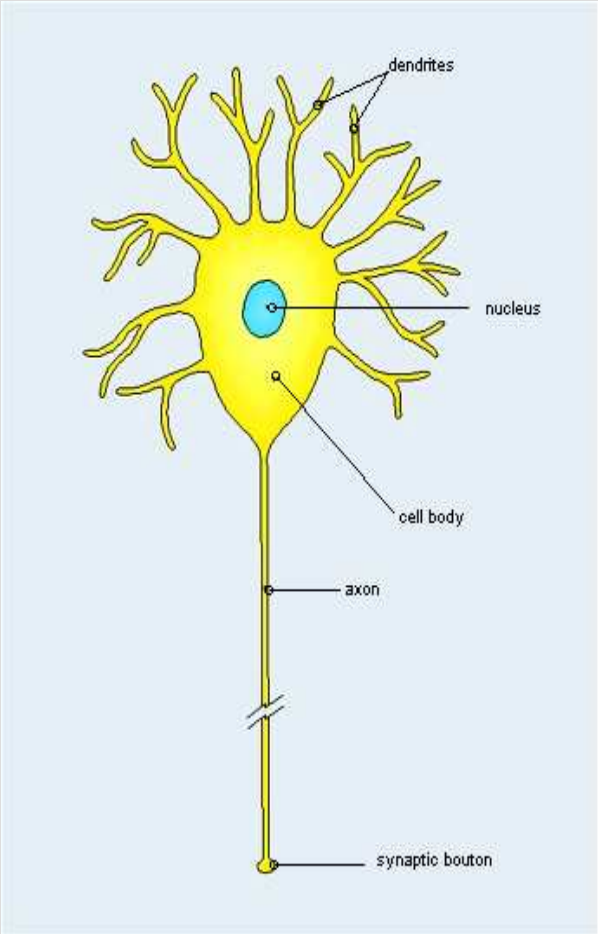
TE nervous system

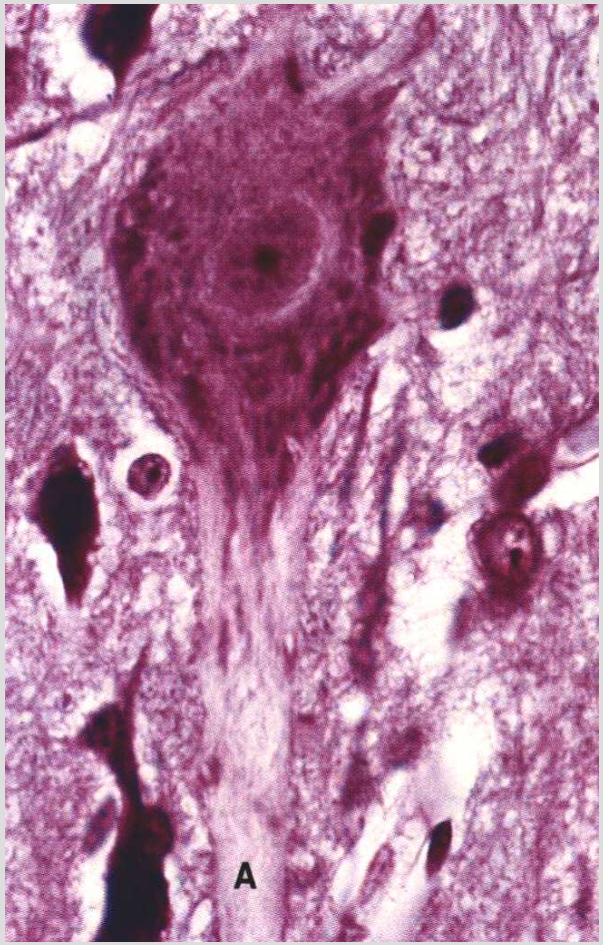
Il sistema nervoso

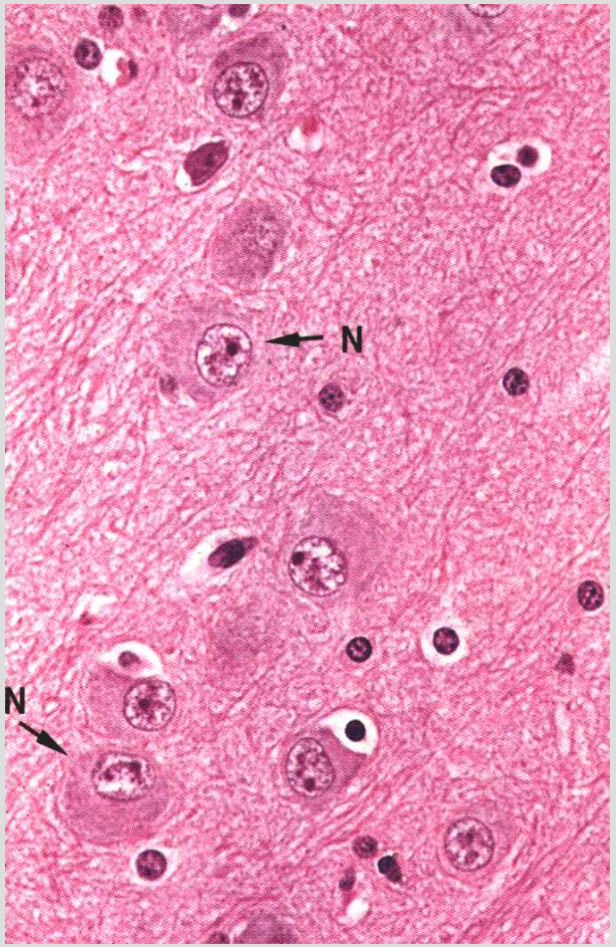
- Centrale
  - Encefalo, midollo spinale
- Periferico
  - Nervi, gangli

- Vie afferenti
- Vie efferenti

Il neurone







La glia (SNC)

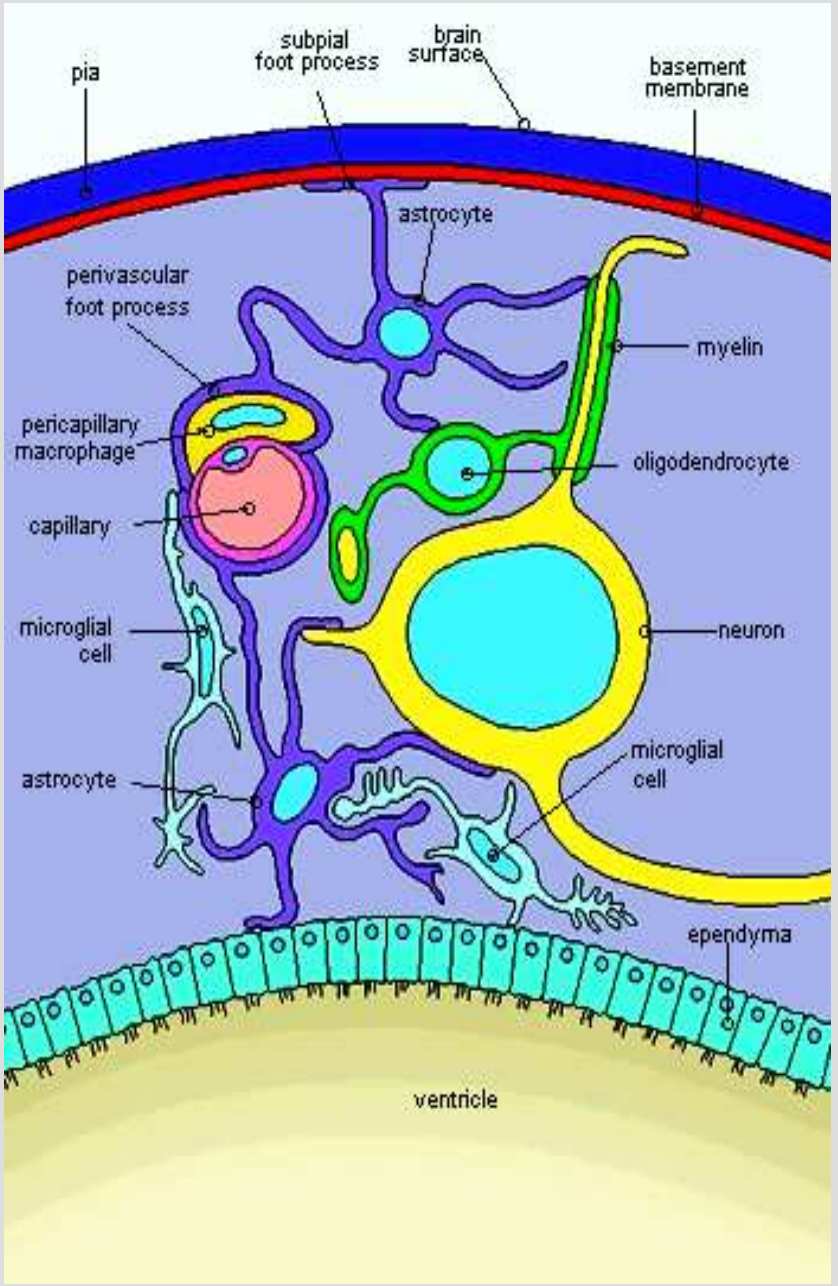
- 4 tipi cellulari:

  oligodendrociti

  astrociti

  microglia

  ependima



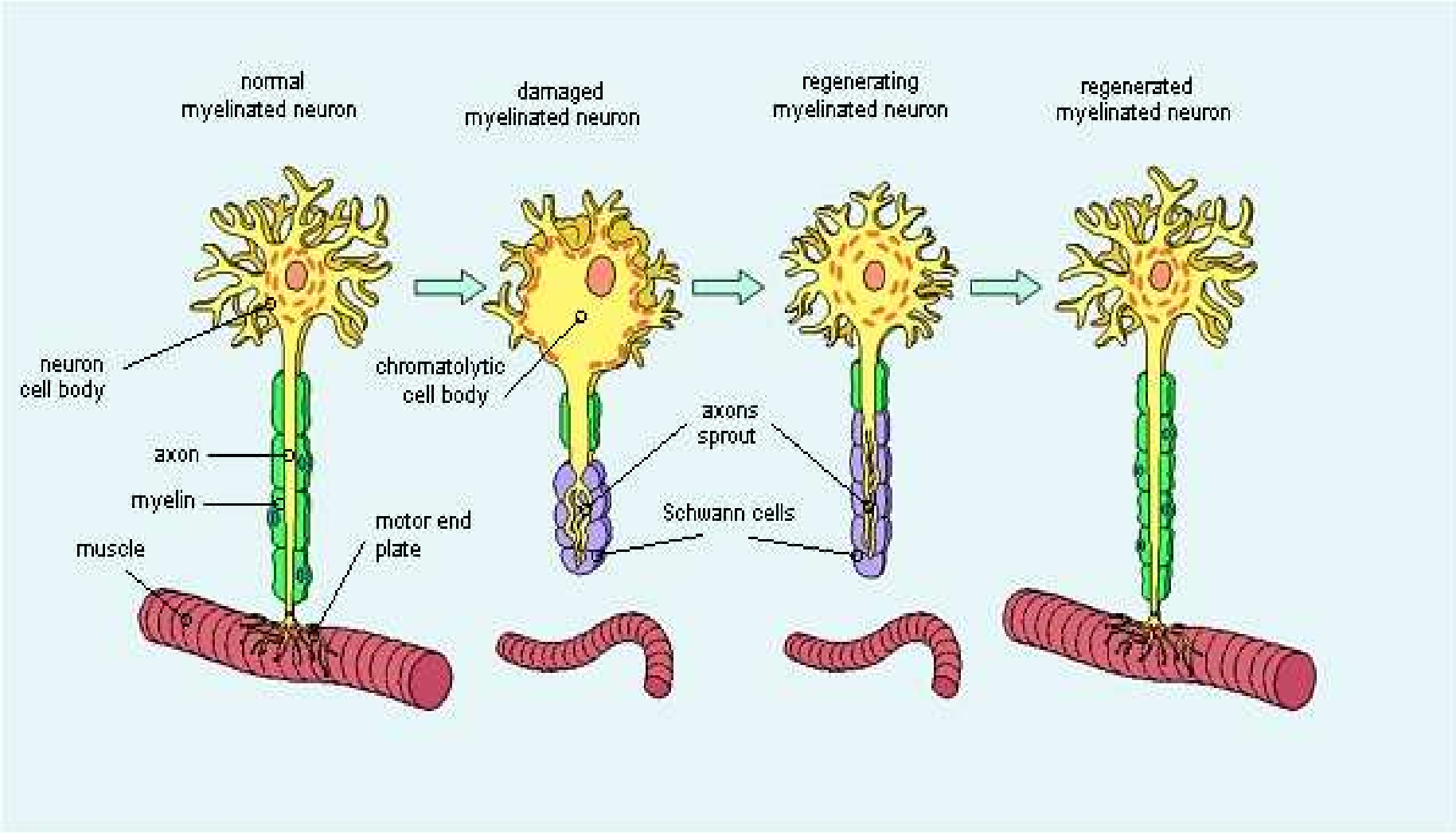
Peripheral nerve regeneration

- challenging scientific field with relevant clinical implications since nerve injuries are much more frequent than spinal cord injuries.
- Peripheral nerve lesions are common and serious injuries affecting 2.8% of **trauma** patients annually
- may result in **loss of motor function and sensory function** that generally lead to lifelong disability
- Peripheral nerve lesions are caused primarily by traumatic accidents, tumour resection, or iatrogenic side effects of various types of surgery, including orthopaedic intervention, intravenous aspiration, and cosmetic facial surgery

repair of peripheral nerve lesions

- attempted in many different ways, which have in common the goal of directing the regenerating nerve fibers into the proper distal endoneurial tubes.
- 2 strategies
  - bridging, which includes grafting and tubulization techniques (autologous nerve graft with its Schwann cells (SCs) and its basal lamina → GFs & adhesion Fs. Possibly with artificial conduits for reconstruction of nerve gaps.
  - end-to-end suturing of the nerve stumps.

Ruolo delle cellule di Schwann nella rigenerazione di un nervo leso



Artificial nerve guidance channels (NGCs)

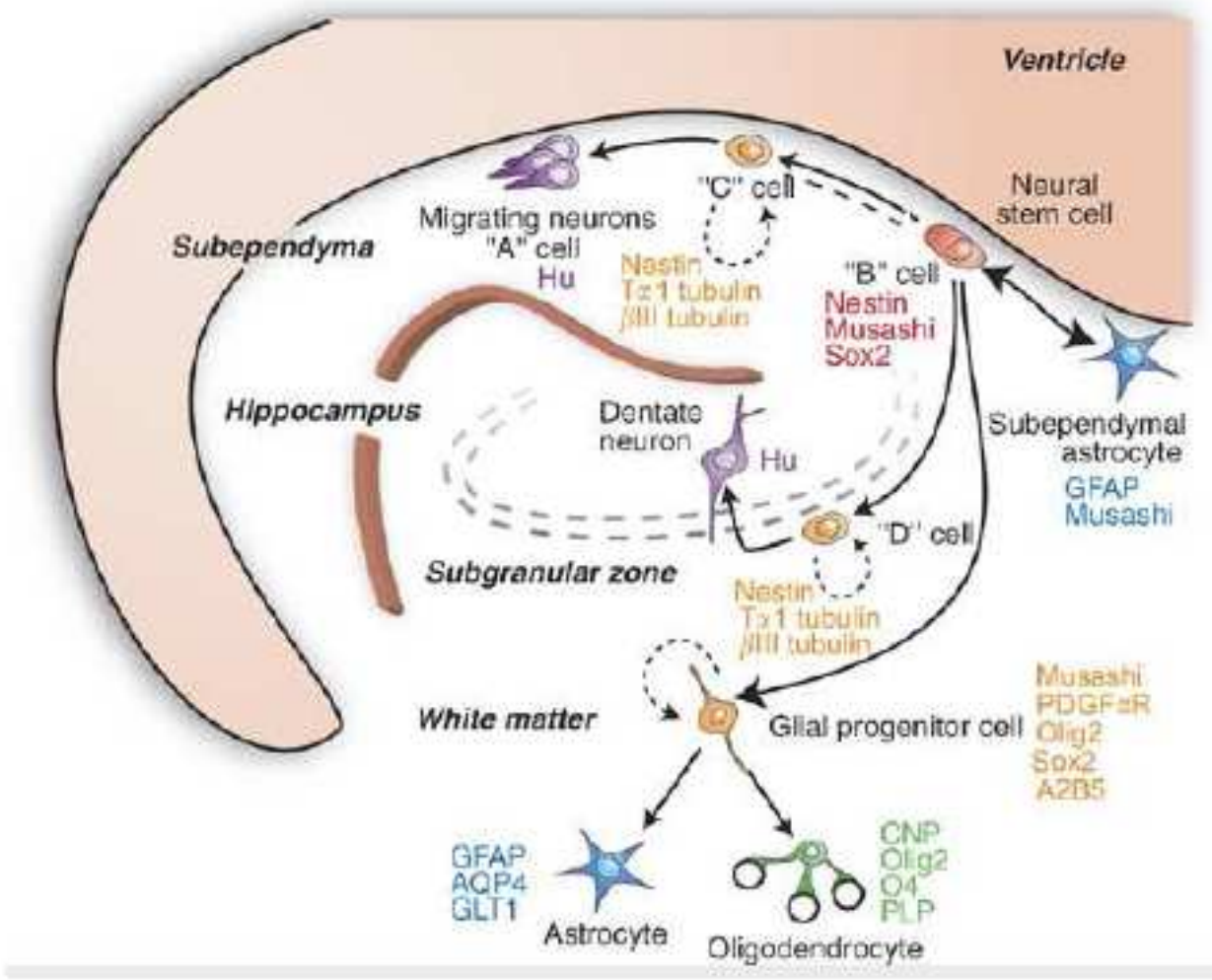
- No sacrifice of a healthy nerve and supports
- guides the axons during their growth
- avoids scar tissue infiltration in the gap
- Can be functionalized
- should ideally be biocompatible, sufficiently mechanically stable, flexible (to prevent compression of the regenerating nerve), porous to ensure supply of nutrients, degrade into nontoxic products to prevent long-term irritation
- Various materials: …possibly functionalized

Cues in the NS

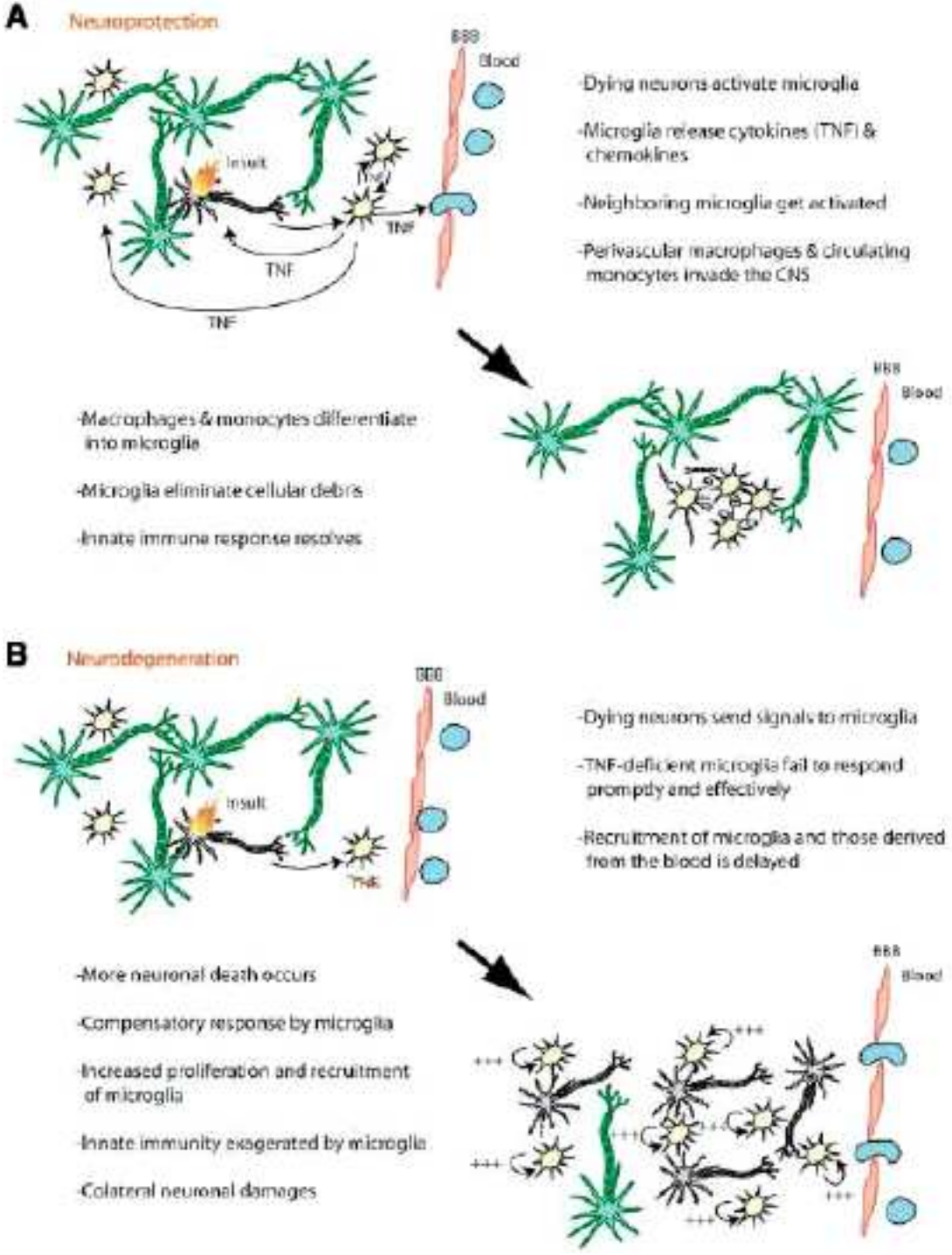
- Haptotactic = molecules in direct contact
  - Molecules mediating adhesion (laminin,…)
- Chemotactic molecules & GFs
  - NGF, GDNF, NT family
  - Spatially and temporally regulated
  - GF therapy is a difficult task due to the high biological activity, pleiotrophic effects, short biological half-life

Delivery of GFs

Stem and progenitor cells of the adult human brain

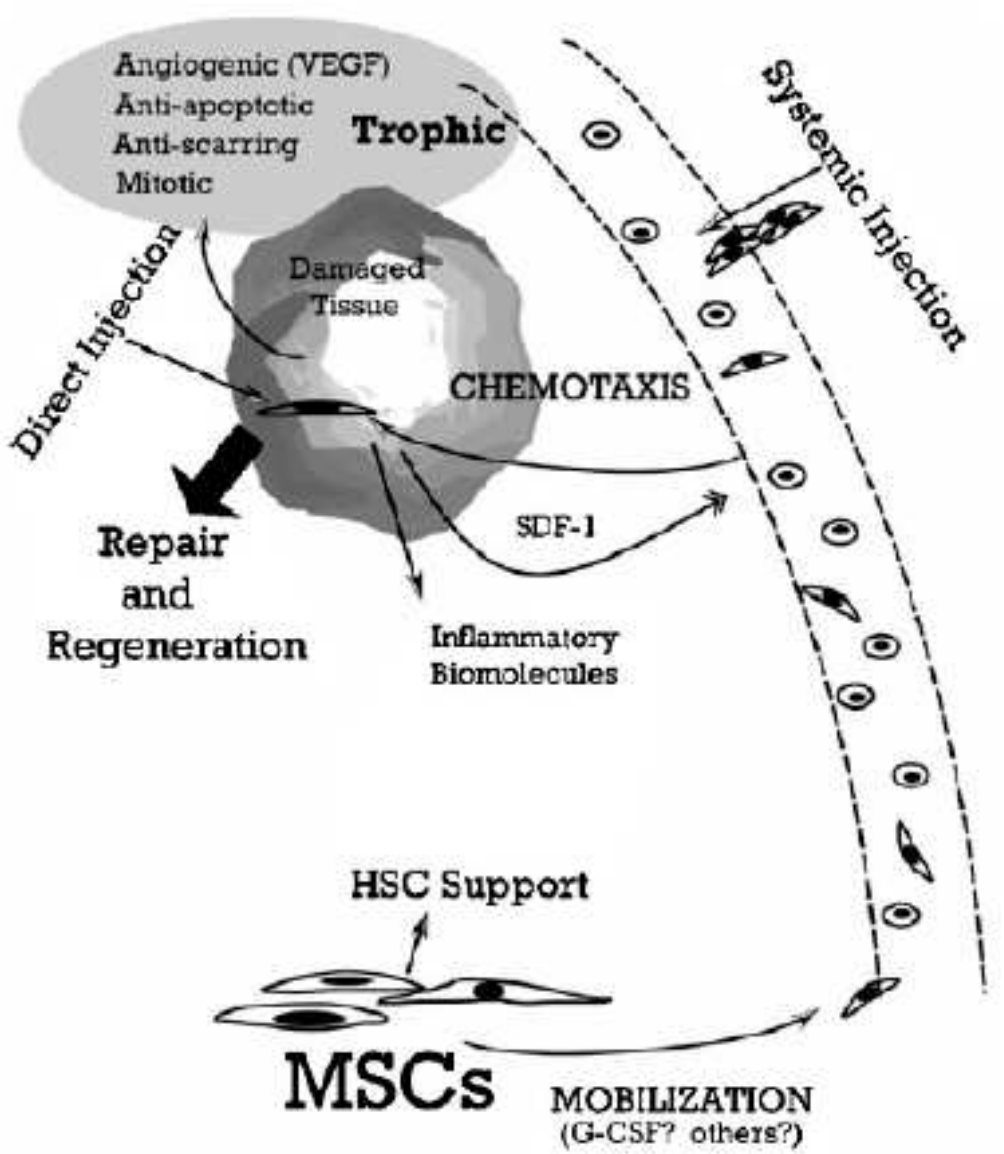


Goldman, Clin Pharmacol Ther 2007



Turrin and Rivest. Mol Neurobiol.. 200

Trophic mechanism of tissue repair



Caplan and Dennis, J Cell Biochem. 2006

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Melanoma	Mouse	Skin	Inhibition of tumour-specific T cells by $CD8^+$ T cells	Local	91
Acute renal failure	Rat	Kidney	Inhibition of pro-inflammatory cytokine production and induction of anti-apoptotic and trophic factors	Systemic	87
EAE	Mouse	CNS	Inhibition of myelin-specific T cells and induction of peripheral tolerance	Systemic	38
Diabetes	Mouse	Pancreas and renal glomeruli	Induction of local progenitor cells and inhibition of macrophage infiltration	Systemic	85
EAE	Mouse	CNS	Inhibition of production of myelin-specific antibodies and encephalitogenic T cells; decreased axonal loss	Systemic	59
Rheumatoid arthritis	Mouse	Joint	Inhibition of T cells and of production of pro-inflammatory cytokines; induction of regulatory T cells	Systemic	105
Retinal degeneration	Rat	Eye	Decreased retinal degeneration through anti-apoptotic and trophic molecules	Local	117
Acute lung injury	Mouse	Lung	Inhibition of production of pro-inflammatory cytokines	Systemic	88
Acute lung injury	Mouse	Lung	Inhibition of production of pro-inflammatory cytokines and increased production of IL-10	Local	118
Acute renal failure	Mouse	Kidney	Tubular-cell regeneration through IGF1 secretion	Systemic	119
Myocardial infarction	Rat	Heart	Anti-apoptotic and mitogenic effect by the WNT-related molecule SFRP2	Local	89
Hepatic failure	Rat	Liver	Inhibition of leukocyte invasion through the release of cytokines and chemokines	MSC-conditioned medium	120
Diabetes	Mouse	Pancreas	Induction of local progenitors and inhibition of β-cell-specific T cells	Systemic	86

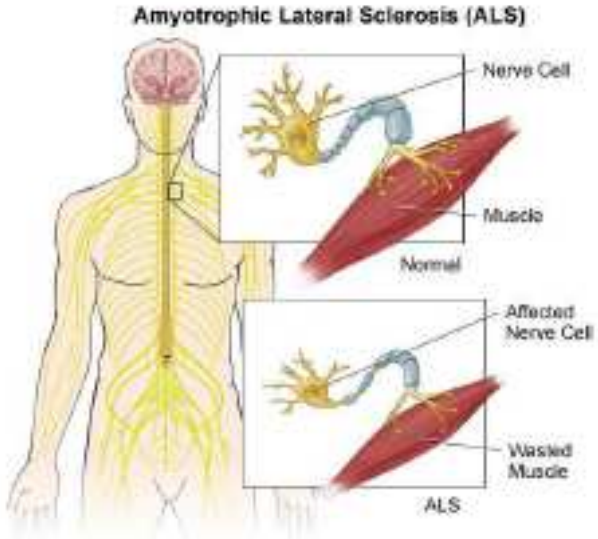
CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; HSC, haematopoietic stem cell; IGF1, insulin-like growth factor 1; IL, interleukin; MSC, mesenchymal stem cell; SFRP2, secreted frizzled-related protein 2.

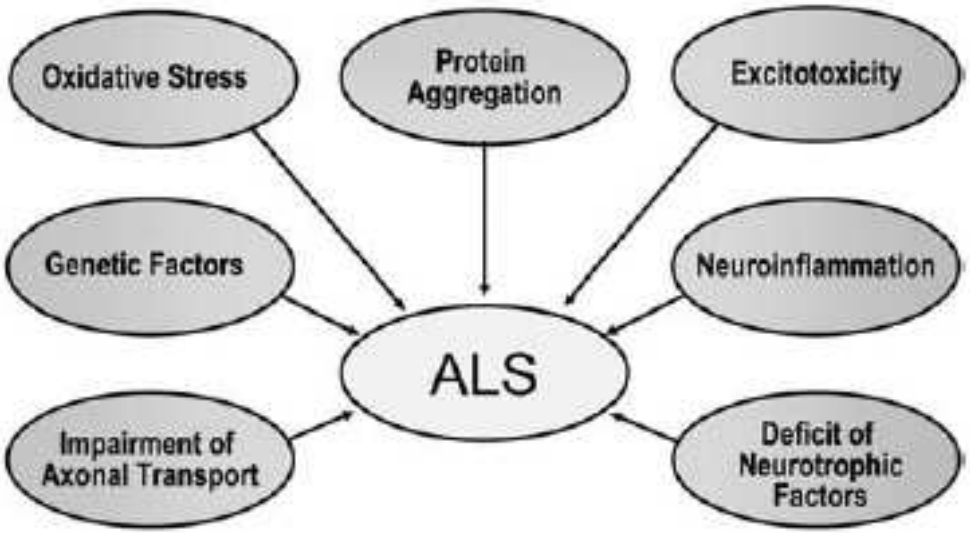
Uccelli *et al.*, Nat. Rev. Immunol., 2008

SLA

AMYOTROPHIC LATERAL SCLEROSIS

- Progressive late-onset motoneuron disease
- Degeneration and death of upper and lower motoneurons
- Weakness, muscle atrophy, fasciculations, spasticity
- Death caused by respiratory failure





- ALS is a multifactorial disease

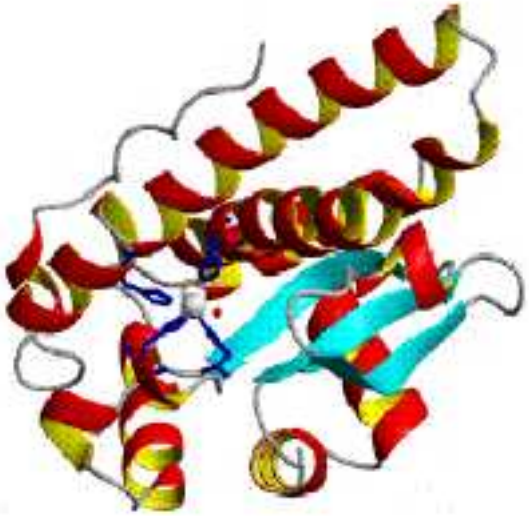
Cozzolino *et al.*, Antioxid Redox Signal, 2008

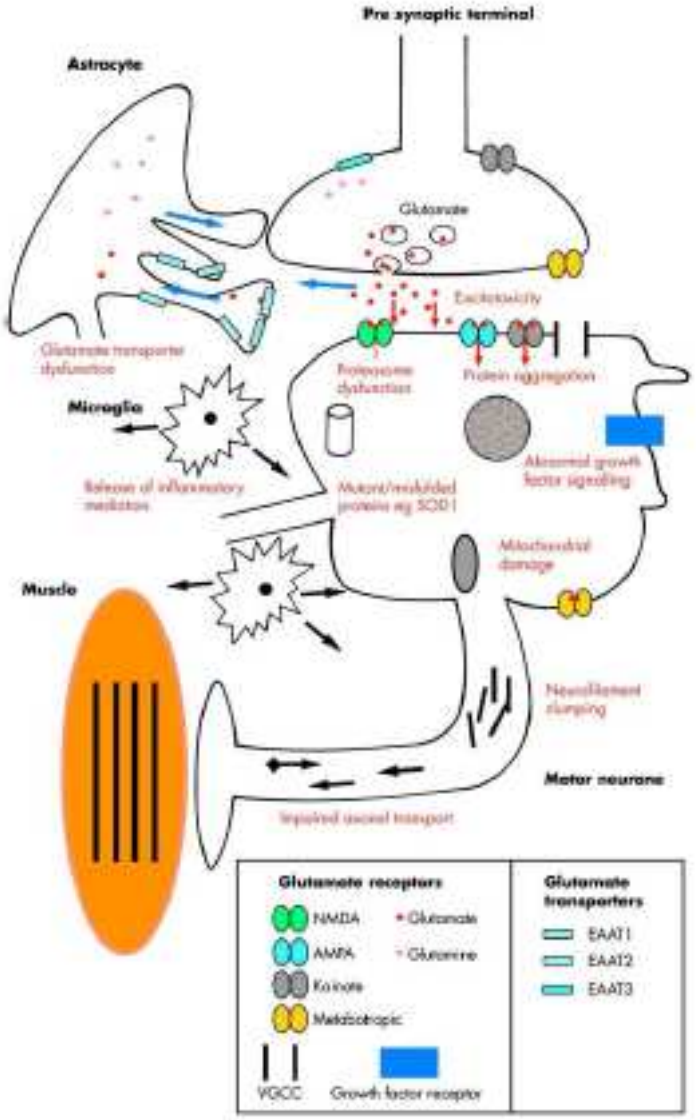
AMYOTROPHIC LATERAL SCLEROSIS

Forms of ALS:

- Familial - 5 to 10%
- Sporadic - 90 to 95%

- Mutations in the gene coding for Cu/Zn superoxide dismutase (SOD1)

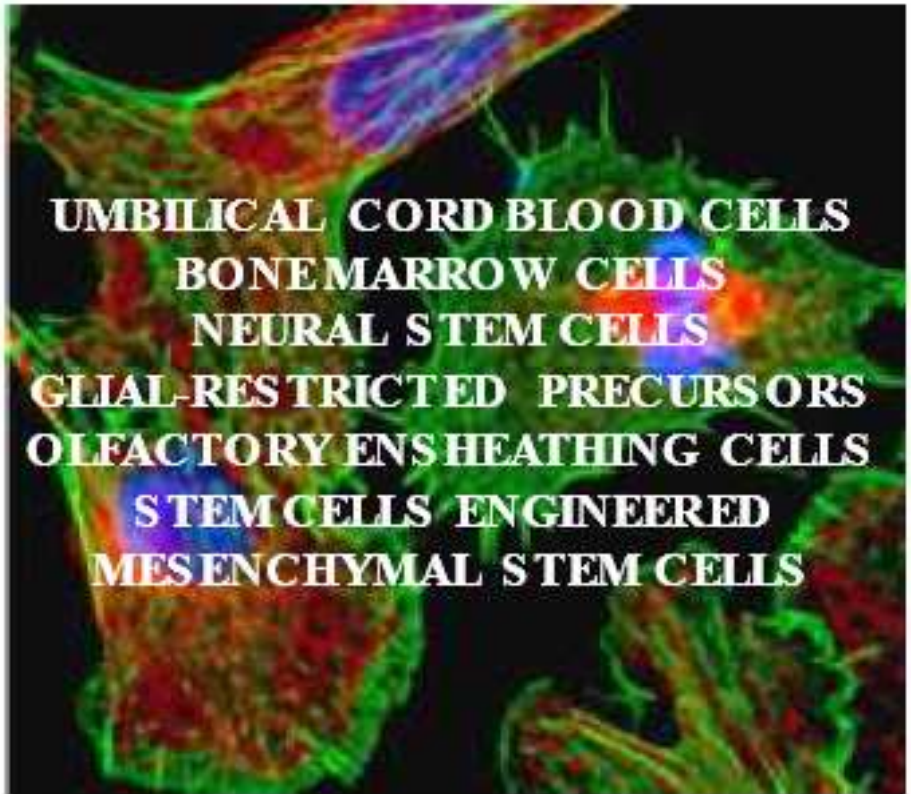




Shaw , J. Neurol. Neurosurg. Psychiatry, 2009

THERAPEUTIC APPROACHES IN ALS

- Pharmacologic therapies (antioxidants, antiapoptotics, antiinflammatories, antiexcitotoxic)
- Growth factor therapies (VEGF, CNTF, GH, thyrotropin-releasing hormone, IGF-1, BDNF)
- Gene therapies in ALS (silencing toxic genes, delivering trophic factors)
- Stem cell therapies



hMSCs TRANSPLANTATION IN SOD1 MICE

Human bone marrow

Percoll 1,073 g/l

a-MEM + 10% FBS

8000 cells/cm^2

Labeling with Bisbenzimide

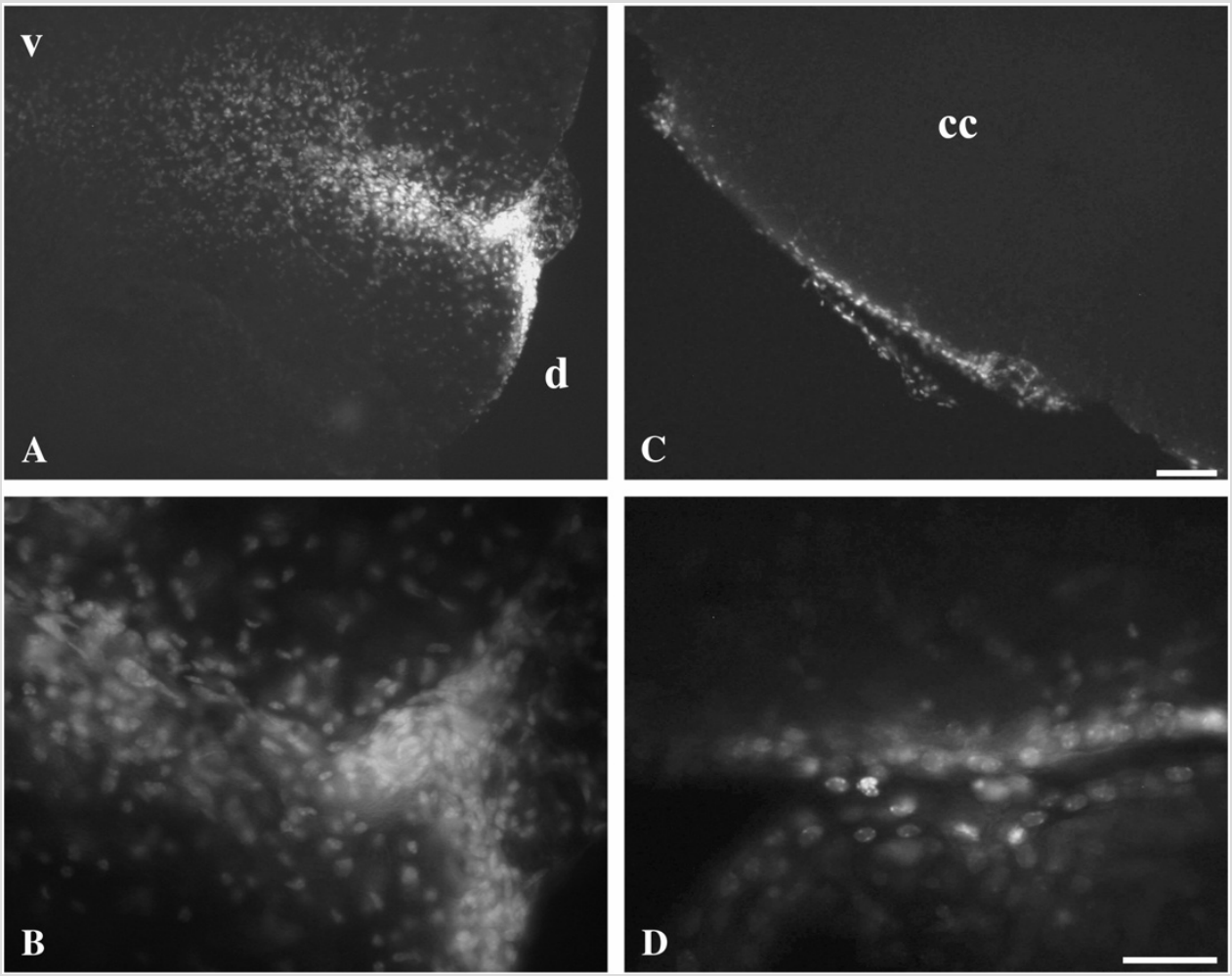
MSC injection
2 µl/site
50,000 cells/µl

SOD1
[B6SJL-TgN(SOD1-G93A)dl1Gur/J mice]

Transplantation at 28 wks into the lumbar spinal cord (T13-L4)

- Clinical tests
  Motor score, Rotarod task, Paw Grip Endurance
- Sacrifice at 38 wks
- Tissue analysis:
  - Quantification of a-motoneurons
  -Immunofluorescence for reactive astrogliosis (GFAP)
  - Immunohistochemistry for microglial antigen (CD11b)

Localization of transplanted hMSCs (Bisbenzimide-stained nuclei)



lumbar spinal cord

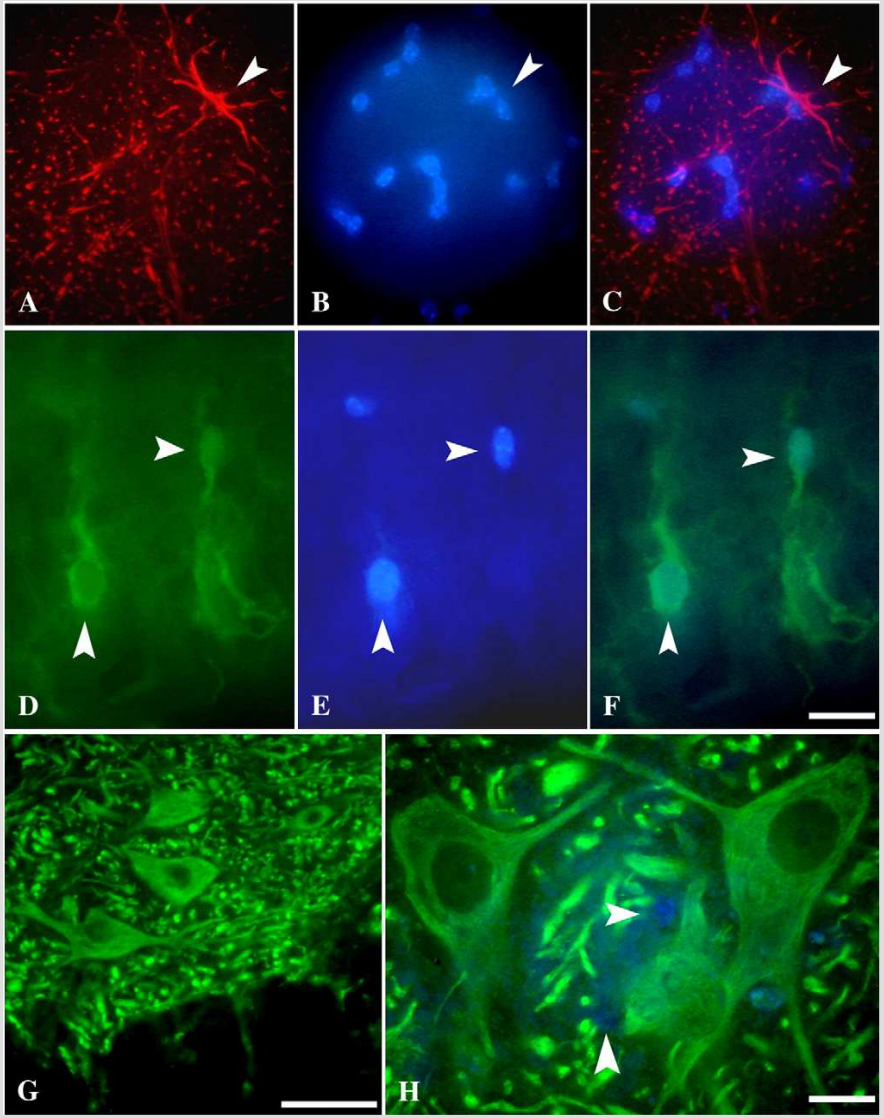
cerebral cortex

Expression of neural markers by transplanted hMSCs (bisbenzidimide)

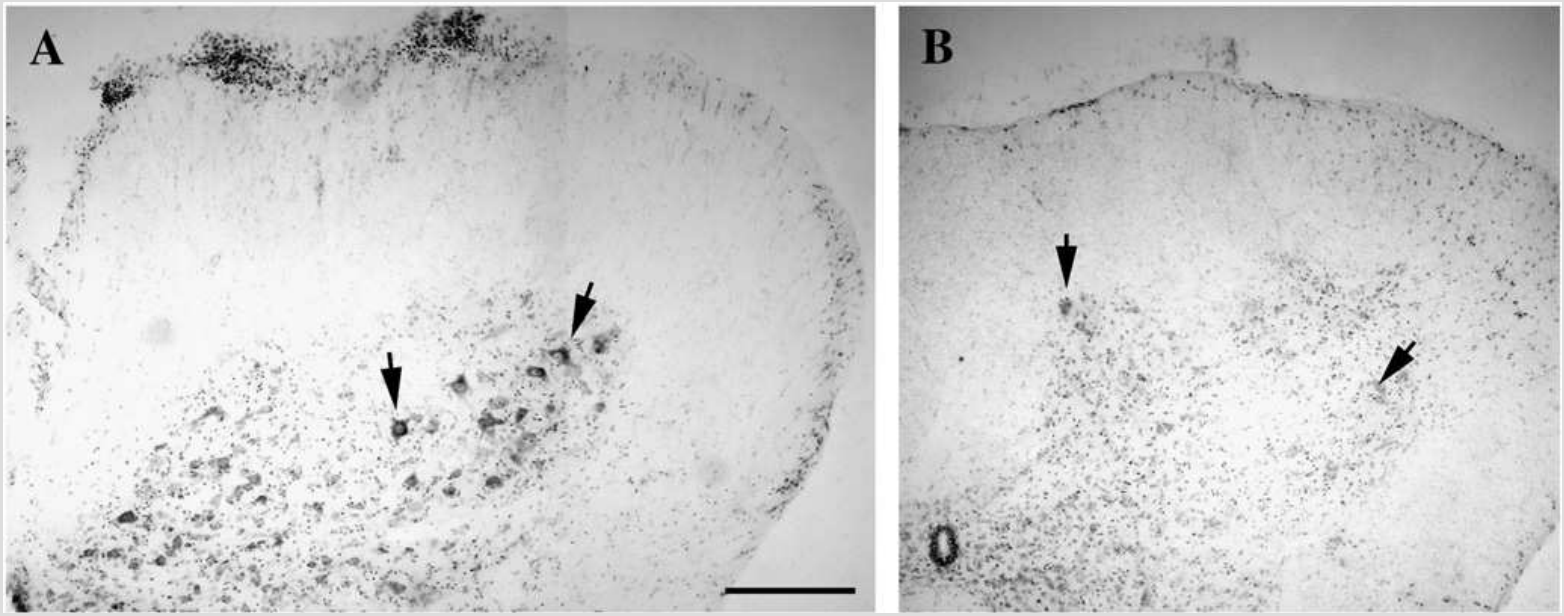
GFAP

MAP-2

Localization close to motoneurons



Motoneurons in the lumbar spinal cord (Transverse sections)

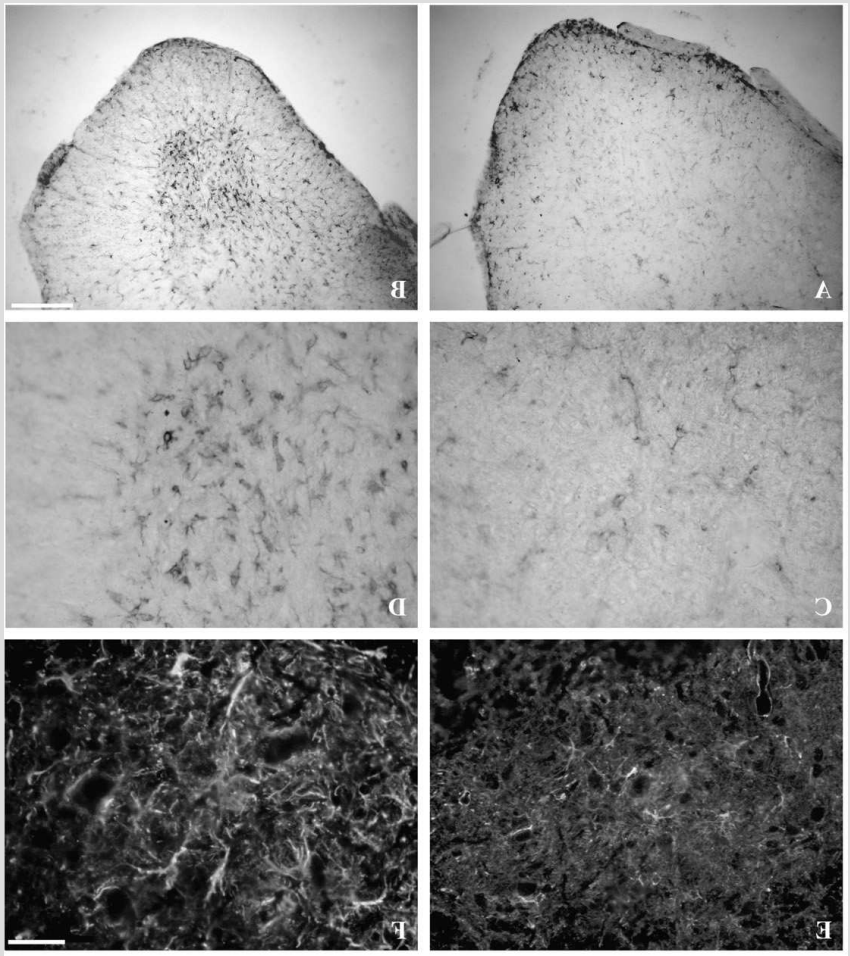


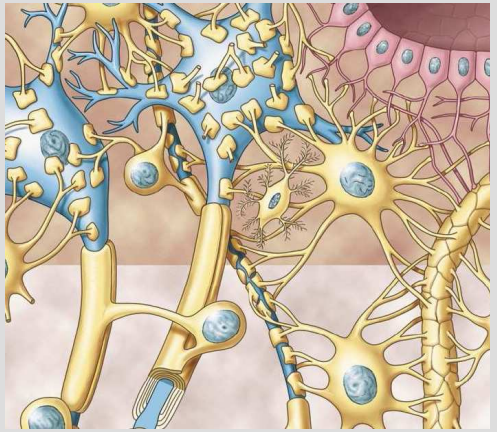
(A) and sham-operated (B) female SOD1G93A mice at 38 weeks of age

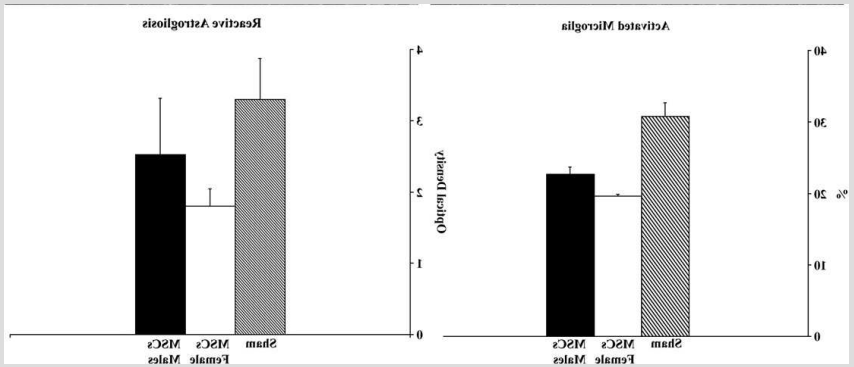
NUMBER OF MOTONEURONS

Transplanted females 5458 ± 682	•	Sham-operated females 3549 ± 607
Transplanted males 3766 ± 980	•	Sham-operated males 1489 ± 446

Quantification of microglial activation and of reactive astrogliosis.







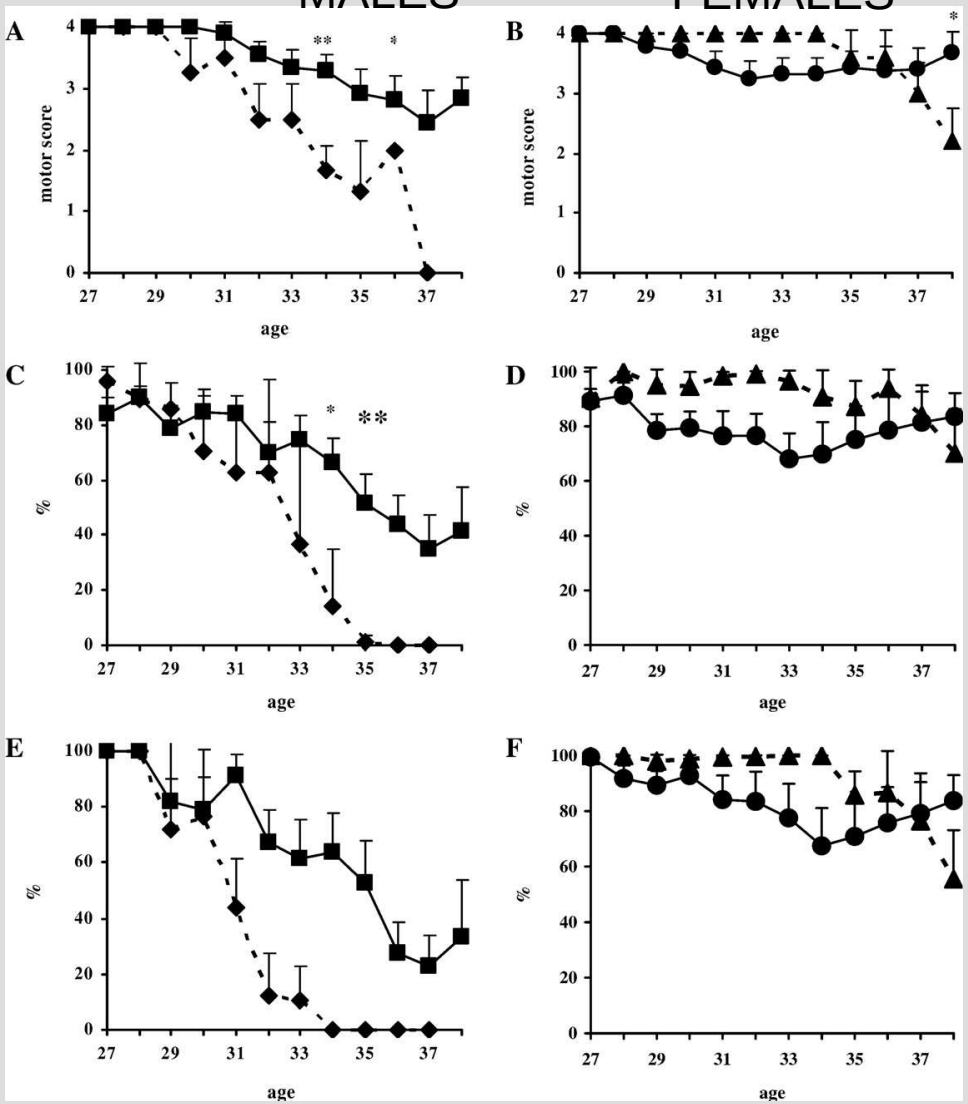
Behavioral tests

MALES FEMALES

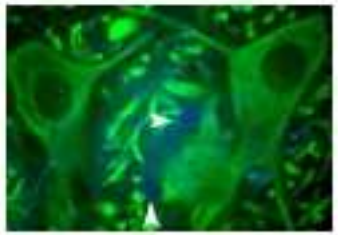
neurologic

Rotarod test

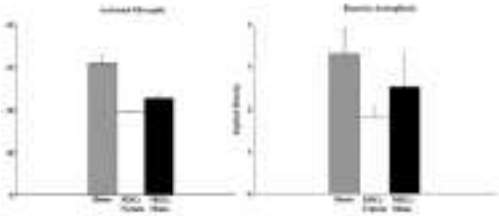
PaGE



CONCLUSIONS



• hMSCs were found in the spinal cord 10 weeks after, sometimes close to motoneurons and were rarely GFAP- or MAP2-positive.



• Astrogliosis and microglial activation were reduced and motoneuron counts were higher following transplantation.

• Motor tests (Rotarod, Paw Grip Endurance, neurological examination) were significantly improved in transplanted mice.

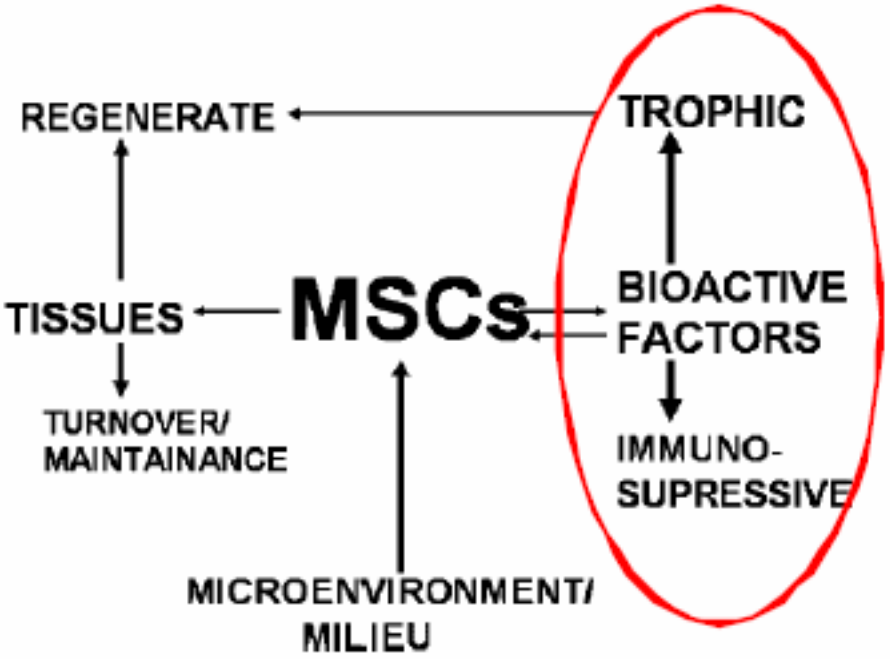
• Differences in our results between males and females suggested a more rapid progression of the disease in males: it is known that estrogen represents a neuroprotectant in both the adult and the aging brain, both in vitro and in vivo.

• hMSCs are good candidates for ALS cell therapy.

MSCs can be considered as *biologic minipumps* migrating close to motoneurons affected by the disease, delivering *trophic factors* and *immunomodulatory molecules*.

MSC therapy could be exploited by inserting genes coding for specific neurotrophic factors or immunomodulatory molecules.

MSCs AS TROPHIC MEDIATORS



- MSCs produce VEGF (Caplan and Dennis, 2006) and BDNF (Crigler, 2006)
- Immunosuppressive role in multiple sclerosis (Uccelli *et al.*, 2006)
- MSCs produce GDNF (Suzuki *et al.*, 2008)

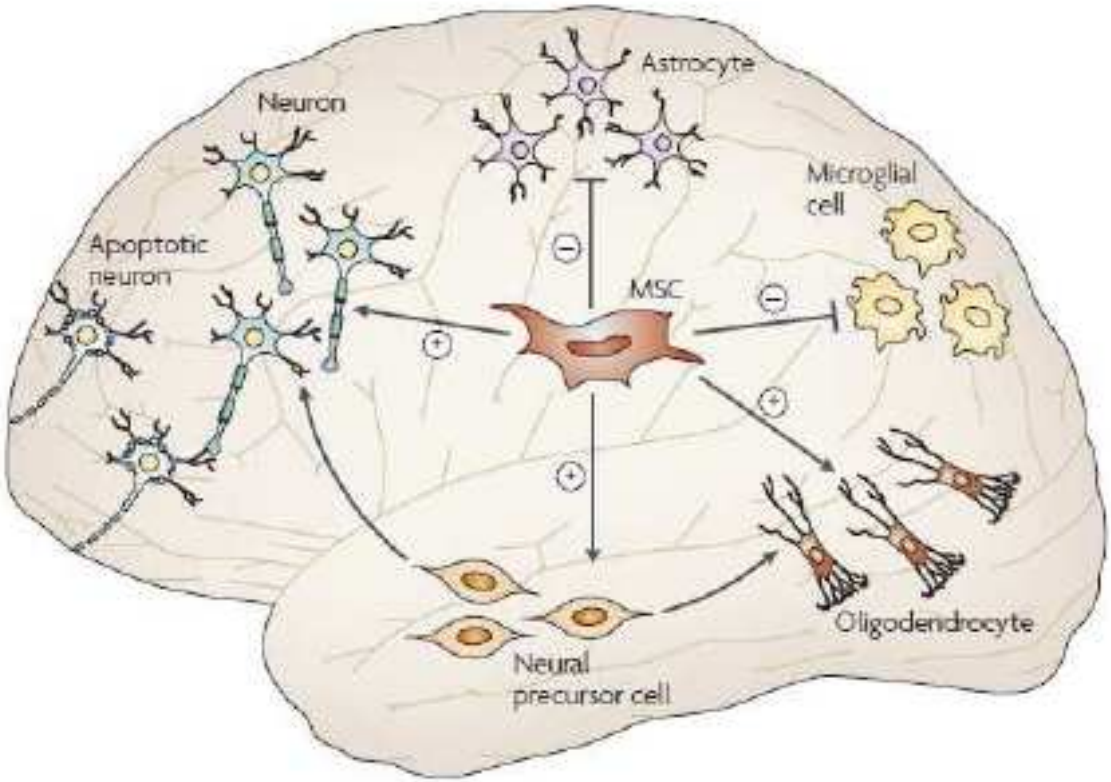
Caplan and Dennis, J Cell Biochem. 2006

BYSTANDER EFFECTS

• MSCs can rescue neurons and oligodendrocytes from apoptosis through the release of trophic and anti-apoptotic molecules.

• MSCs can have anti-inflammatory and anti-proliferative effects on microglial cells and astrocytes, determining a neuroprotective microenvironment.

• MSCs can promote the proliferation and maturation of local neural precursors cells, leading to their differentiation into mature neurons and oligodendrocytes.



Uccelli *et al.*, Nat. Rev. Immunol., 2008

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CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; HSC, haematopoietic stem cell; IGF1, insulin-like growth factor 1; IL, interleukin; MSC, mesenchymal stem cell; SFRP2, secreted frizzled-related protein 2.

Uccelli *et al.*, Nat. Rev. Immunol., 2008

EAE = modello sp
erimentaledi Sclerosi Multipla

Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis

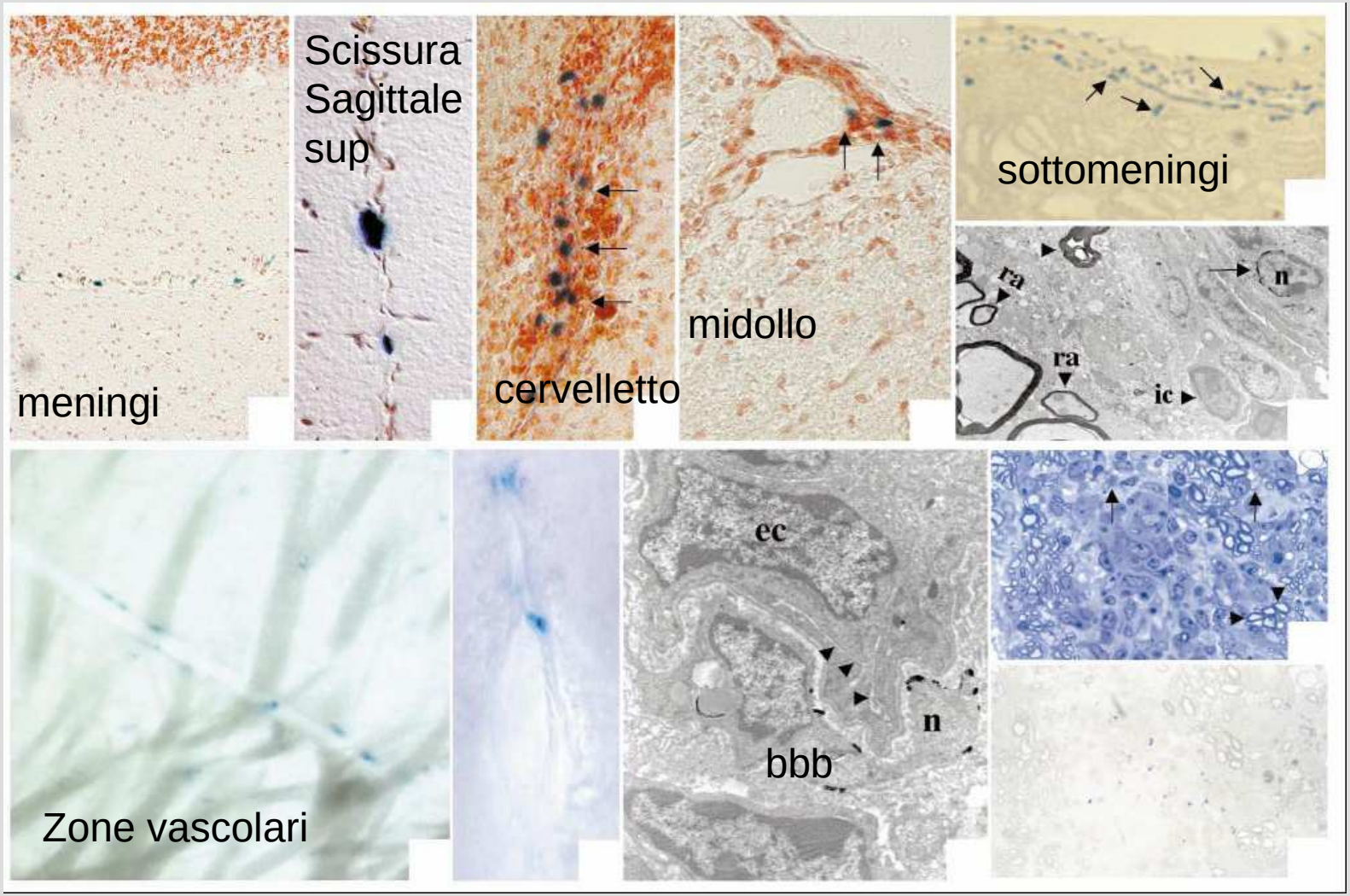
NATURE | 2003; 422: 688-

- Multiple sclerosis = Widespread demyelination and axonal loss
- syngenic adult neural stem cell cultures and injected them into an animal model of multiple sclerosis—experimental autoimmune encephalomyelitis (EAE) in the mouse—either intravenously or intracerebroventricularly

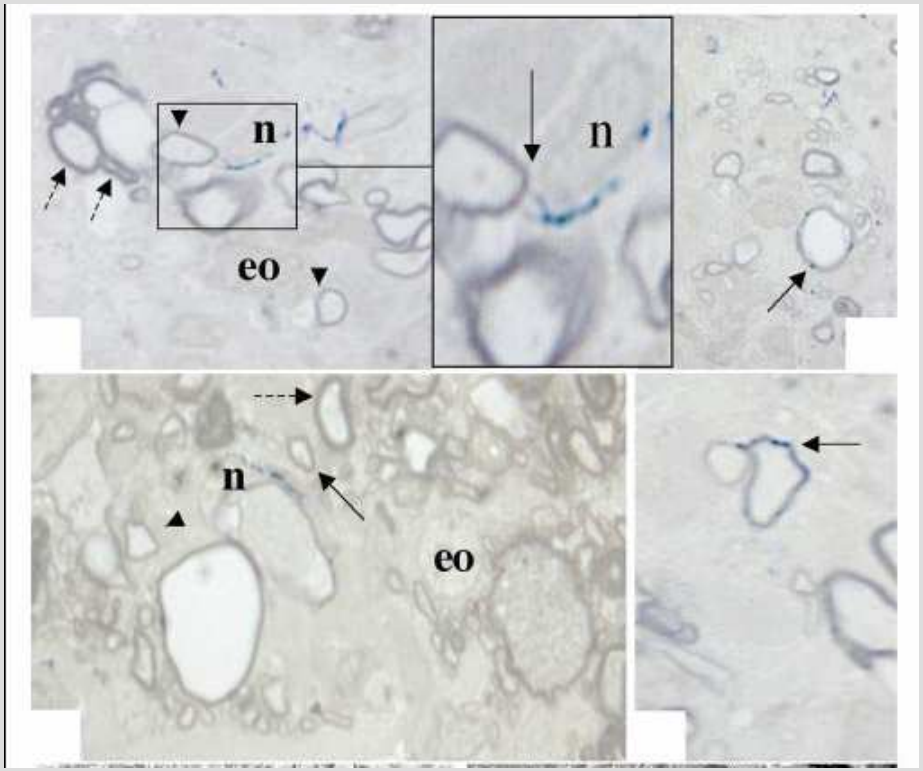
- donor cells entered into demyelinating areas of the central nervous system and differentiated into mature brain cells
- oligodendrocyte progenitors markedly increased, with many of them being of donor origin and actively remyelinating axons
- reduction of astrogliosis and a marked decrease in the extent of demyelination and axonal loss in transplanted animals.

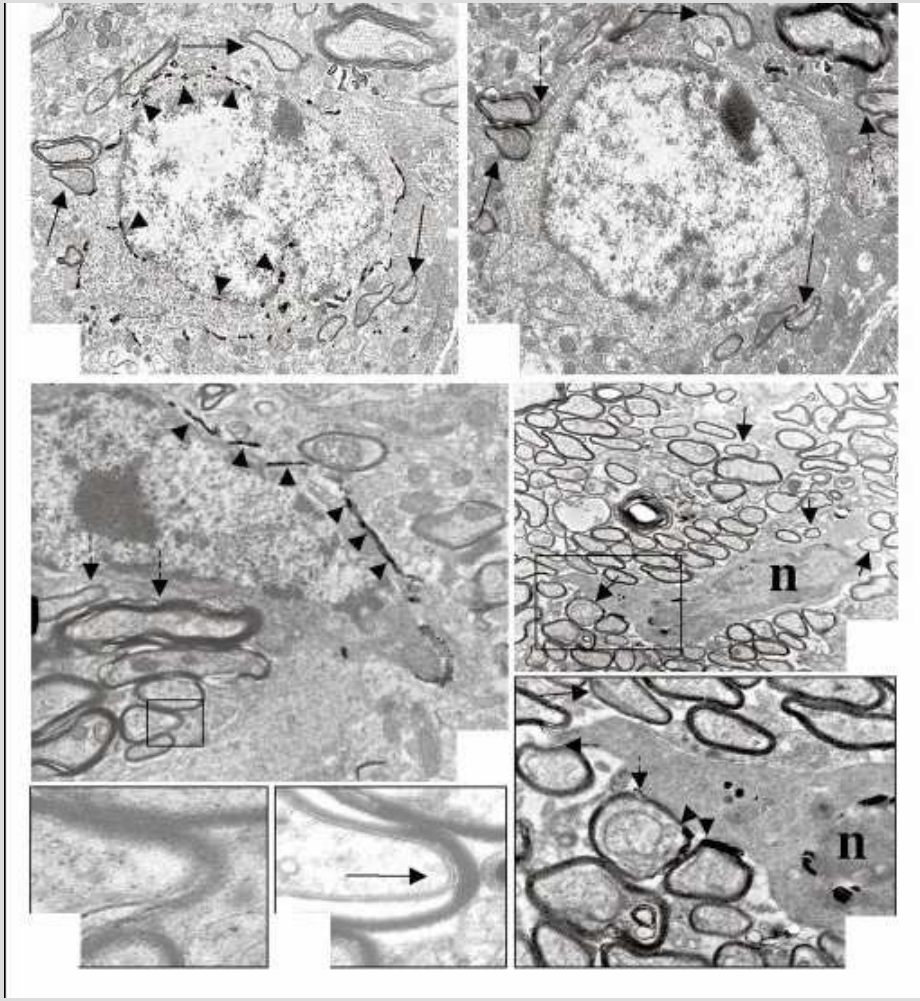
- functional impairment caused by EAE was almost abolished in transplanted mice, both clinically and neurophysiologically
- → adult neural precursor cells promotemultifocal remyelination and functional recovery after intravenous or intrathecal injection in a chronic model of multiple sclerosis.

Distribution of nls-lacZ-labelled, syngenic neural precursors (BBB forced)



Neural precursors contribute to remyelination of demyelinated axons in EAE mice



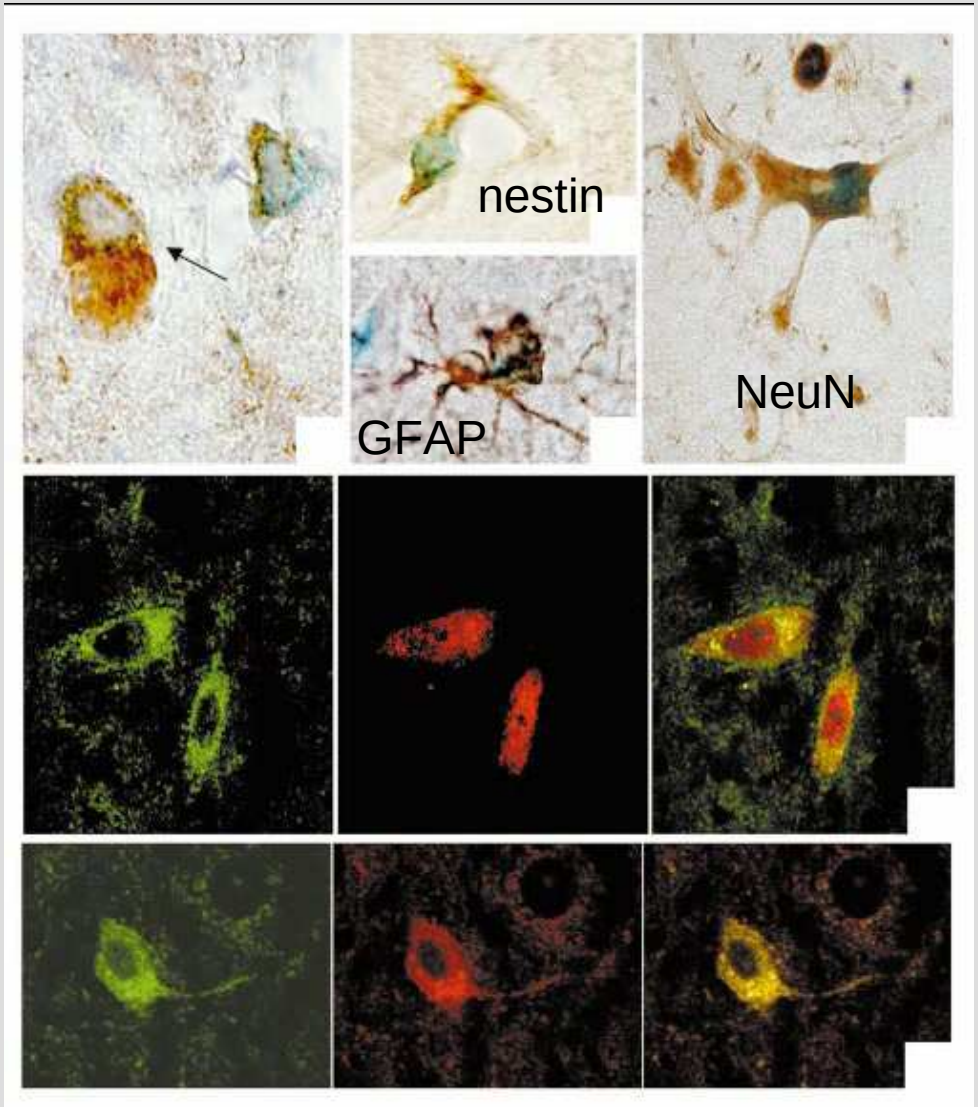


Differentiation of engrafted NP into mature neural cells in EAE mice

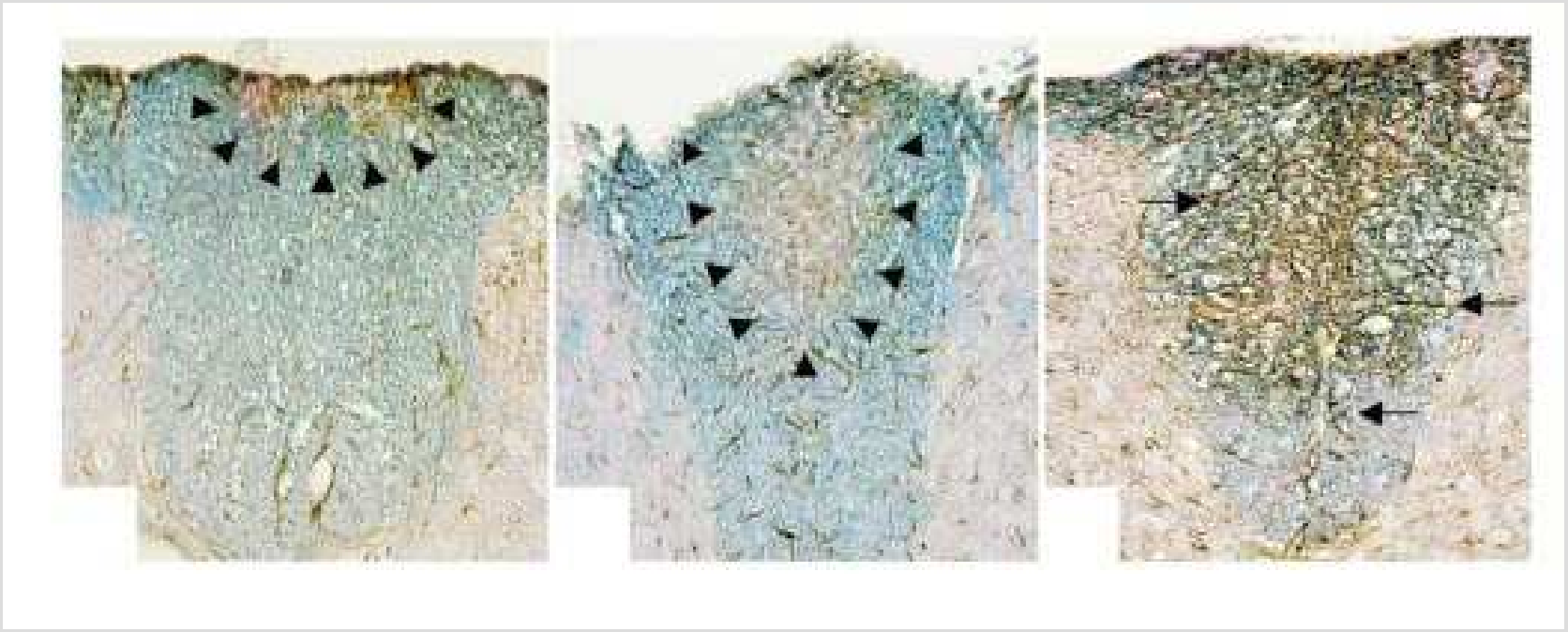
PDGFa receptor

eGFP (green) / the neuronal marker NeuN

PDGF-a-R (green) / neuronal marker NeuN



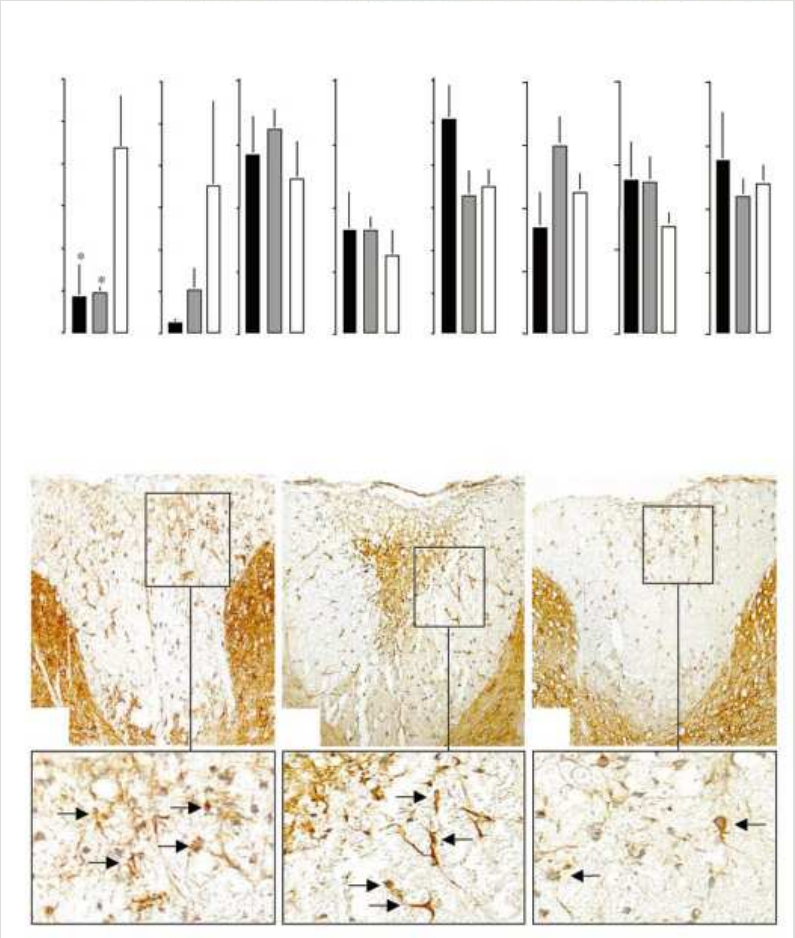
I.c. or i.v. injection of NP reduces glia scarring within the CNS.



i.c. or i.v. injection of NP modulates neurotrophic growth factor mRNA expression within the CNS.

FGF-II
TGF-b
CNTF
NT-3
GDNF
BDNF
LIF

Black = i.c
Grey = i.v.
White =control

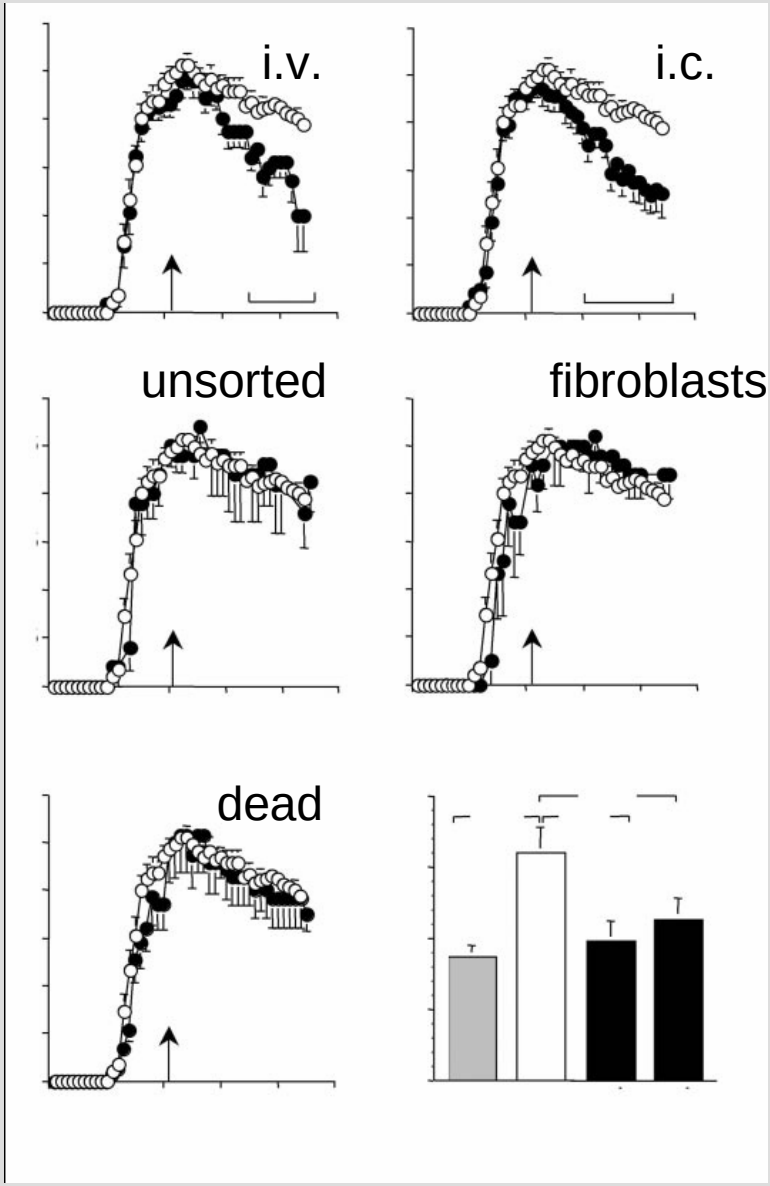


Increase in PDGFa receptor expressing oligodendrocyte progenitors (brown stain) within the posterior columns of the spinal cord of EAE mice transplanted with neural precursors either by i.c. or i.v injection, as compared with sham-treated animals

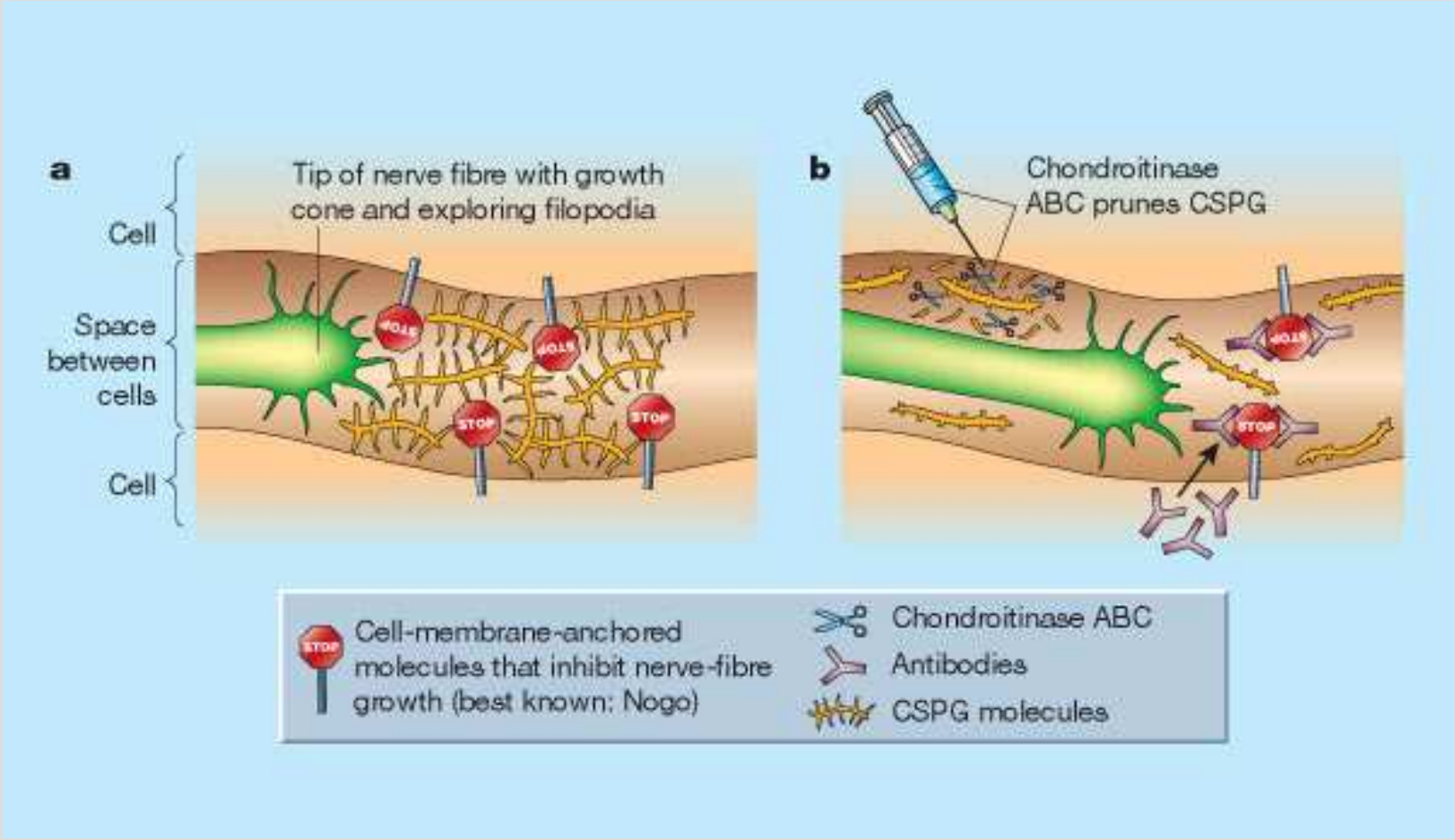
i.v. and i.c. injection of neural precursors after disease onset (arrow) significantly improves clinical features (movement) in EAE mice

myelin conduction velocity

Grey = naive
White = EAE
Black = i.v. or i.c. PN injected



Condroitinasi ABC promuove il ricupero di midollo spinale lesionato



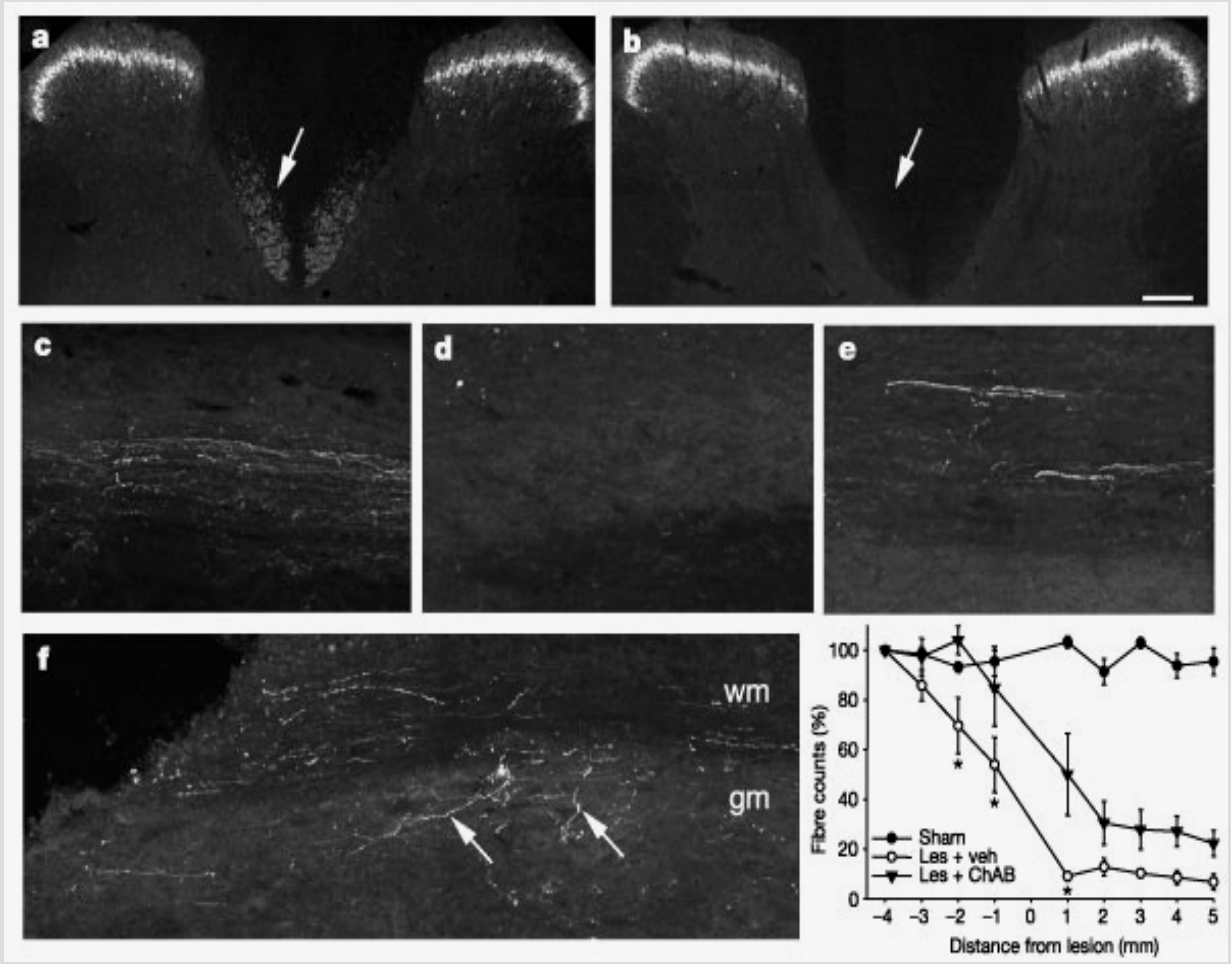
Nature, apr 11, 2002

Condroitinasi ABC promuove il ricupero di midollo spinale lesionato

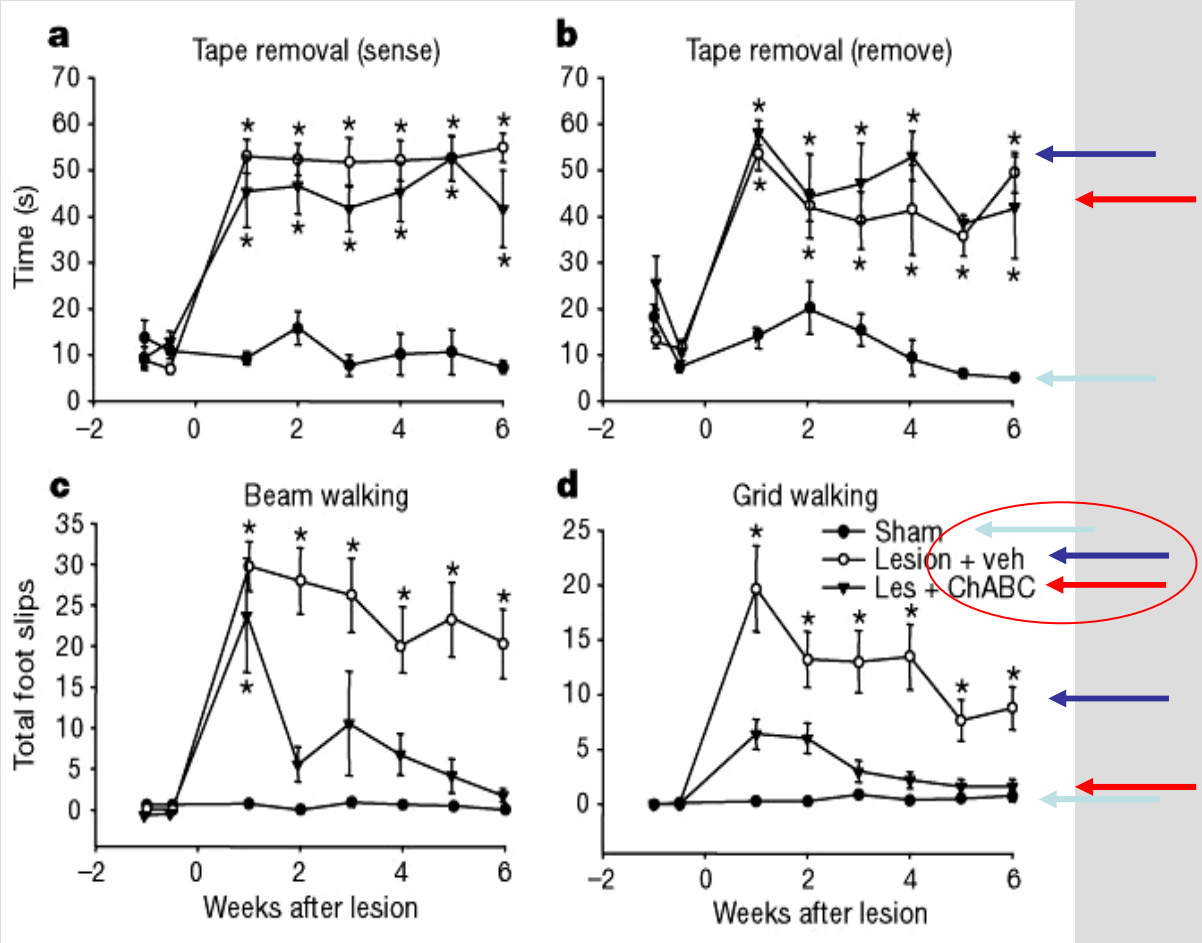
- Fibre nervose delle colonne dorsali (via afferente sensitiva e via efferente motoria) compresse fino al taglio
- Trattamento intratecale con condroitinasi ABC
- Rigenerazione del nervo/fibra (4 mm)
- Ricupero motorio (riscontro elettrofisiologico e comportamentale)
- Non gran ricupero della sensibilità

Nature, apr 11, 2002

Trattamento con condroitinasi induce rigenerazione degli assoni del tratto cortico-spinale



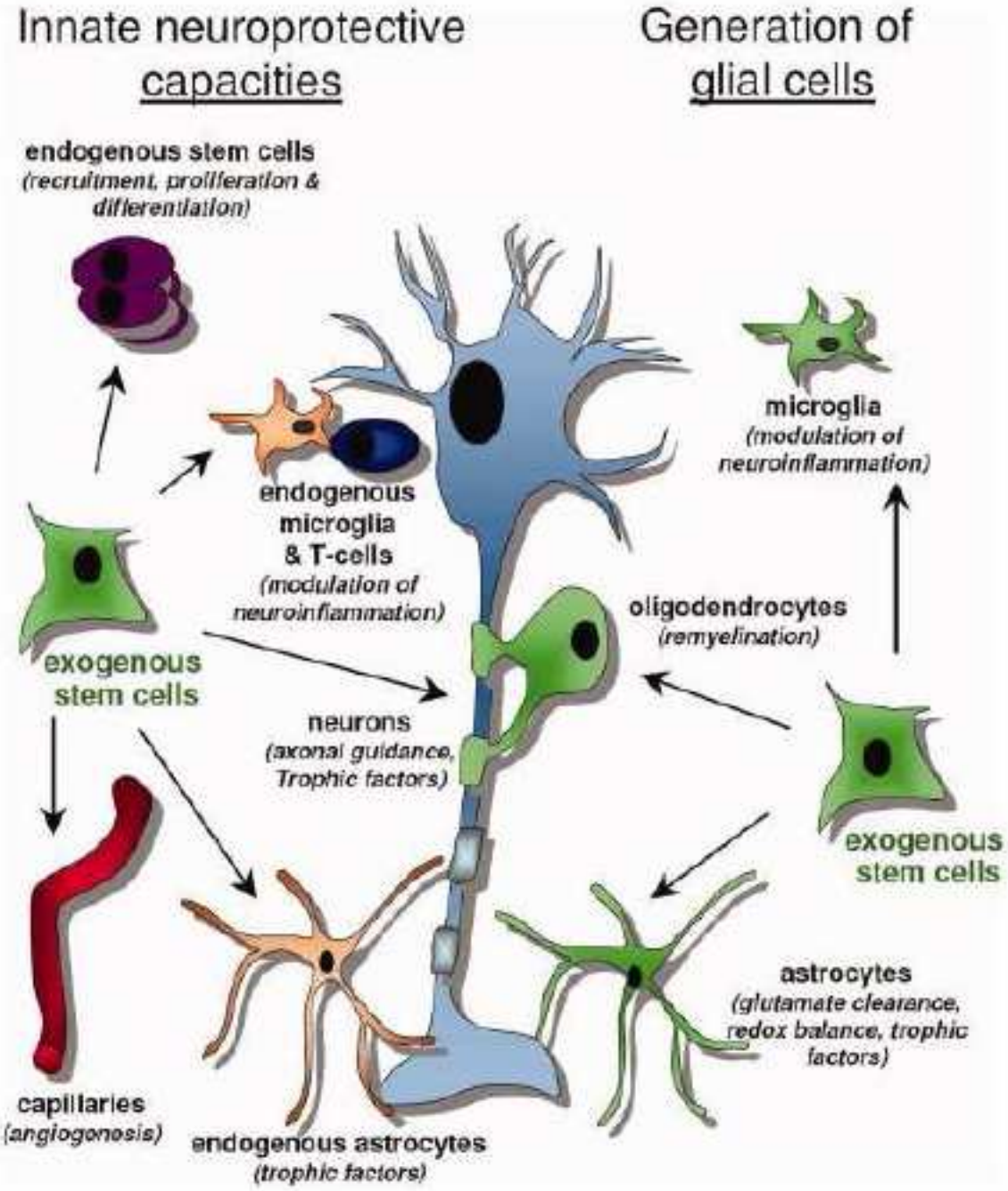
Condroitinasi induce ricupero funzionale motorio, ma non sensitivo



Nuove strategie per il riparo di midollo nervoso lesionato

- Nessuno dei trattamenti da solo funziona pienamente
- → usare una combinazione di trattamenti:
- Neutralizzare proteine inibitorie della mielina
- Somministrare fattori di crescita (meglio 2-4 settimane dopo il taglio, che non subito!!!)
- Trapiantare cellule nervose o cellule che producano fattori

SCARTI



Boucherie and Hermans, J. Neurosci. Res, 2009