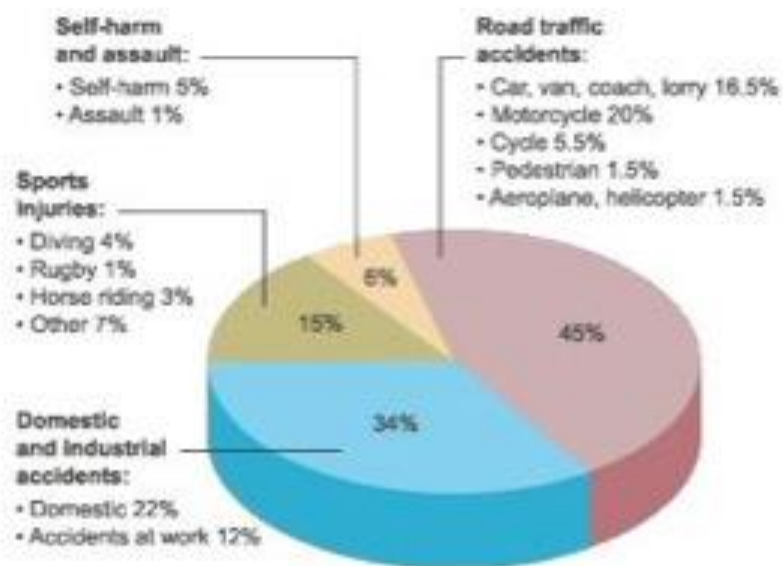




Experimental Neuroprotection for Spinal Cord Injury

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Epidemiology of SCI



U.S. Statistics on Spinal Cord Injuries

- Approximately 11,000 new cases of SCI/yr
- Over 200,000 patients living with chronic SCI
- 60% of SCI patients between ages 16-30, M:F ratio 4:1
- Economic impact: > 4 billion dollars per year

Sources: National Spinal Cord Injury Statistical Center, University of Alabama National Spinal Cord Injury Statistical Center

SCI: Secondary Injury Mechanisms

Vascular

hypotension (neurogenic shock)
loss of autoregulation
hemorrhage
loss of microcirculation
vasospasm, thrombosis
edema

Inflammatory

neutrophils
macrophages/ microglia
cytokine/ chemokines

Biochemical

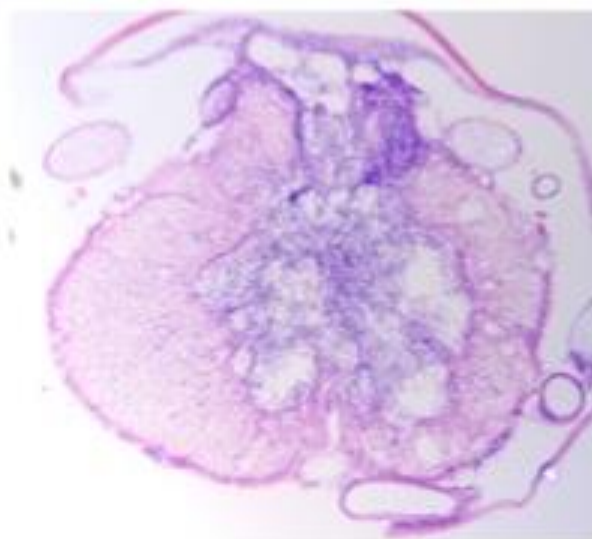
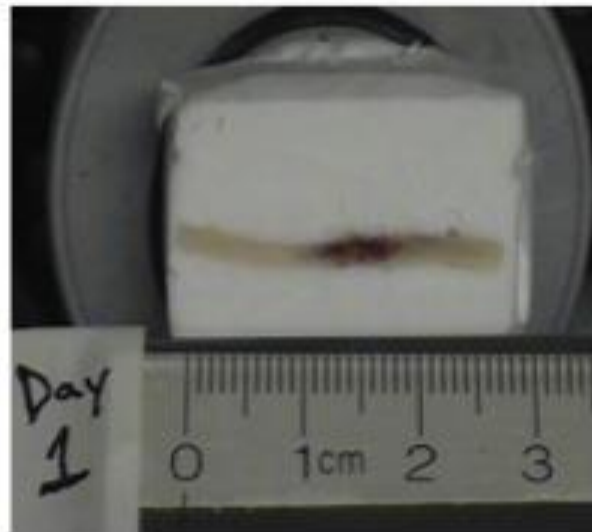
neurotransmitter accumulation
arachidonic acid release
free-radical production
inflammation and cytokine release
lipid peroxidation

Ion Permeability

Increased intracellular calcium
Increased extracellular potassium
Increased sodium permeability



Spinal cord contusion



Current Experimental SCI Therapies

Neuroprotection

- surgical decompression/ stabilization
- methylprednisolone
- systemic hypothermia / epidural cooling
- erythropoetin
- neurotrophic factors
- X-irradiation
- **immune-based therapies**

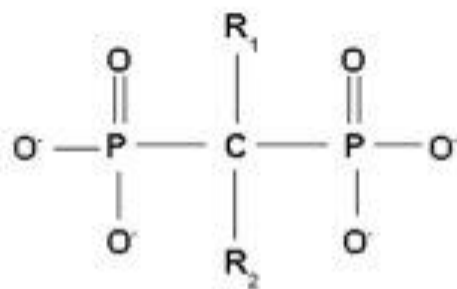




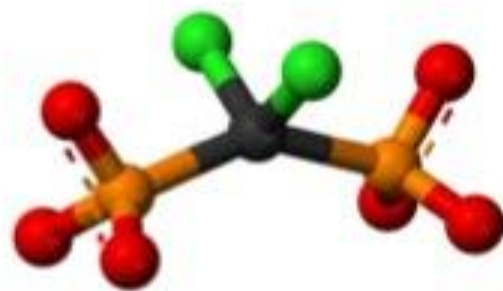
Immunomodulation Using Macrophage Depletion
and cAMP Elevation Promotes Neuroprotection
and Improves Locomotor Recovery
After Contusive SCI


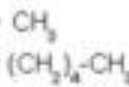


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Cleveland Clinic

Clodronate



 **Bonefos**[®]
CLODRONATE

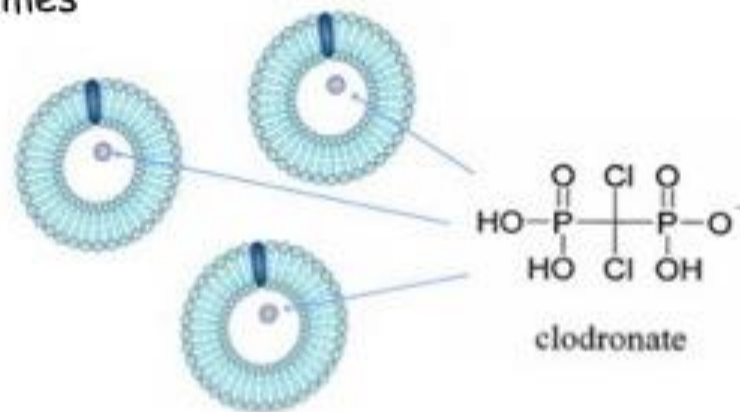


Agent	R ₁ side chain	R ₂ side chain
Etidronate	-OH	-CH ₃
Clodronate	-Cl	-Cl
Tiludronate	-H	-S- 
Pamidronate	-OH	-CH ₂ -CH ₂ -NH ₂
Neridronate	-OH	-(CH ₂) ₆ -NH ₂
Olpadronate	-OH	-(CH ₂) ₂ N(CH ₃) ₂
Alendronate	-OH	-(CH ₂) ₃ -NH ₂
Ibandronate	-OH	-CH ₂ -CH ₂ N 
Risedronate	-OH	
Zoledronate	-OH	

- first generation non-nitrogenous bisphosphonate
- FDA-approved for osteoporosis & hypercalcemia of malignancy
- used experimentally to deplete hematogenous peripheral macrophages in animals

Liposomal Clodronate

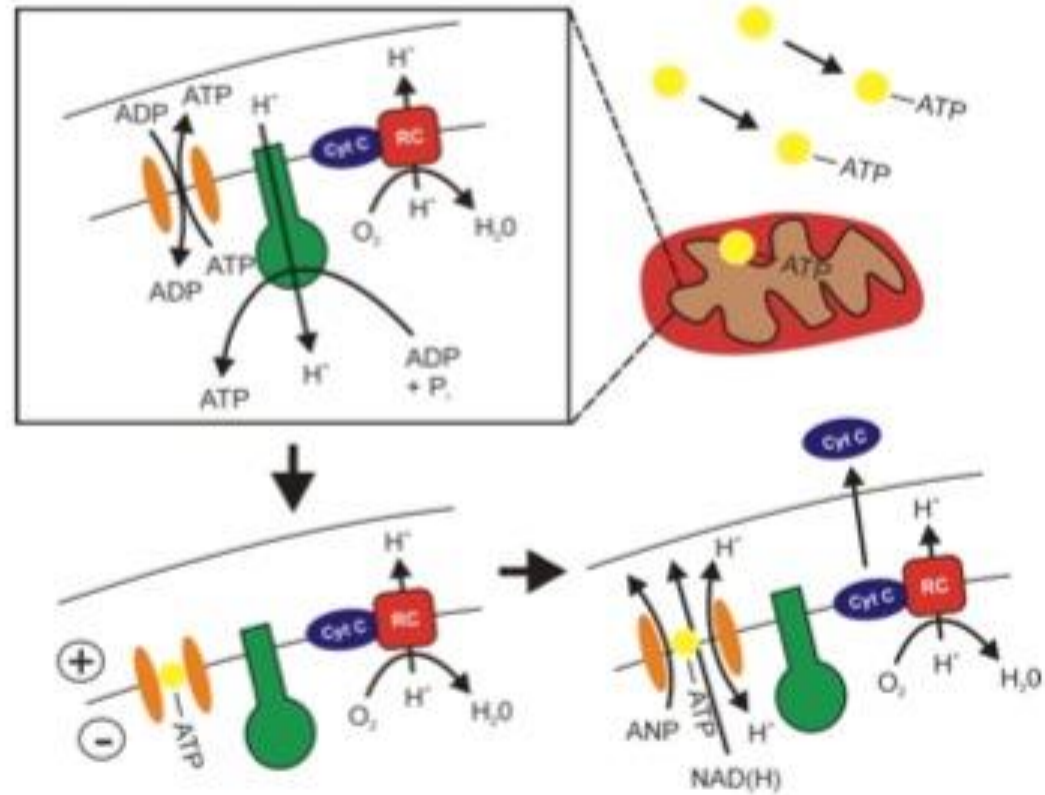
- clodronate is non-toxic
 - liposomes prepared with phosphatidylcholine and cholesterol (non-toxic)
 - free clodronate has extremely short $t_{\frac{1}{2}}$ (15 min)
 - does not cross phospholipid bilayers of liposomes and cell membranes
-
- i.p. injection \rightarrow intravenous circulation
 - clodronate-liposomes phagocytosed by peripheral macrophages
 - lysosomal degradation
 - intracellular release / accumulation of clodronate
 - induction of macrophage apoptosis



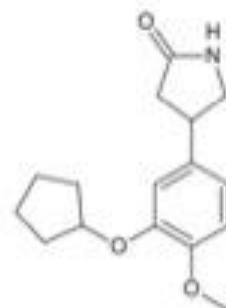
(*J Immunol Meth* 193: 93-99, 1996)

Clodronate-mediated Macrophage Apoptosis

- Converted to non-hydrolyzable ATP analog
- Blocks ATP-ADP translocator in inner mitochondrial membrane
 - disrupts proton gradient
 - release of cytochrome c
 - apaf-1/ caspase-activation
 - macrophage apoptosis



Rolipram: A Phosphodiesterase Inhibitor



Effects of Rolipram:

- Neuroprotection / Axonal regrowth
 - cAMP elevation
 - Potentiation of neurotrophins
 - Prevention of Ca^{2+} influx and overload (PKA-mediated)
 - Anti-apoptotic effects (MAPK/CREB pathway)
- Anti-inflammatory / Immunomodulation
 - decreases TNF- α production (inhibits NF- κ B activation)
 - decreases ICAM-1 (neutrophil adhesion)
 - decreases MMP-9 expression (cell migration)

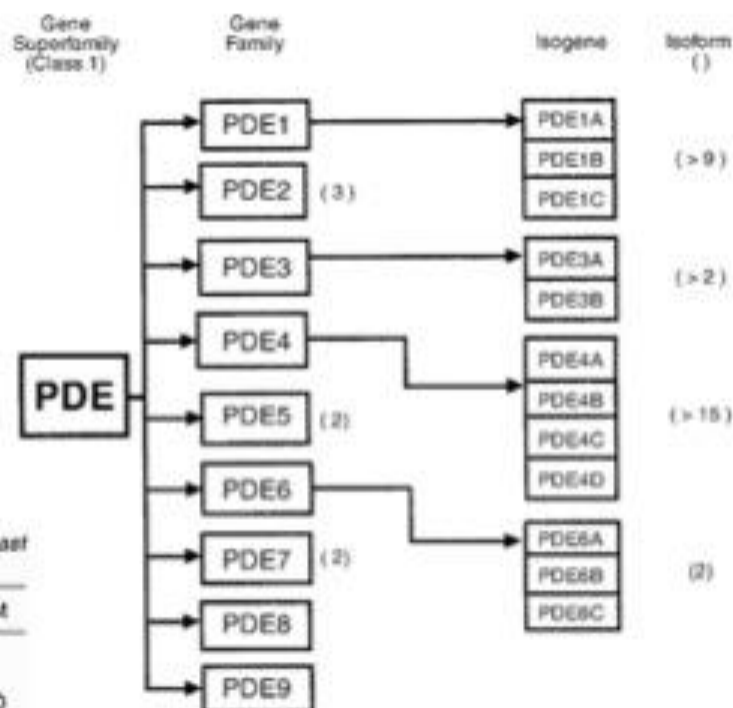
A specific phosphodiesterase (PDE4) inhibitor

Table 1: Comparison of the biochemical potency and selectivity of roflumilast, its major in vivo metabolite roflumilast N-oxide, rolipram and cilomilast.

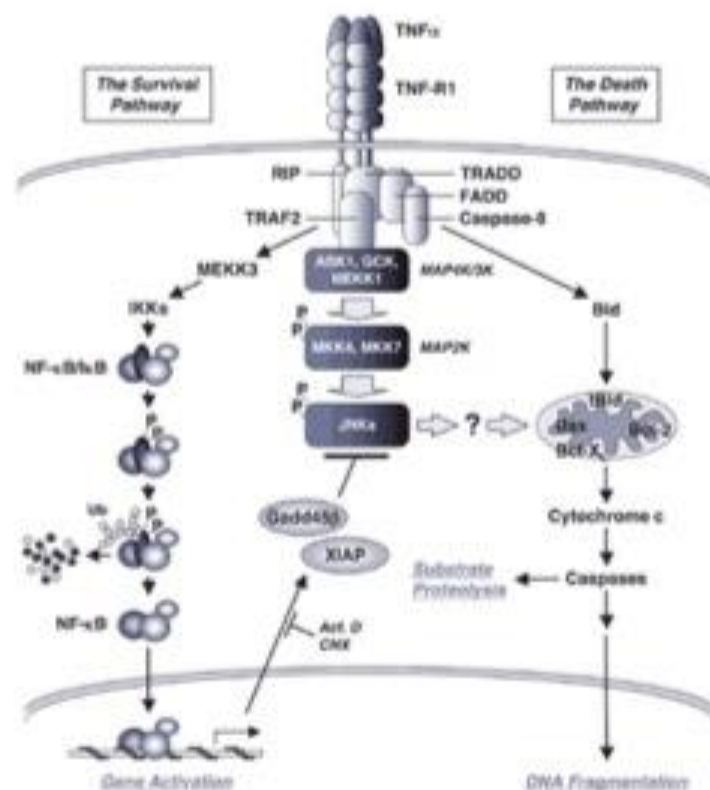
	Roflumilast	Roflumilast N-oxide	Rolipram	Cilomilast
PDE1	>10,000	>10,000	>100,000	74,000
PDE2	>10,000	>10,000	>100,000	65,000
PDE3	>10,000	>10,000	>100,000	>100,000
PDE4	0.8	2	210	120
PDE5	8,000	>10,000	>100,000	83,000

Data represent IC_{50} values given in nM and are taken from ref. 46.

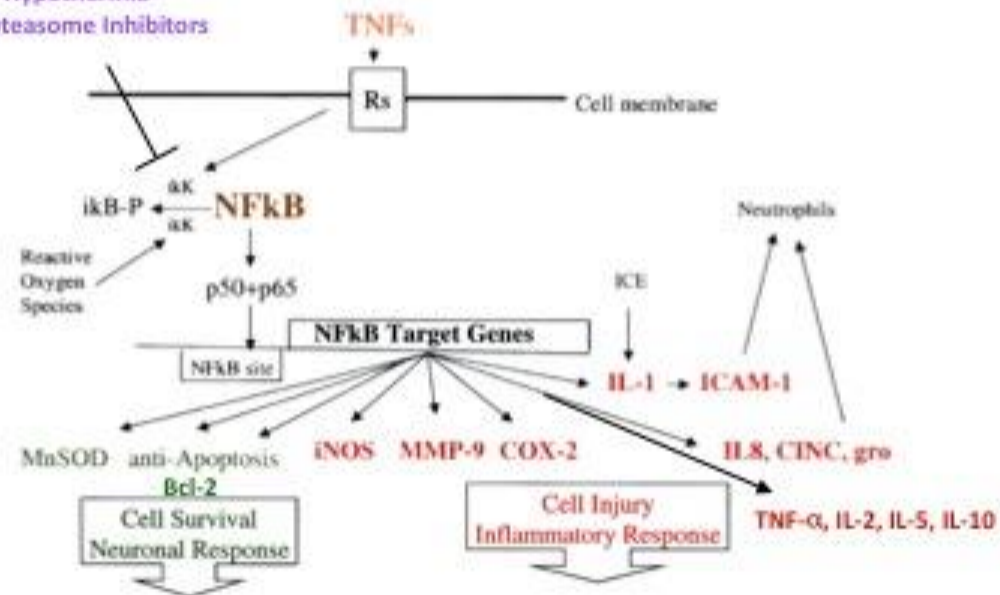
PDE gene family



Rolipram-Mediated Inhibition of NF- κ B Binding: Target Genes

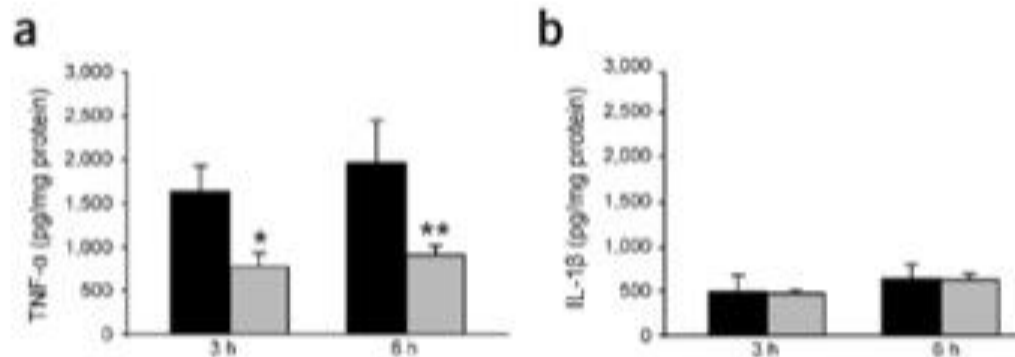


- Rolipram
- Hypothermia
- Proteasome Inhibitors

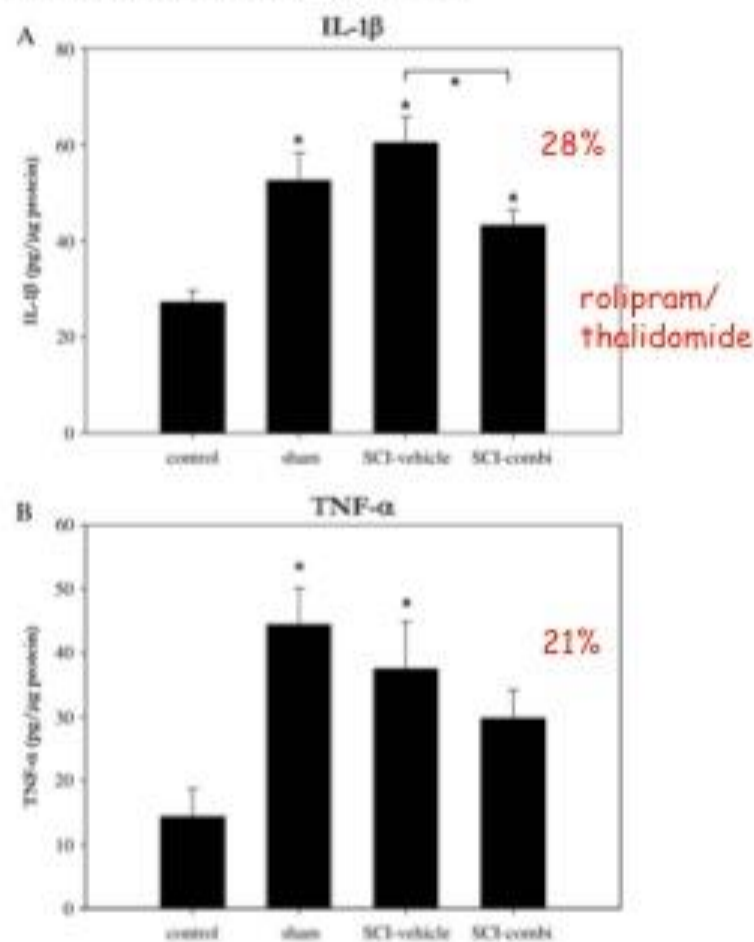


Sharp FR et al., 2000. Multiple Molecular Penumbra After Focal Cerebral Ischemia. *Journal of Cerebral Blood Flow & Metabolism* 20, 1011-1032

Rolipram decreases pro-inflammatory cytokine production following contusion SCI



Pearse et al. (2004) cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. Nat Med 10:610-616



Koopmans et al. (2009) Acute rolipram/thalidomide treatment improves tissue sparing and locomotion after experimental spinal cord injury. Exp Neurol 216:490-498

Methods

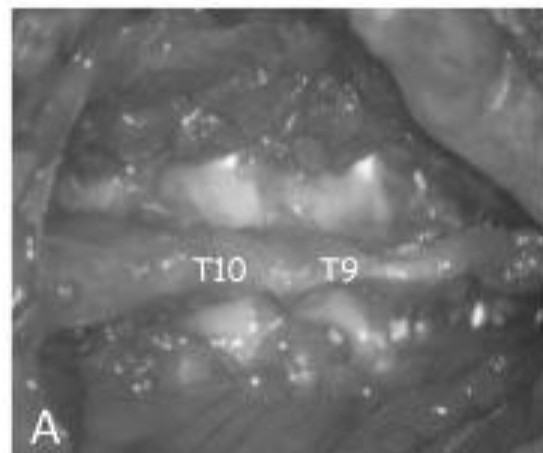
- Female SD rats (220- 250 grams)
- Moderate contusive SCI at T8 using NYU/ MASCIS II impactor
- **Animal groups** (n=10 per group):
 - 1) SCI controls: mini-osmotic pumps w/ DMSO (n=10 per group)
 - 2) liposomal clodronate (0.25 mg/mL; 2 mL i.p.) delivered immediately after injury and on post-injury days 1, 3, and 6
AND / OR
 - 3) rolipram via implanted mini-osmotic pumps (Model 1007D; 0.5 μ L/hr, (0.5 mg/kg/day, 2 week delivery) in DMSO
- **BBB open-field testing:**
 - performed on post-injury days 1, 7, 14, 21, and 28
 - blinded
-

Methods

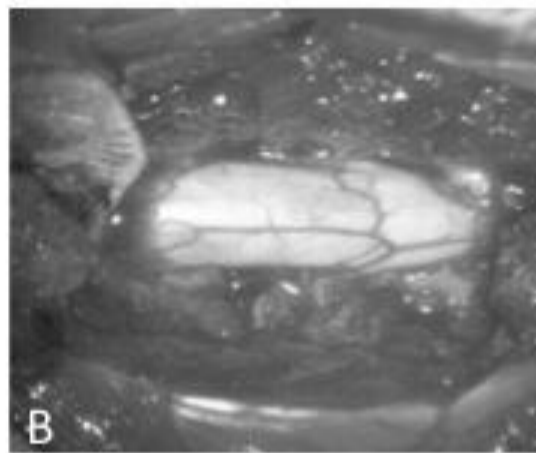
- **Retrograde Tracing** (n=4 per group):
 - 4% Fluorogold injected into rostral lumbar enlargement (L1 cord)
 - survival for additional week to allow for retrograde transport
 - sacrifice at five weeks
- **Histology**
 - Modified Eriochrome-Cyanine (EC) staining (myelin)
 - Lesion Volume
 - % Lesion Area at Epicenter
 - Lesion Area, spared WM, spared GM
 - plotted as a function of distance from injury epicenter
- **Immunohistochemistry**
 - ED-1 (CD-68) - macrophages
 - glial fibrillary acidic protein (GFAP) - astrocytes
 - vimentin - fibroblasts/ECM
 - neurofilament (NF)-H - axons
 - myelin basic protein (MBP) - oligodendrocyte myelin

Experimental Contusion SCI

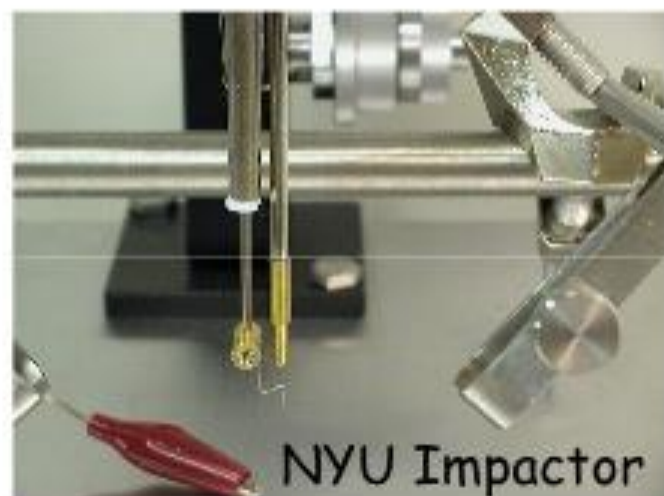
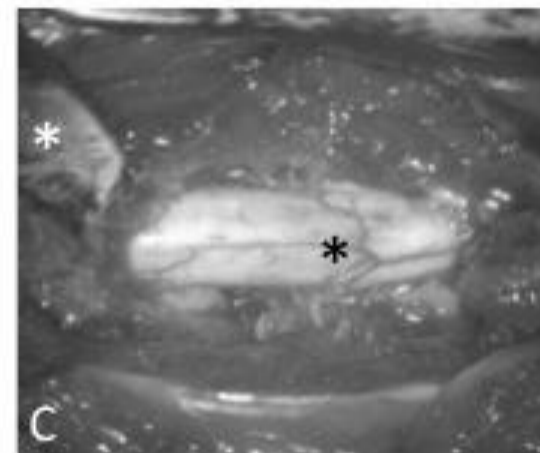
Exposure



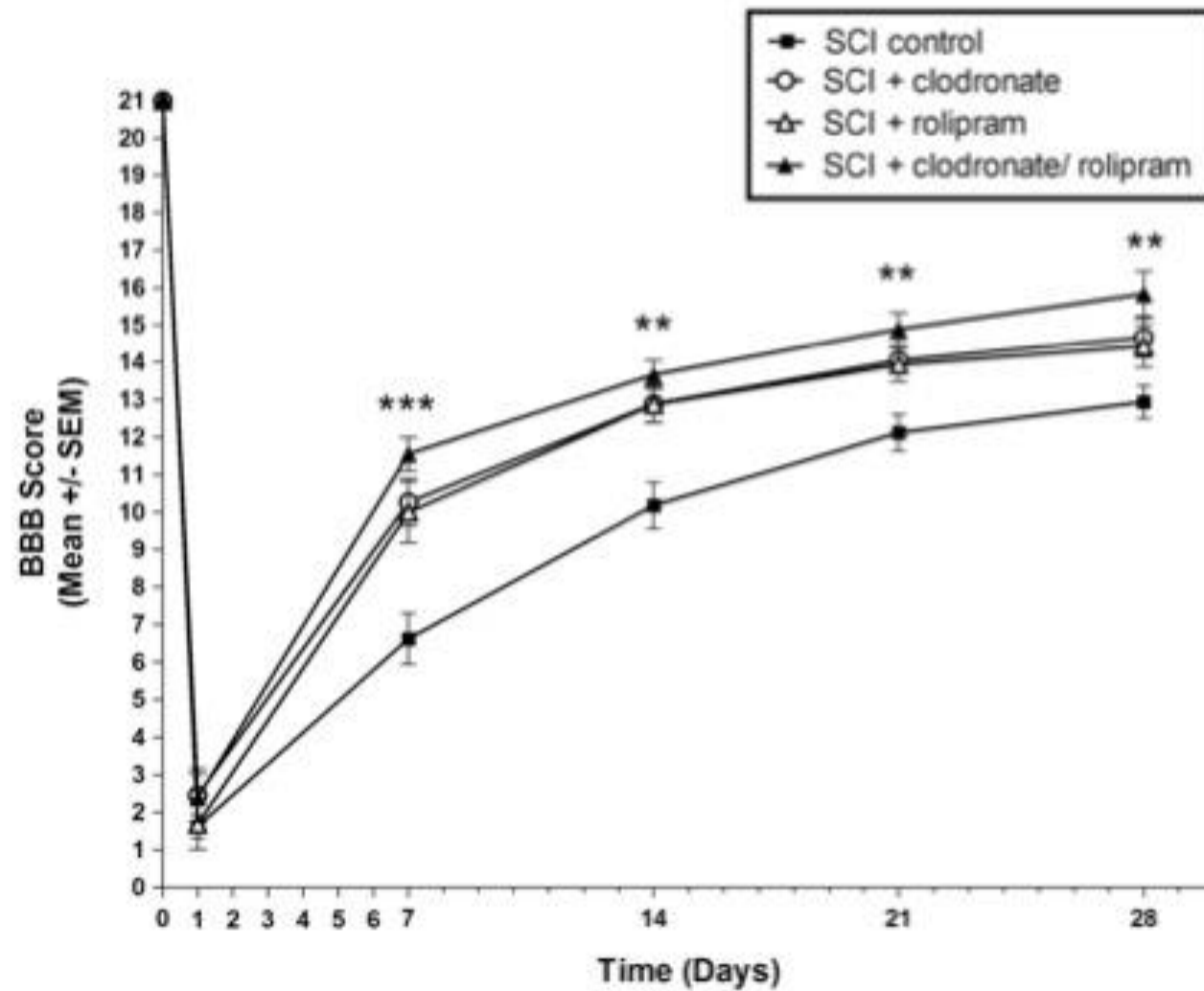
Laminectomy



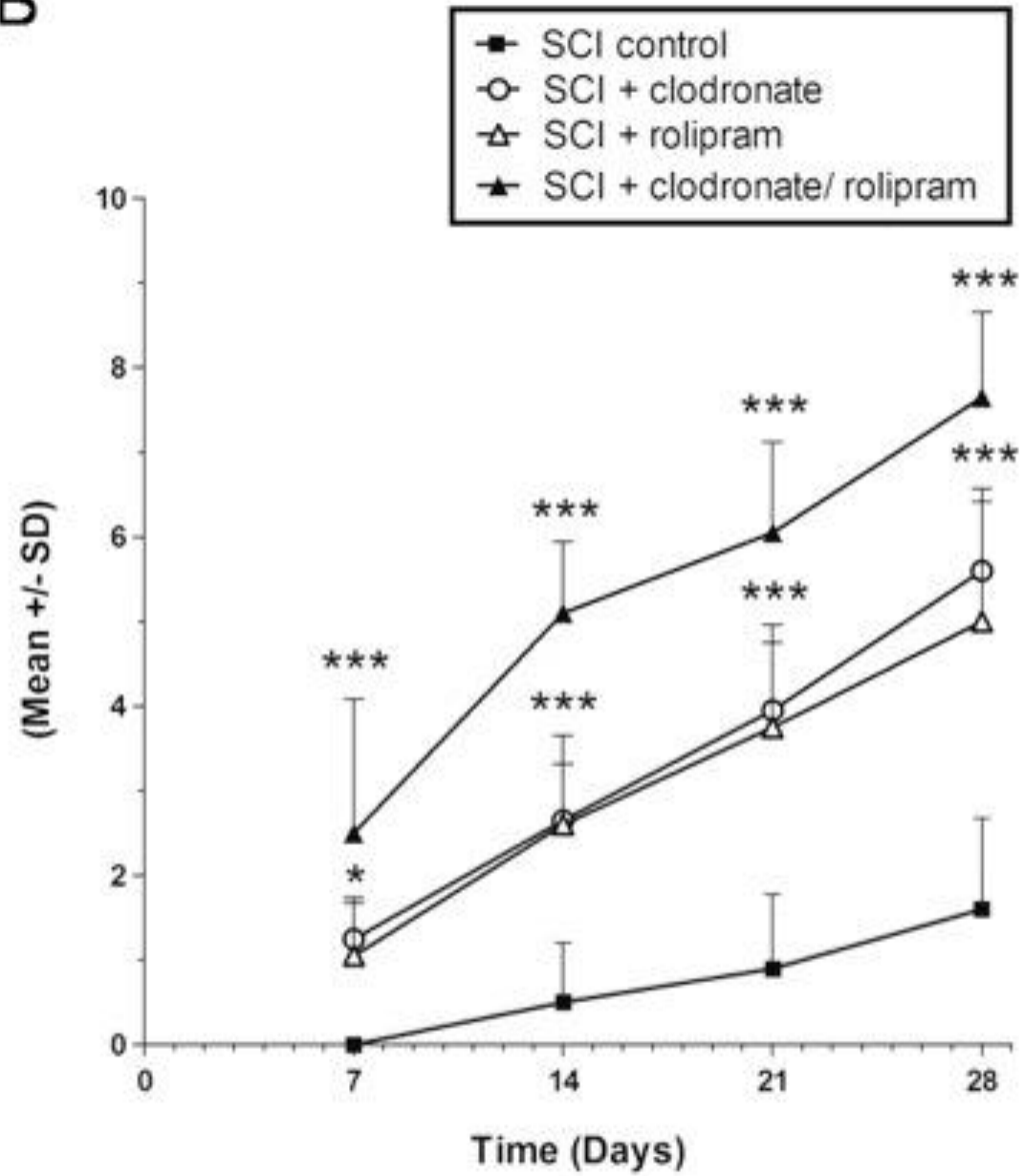
Contusion

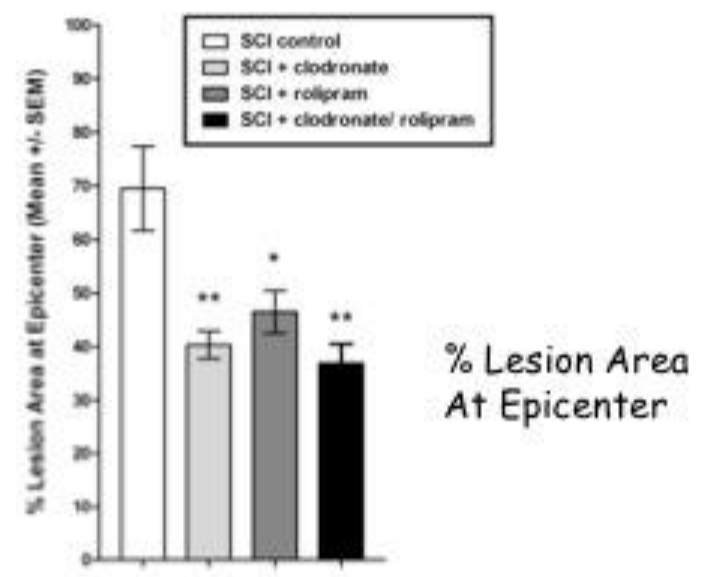
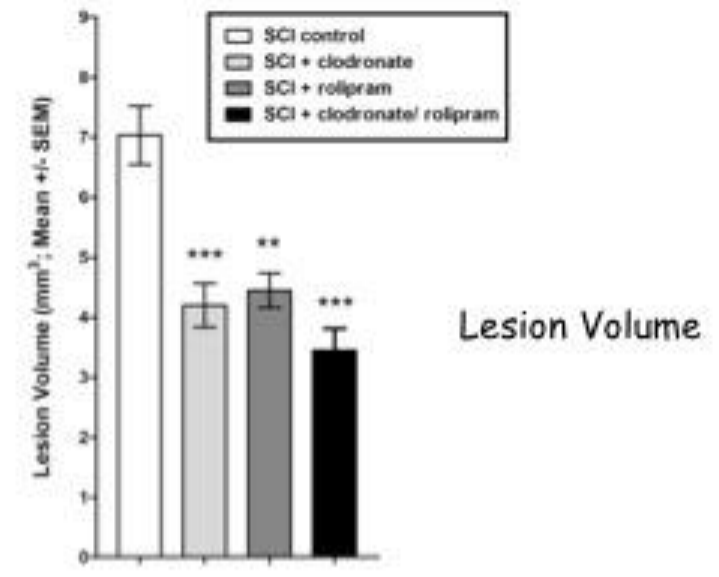
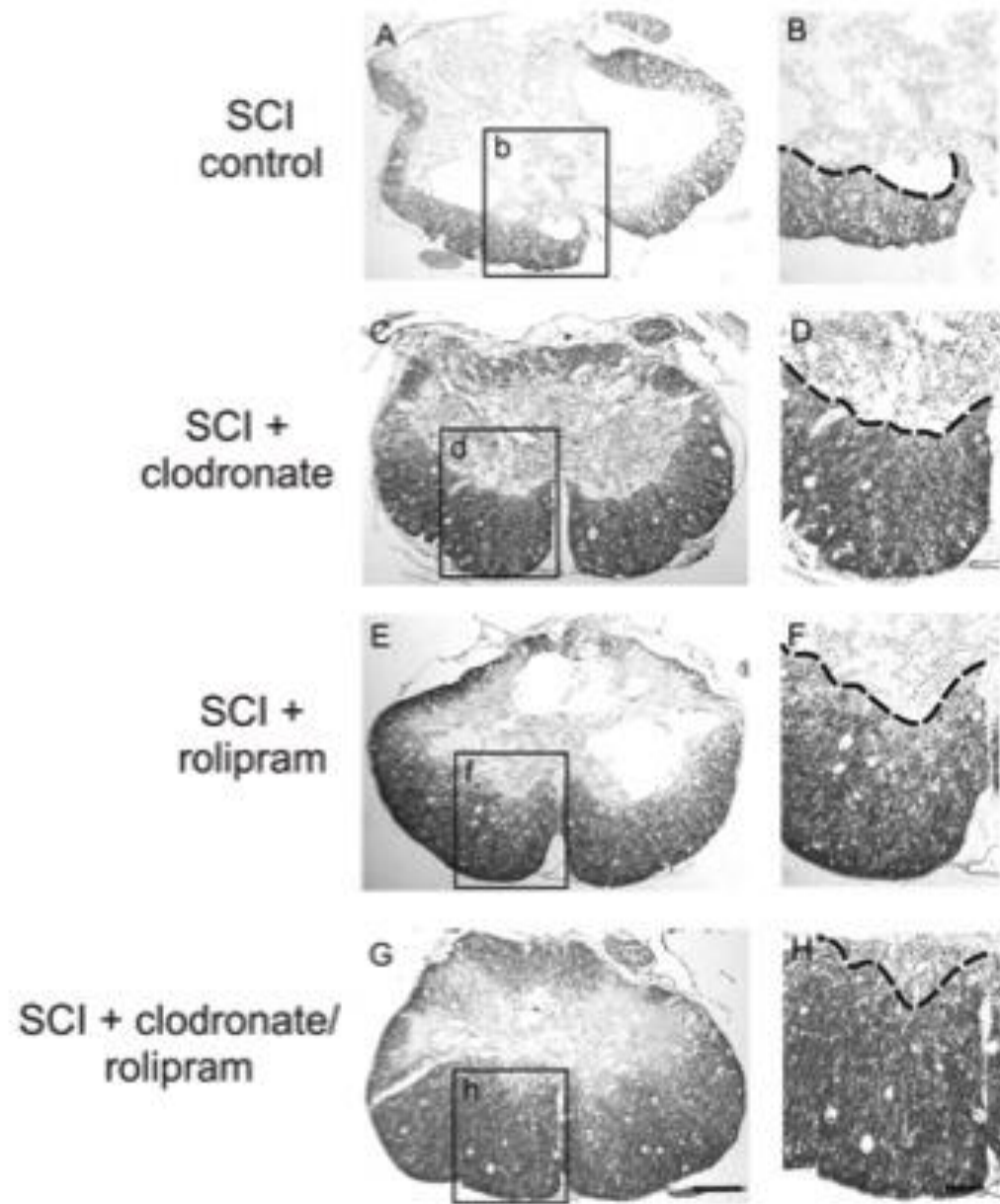


BBB Open-Field Locomotor Testing



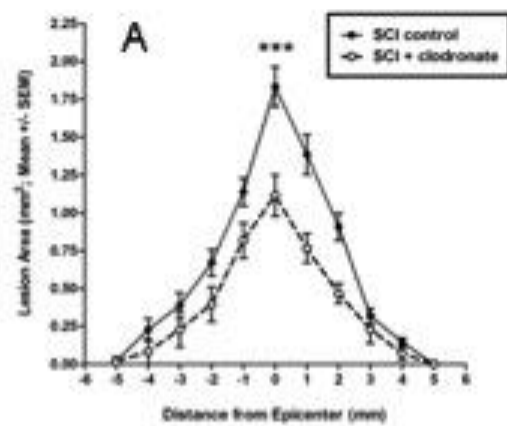
B



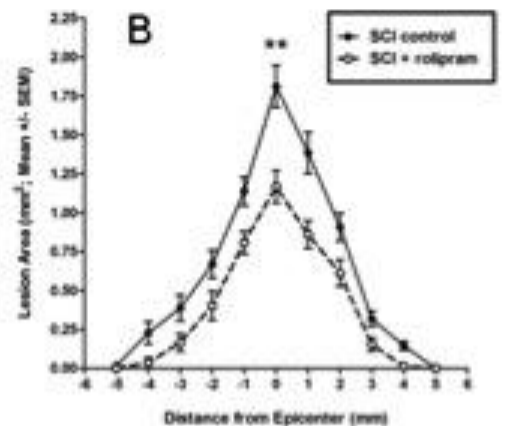


clodronate vs control

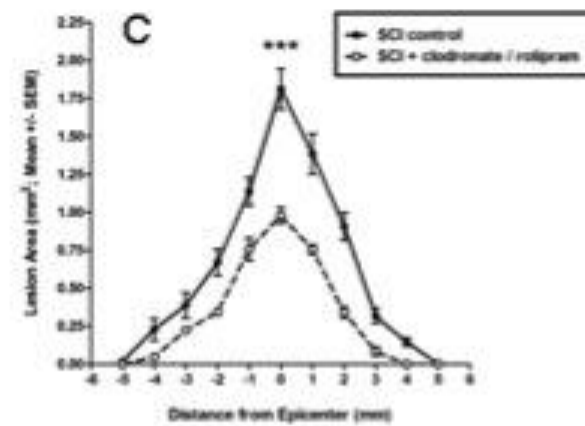
Lesion Area



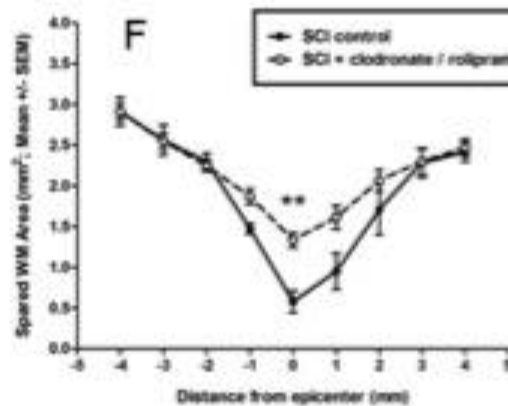
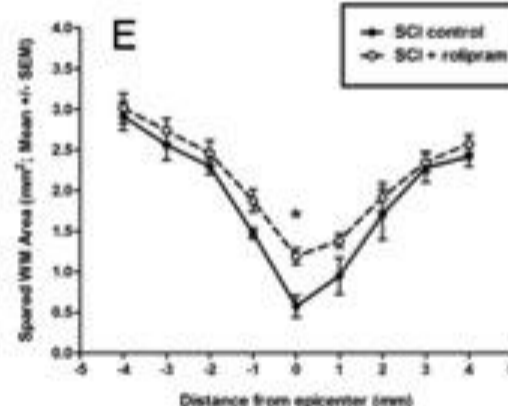
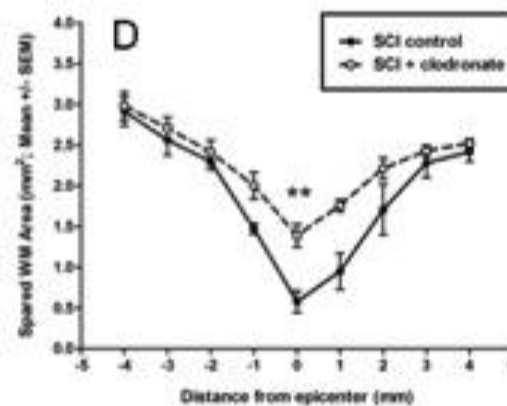
rolipram vs control



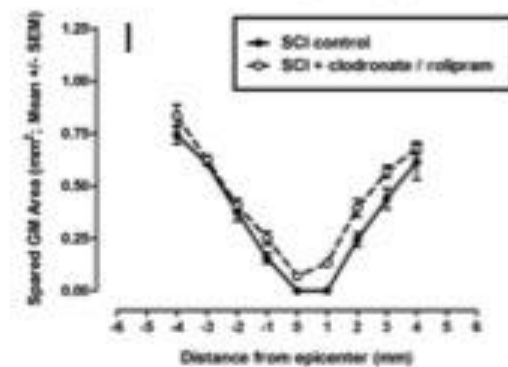
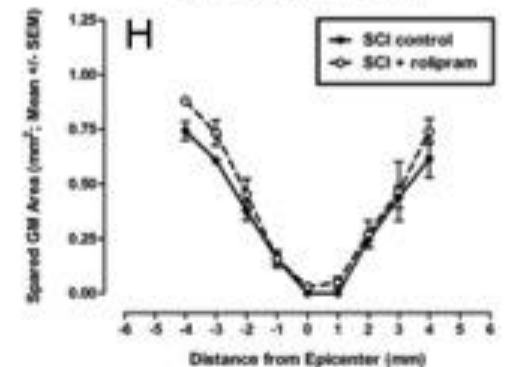
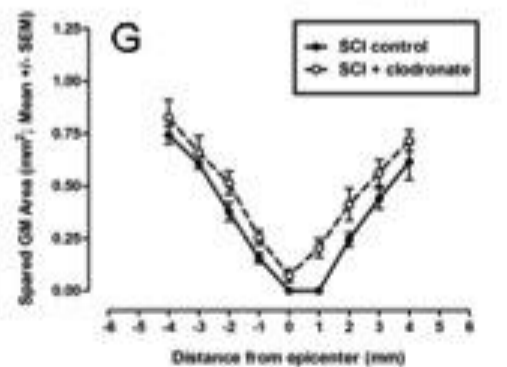
clodronate + rolipram vs control



Spared WM

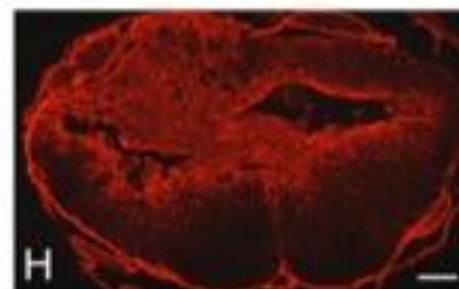
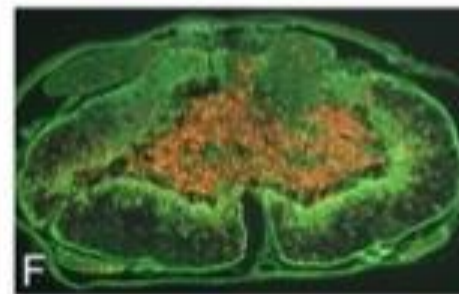
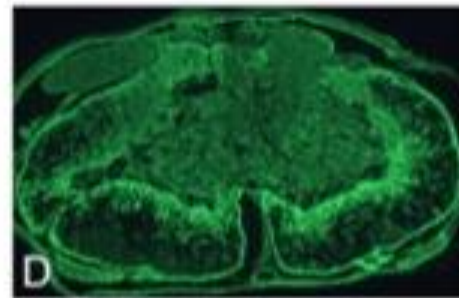
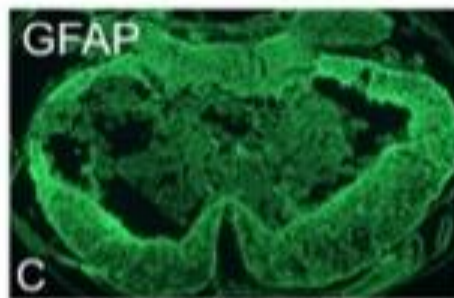
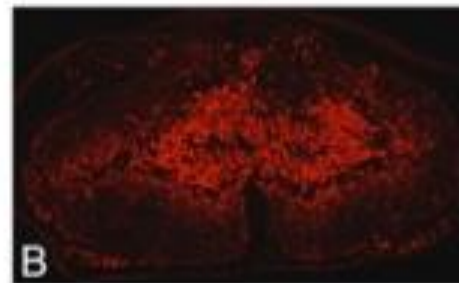
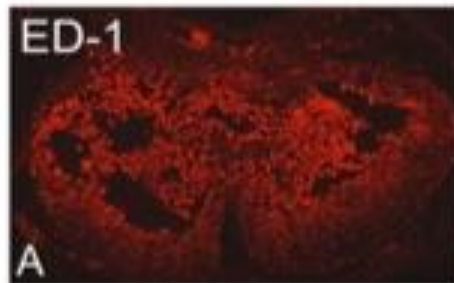


Spared GM



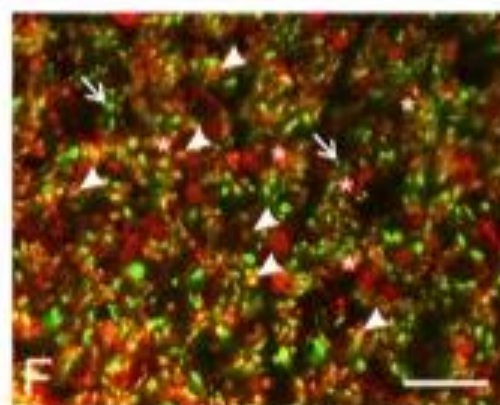
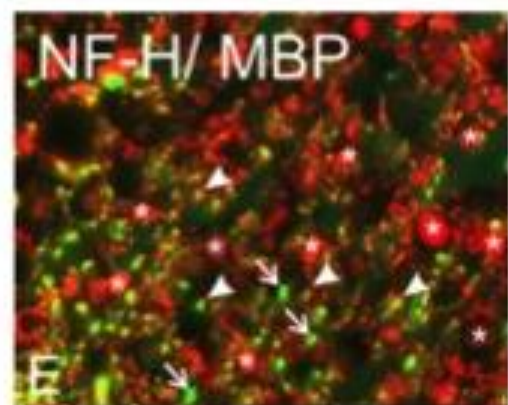
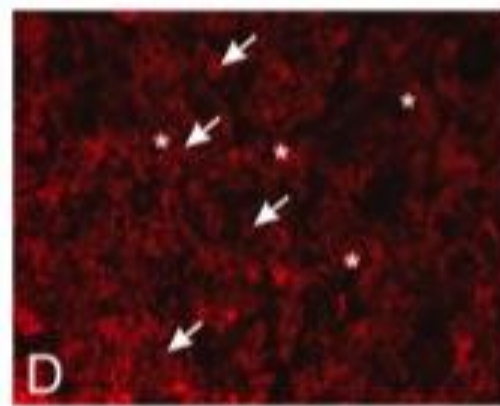
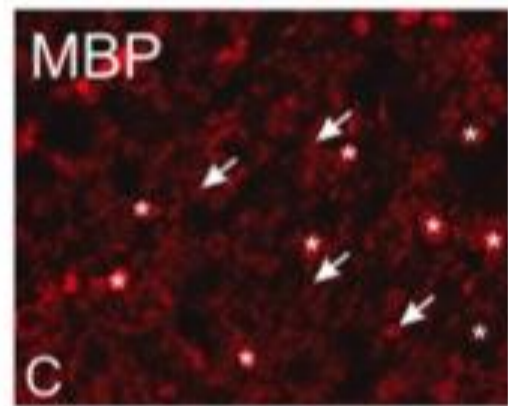
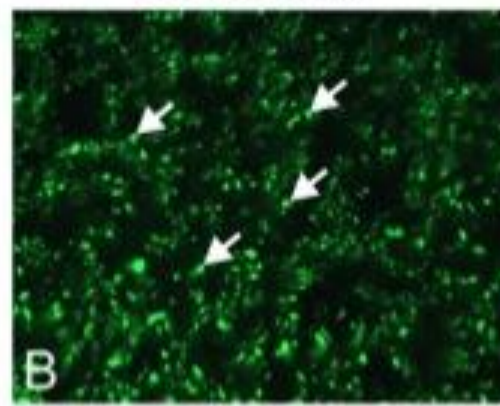
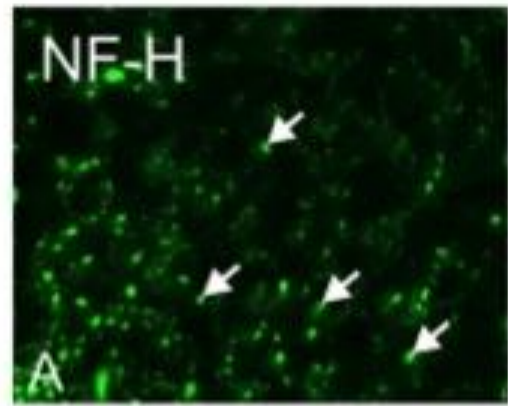
SCI control

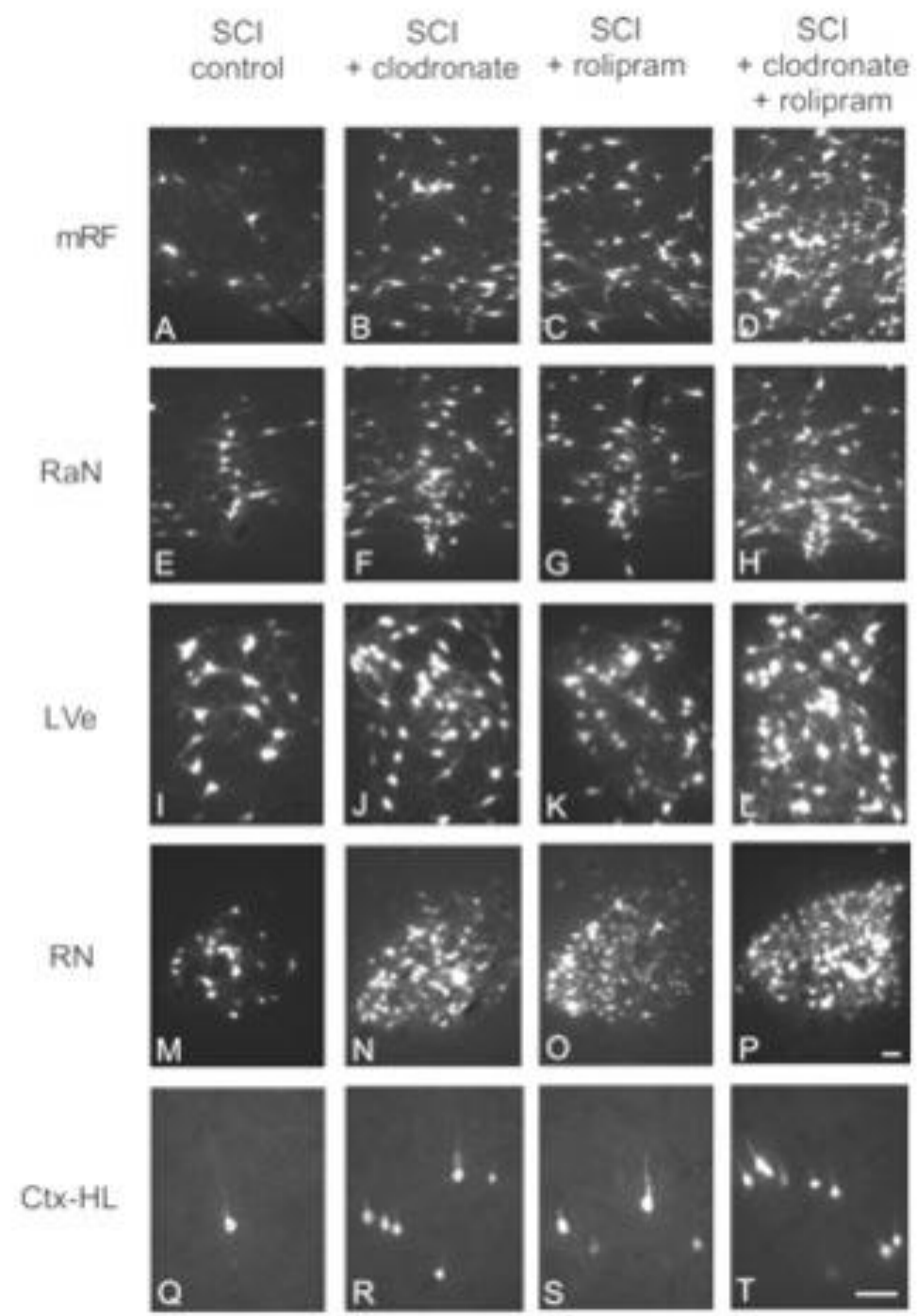
SCI + clodronate
+ rolipram

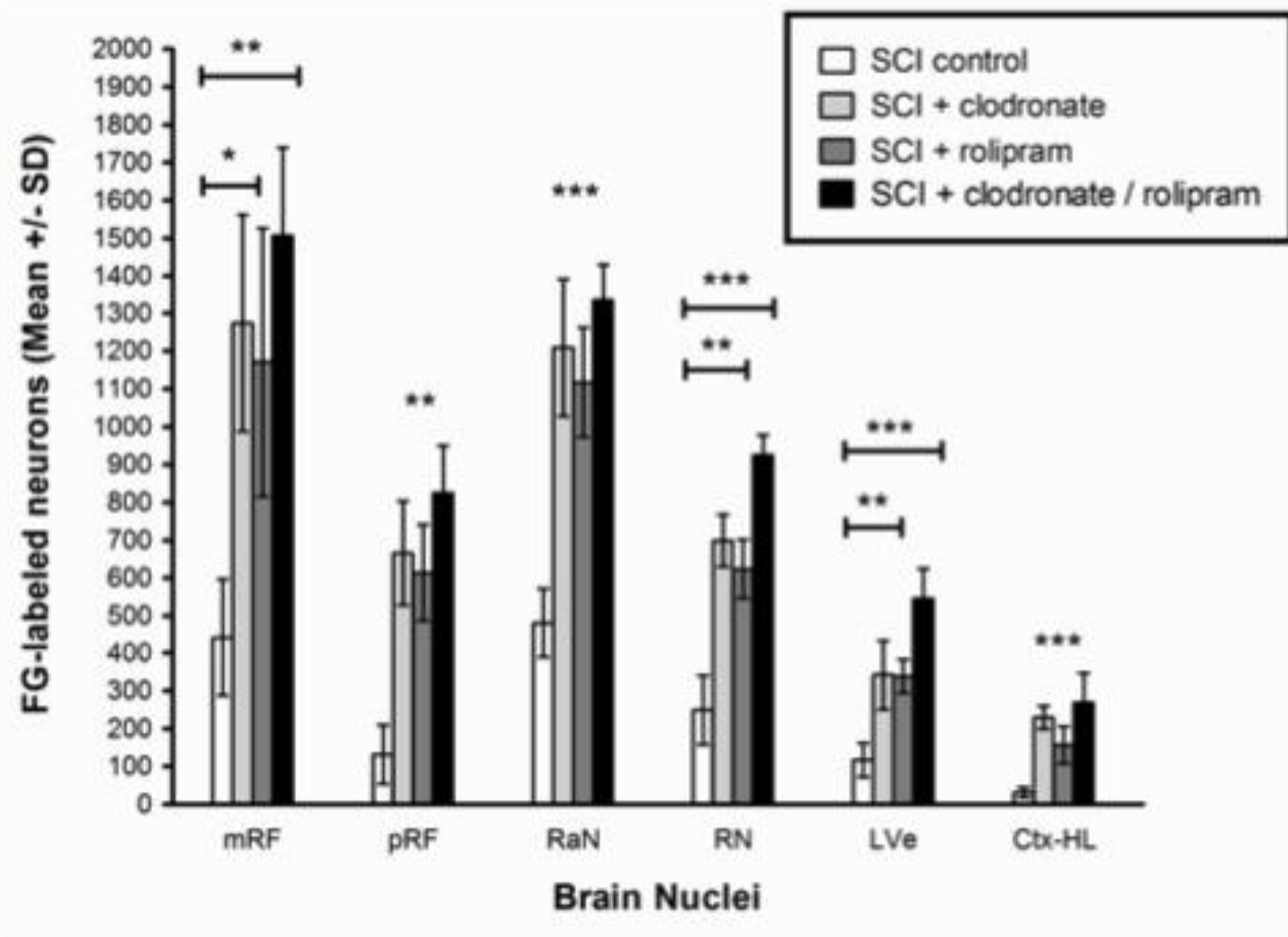


control

clodronate/
rolipram







Conclusions

- Immunomodulation with clodronate and rolipram after contusive SCI:
 - promotes neuroprotection/myelinated tissue sparing
 - promotes sparing of supraspinal axons below the injury
 - decreases ED-1⁺ macrophage infiltration at 5 weeks post-injury
 - promotes significant locomotor recovery, seen as early as one week following injury
 - Neuroprotection within days of treatment