress occurs in the central nervous system (CNS) during aging and disease

Very useful and faithful genetic models exist for SOME aspects of human CNS disease

There is a close, perhaps non-dissociable relationship between oxidative stress and dysfunctional cytokine networks

The G93A-SOD1 mouse is an especially attractive model system for the study of these issues.

Future directions

We need more tools!

Animal genetic manipulations: To dissect the biochemistry

New bioanalytical technologies : To analyze the problem and assess the models

Especially we need attention to the protein chemistry and protein-protein interactions

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References and Recommended Reading

Andrus, P.K.; Fleck, T.J.; Gurney, M.E.; Hall, E.D. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem*. **71**: 2041-2048; 1998

Bruijn, L.I.; Houseweart, M.K.; Kato, S.; Anderson, K.L.; Anderson, S.D.; Ohama, E.; Reaume, A.G.; Scott, R.W.; Cleveland, D.W. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**:1851-1854; 1998.

Crow, J.P.; Sampson, J.B.; Zhuang, Y.; Thompson, J.A.; Beckman, J.S. Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. *J. Neurochem*. **69**: 1936-1944; 1997.

Estevez, A.G.; Crow, J.P.; Sampson, J.B.; Reiter, C.; Zhuang, Y.; Richardson, G.J.; Tarpey, M.M.; Barbeito, L.; Beckman, J.S. Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. *Science* **286**: 2498-2500; 1999.

Gabbita, S.P.; Robinson, K.A.; Stewart, C.A.; Floyd, R.A.; Hensley, K. Redox regulatory mechanisms of cellular signal transduction. *Arch. Biochem. Biophys.* 376: 1-13; 2000. Lim, G.P.; Chu, T.; Yang, F.; Beech, W.; Frautschy, S.A.; Cole, G.M. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J. Neurosci*. **21**: 8370-8377; 2001.

Deng, H.X.; Hentati, A.; Tainer, J.A.; Iqbal, Z.; Cayabyab, A.; Hung, W.Y.; Getzoff, E.D.; Hu, P.; Herzfeldt, B.; Roos, R.P.; Warner, C.; Deng, G.; Soriano, E.; Smyth, C.; Parge, H.E.; Ahmed, A.; Roses, A.D.; Hallewell, R.A.; Pericak-Vance, M.A.; Siddique, T. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science* **20**: 1047-1051; 1993.

Hensley, K**.**, Floyd, R.A., Gordon, B., Mou, S., Pye, Q.N., Stewart, C.A., West, M., Williamson, K.S. Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *J Neurochem*, **82**:365-374, 2002.

Hensley; K.; Maidt; M.L.; Yu; Z.Q.; Sang; H.; Markesbery; W.R.; Floyd; R.A. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation*. J. Neurosci*. **18**: 8126-8132; 1998.

Hensley, K.; Hall, N.; Subramaniam, R.; Cole, P.; Harris, M.; Aksenov, M.; Aksenova, M.; Gabbita, S.P.; Wu, J.F.; Carney, J.M.; Lovell, M.; Markesbery, W.R.; Butterfield, D.A. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J. Neurochem*. **65**: 2146-2156; 1995.

Lino, M.M.; Schneider, C.; Caroni, P. Accumulation of SOD1 mutants in postnatal neurons does not cause motor neuron pathology or motoneuron disease. *J. Neurosci*. **22**: 4825-4832; 2002.

Pramatarova, A.; Laganiere, J.; Roussel, J.; Brisebois, K.; Rouleau, G.A. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci* **21**: 3369-3374; 2001.

Smith, C.D.; Carney, J.M.; Starke-Reed, P.E.; Oliver, C.N.; Stadtman, E.R.; Floyd, R.A.; Markesbery, W.R. Excess protein oxidation and enzyme dysfunction in normal aging and in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **88**: 20540-20543; 1991.

Williamson; K.S.; Gabbita; S.P.; Mou; S.; West; M.; Pye; Q.N.; Markesbery; W.R.; Cooney; R.V.; Grammas; P.; Reimann-Philipp; U.; Floyd; R.A.; Hensley; K**.** The nitration product 5-nitro-gamma-tocopherol is increased in the Alzheimer brain. *Nitric Oxide: Biol Chem*; **6**:221-227; 2002.

Yim, H-S.; Kang, J-H.; Chock, P.B.; Stadtman, E.R.; Yim, M.B. A familial amyotrophic lateral sclerosis-associated A4V Cu,Zn-Superoxide dismutase mutant has a lower Km for hydrogen peroxide. Correlation between clinical severity and the Km value. *J. Biol. Chem*. **272**: 8861-8863; 1997.