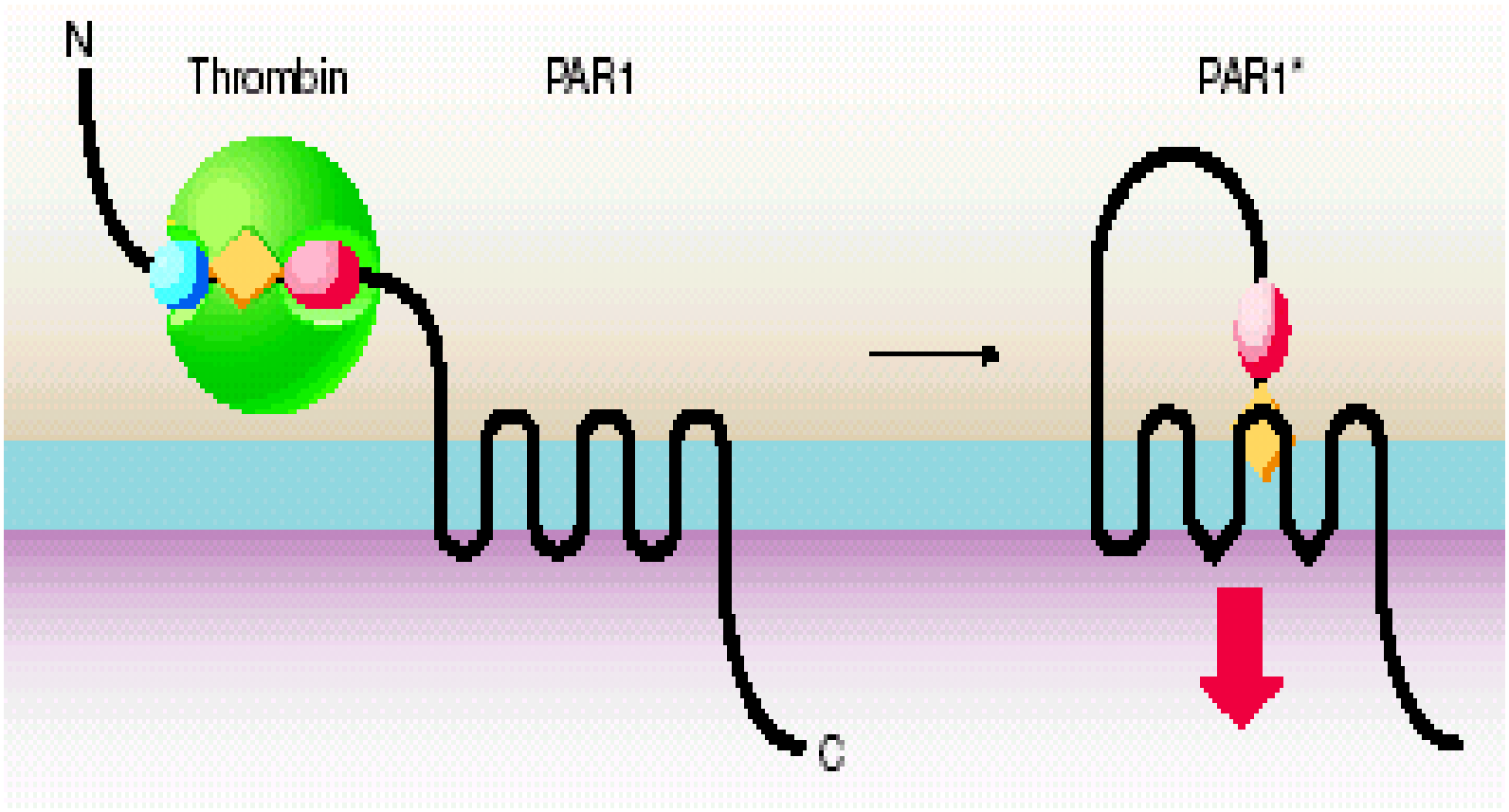
PAR-1 on the Astrocyte End Foot: A New Target for Pharmacological Intervention in Neurological Diseases

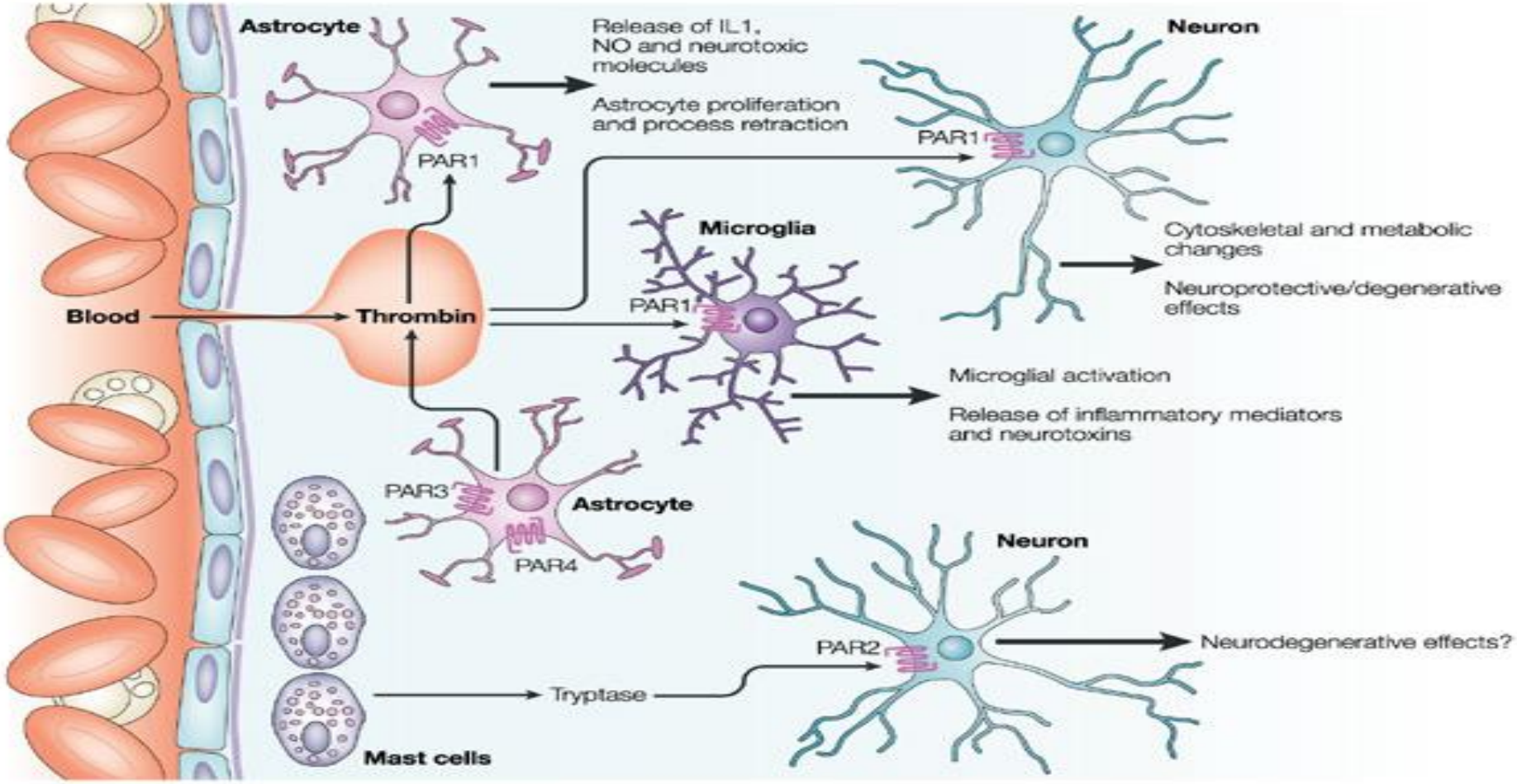
התערבות פרמקולוגית ייחודית במסלול הפעלת הרצפטור לתרומבין – בסיס חדש לטיפול בפגיעה עצבית

Joab Chapman, Efrat Shavit.

PAR: Protease-Activated Receptor

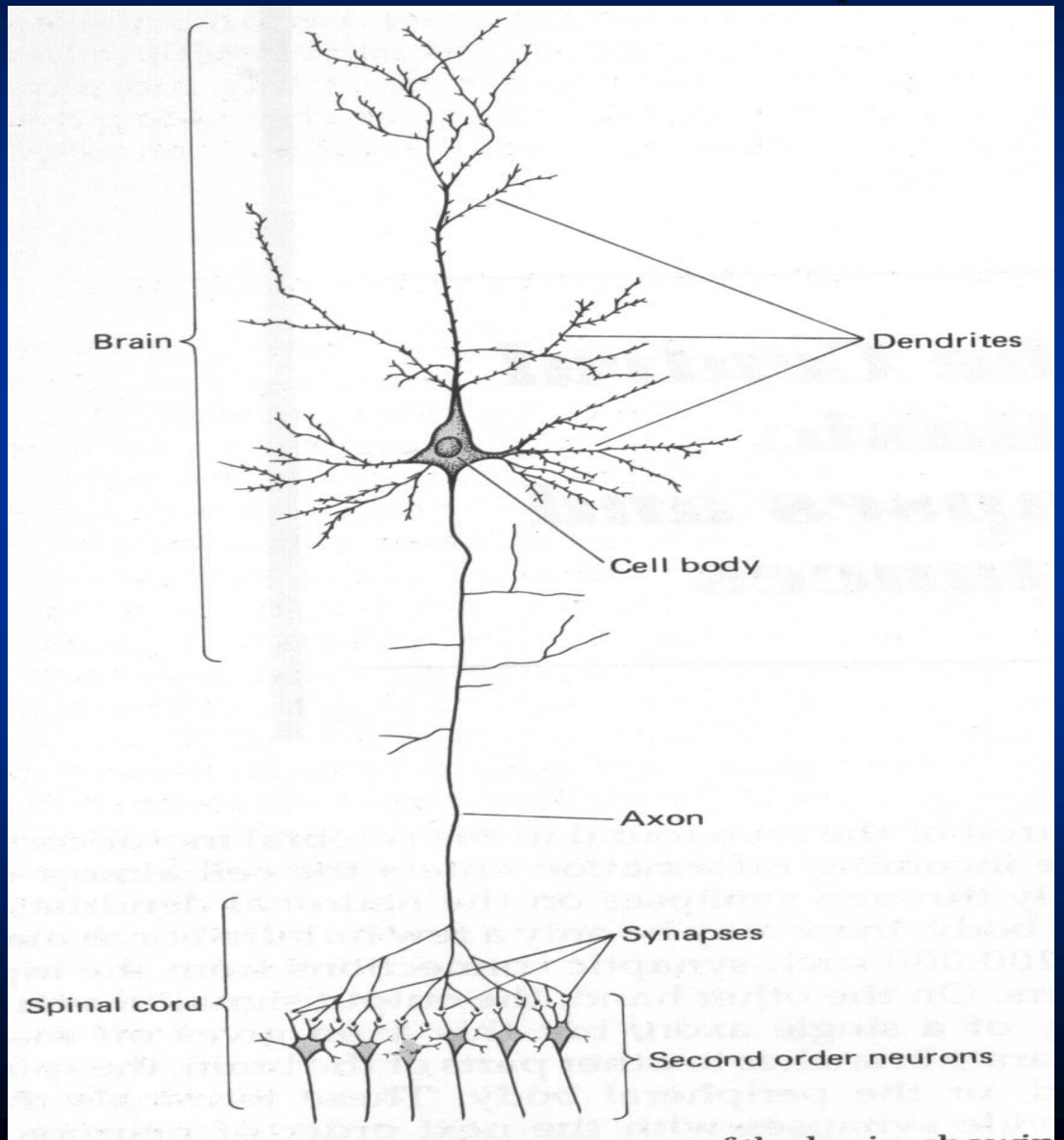


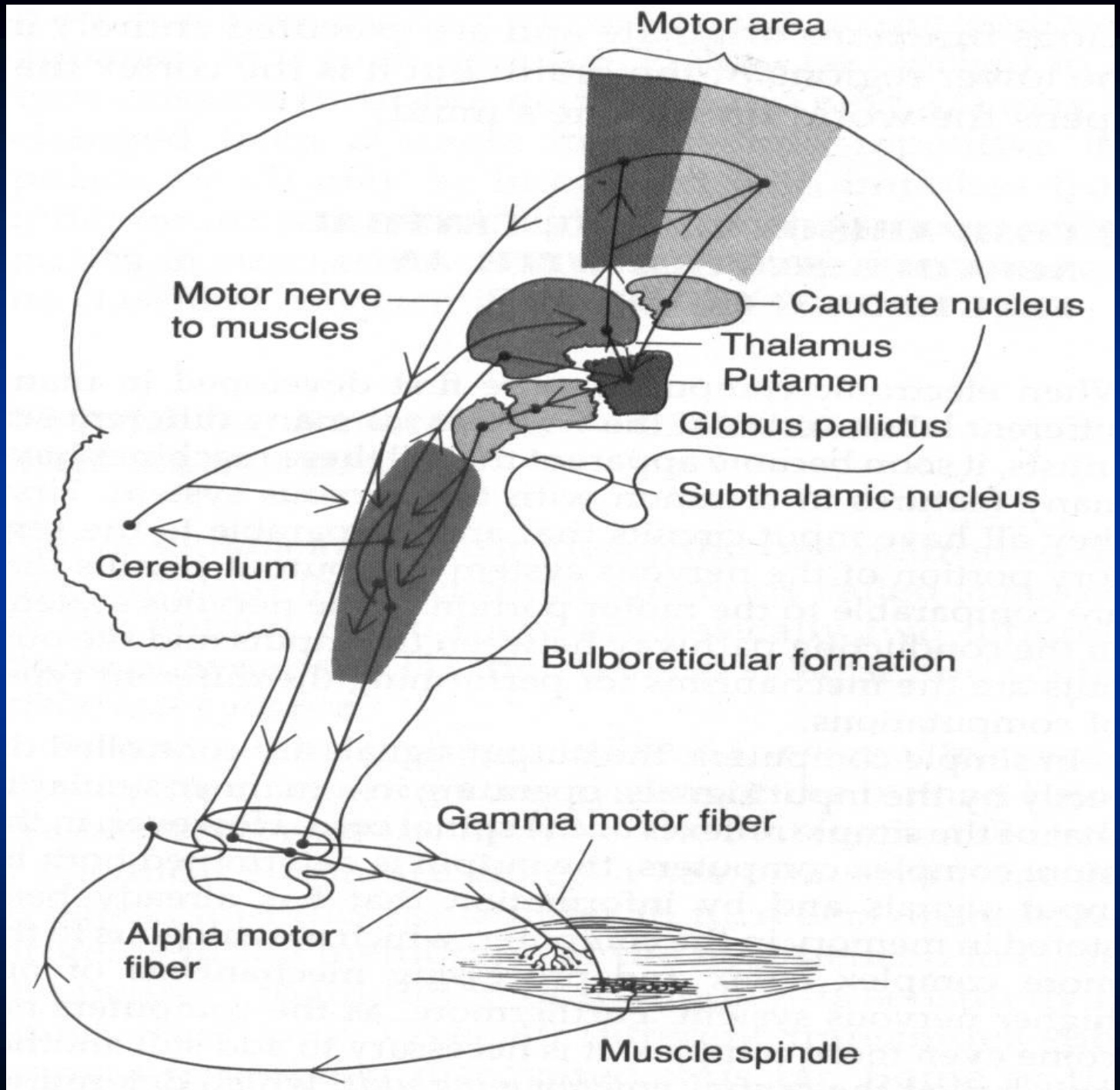
Development of novel PAR-1-based therapeutic compounds for neuro-inflammatory and malignant diseases: diabetic neuropathy and glioblastoma multiforme



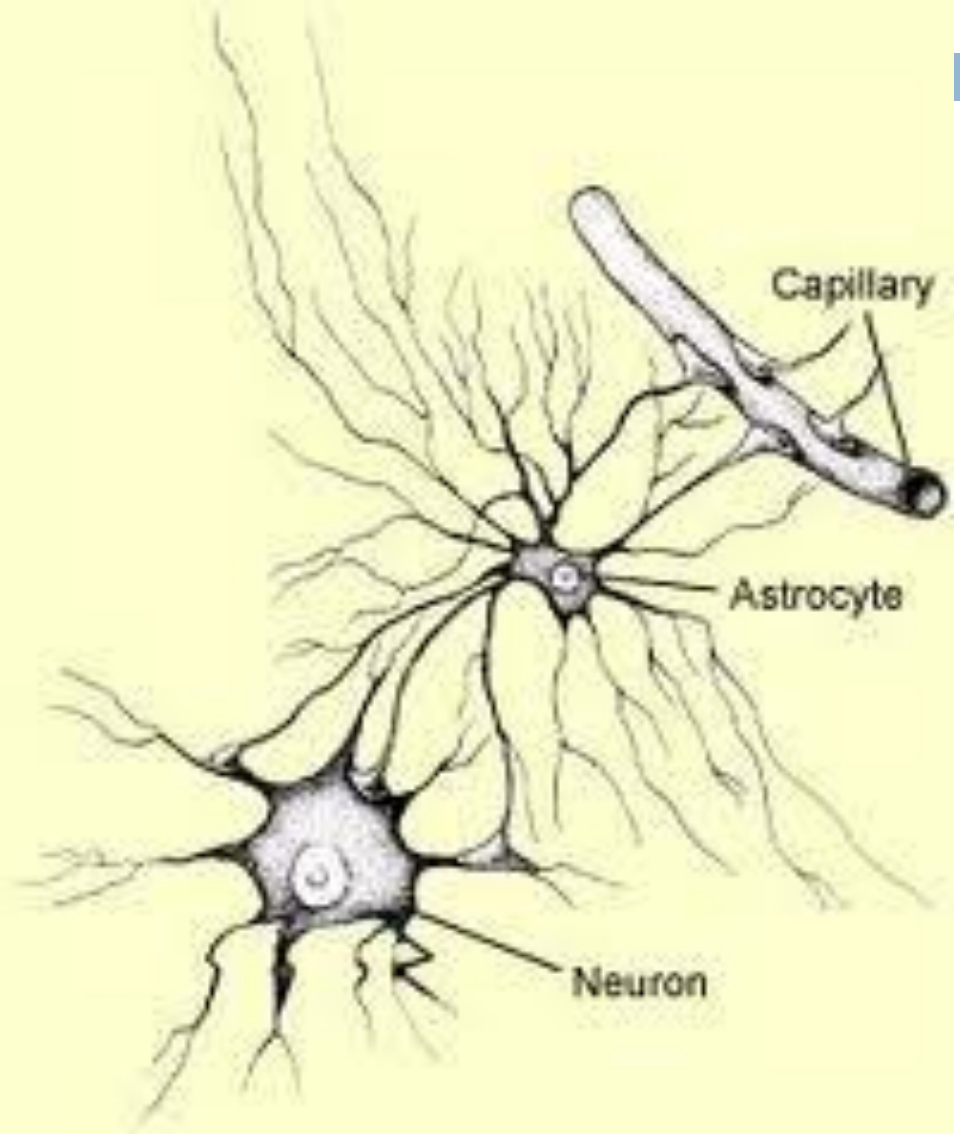
The Neuron

The functional unit of the nervous system

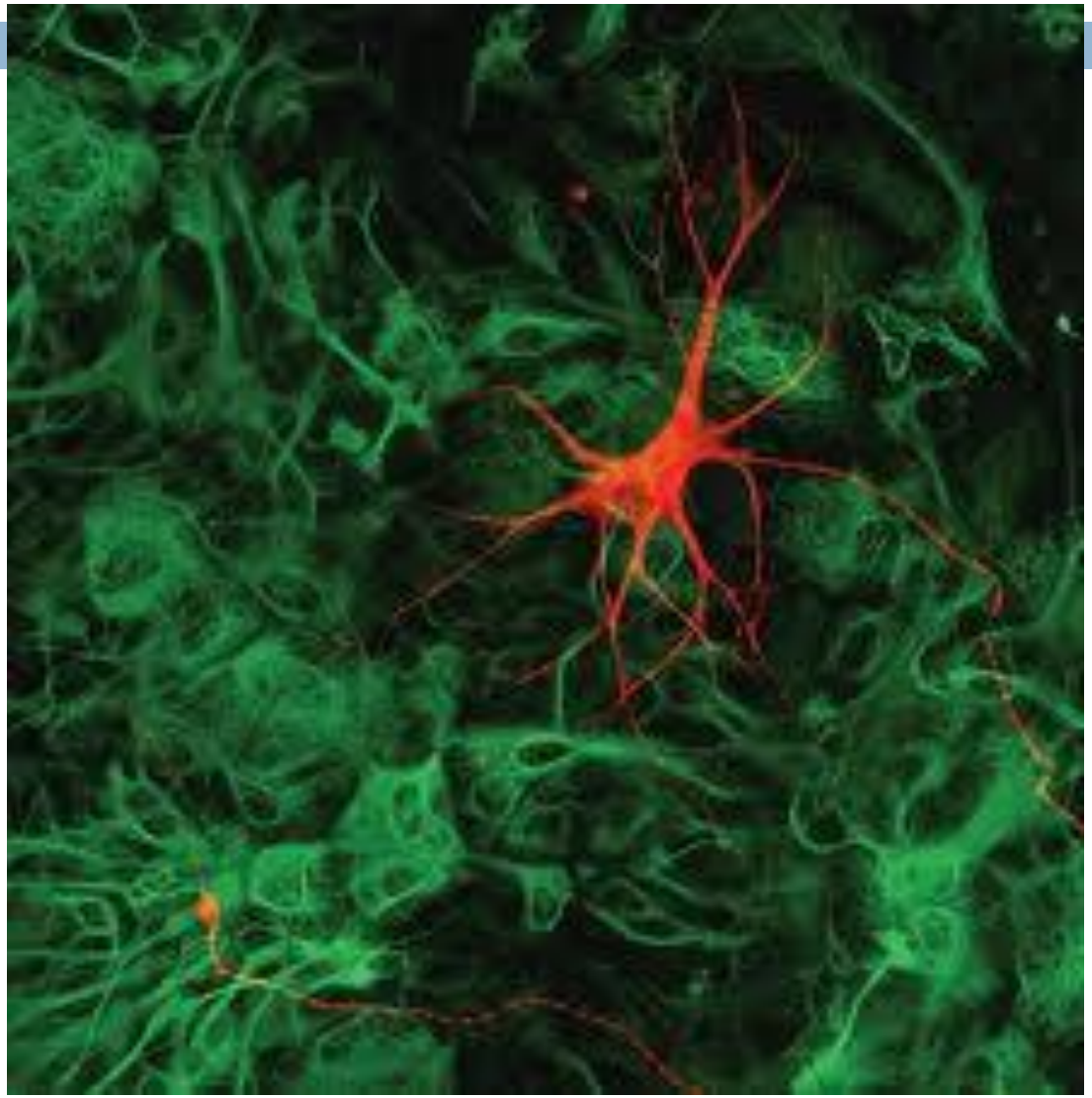



Motor area
Motor nerve to muscles
Caudate nucleus
Thalamus
Putamen
Globus pallidus
Subthalamic nucleus
Cerebellum
Bulboreticular formation
Gamma motor fiber
Alpha motor fiber
Muscle spindle

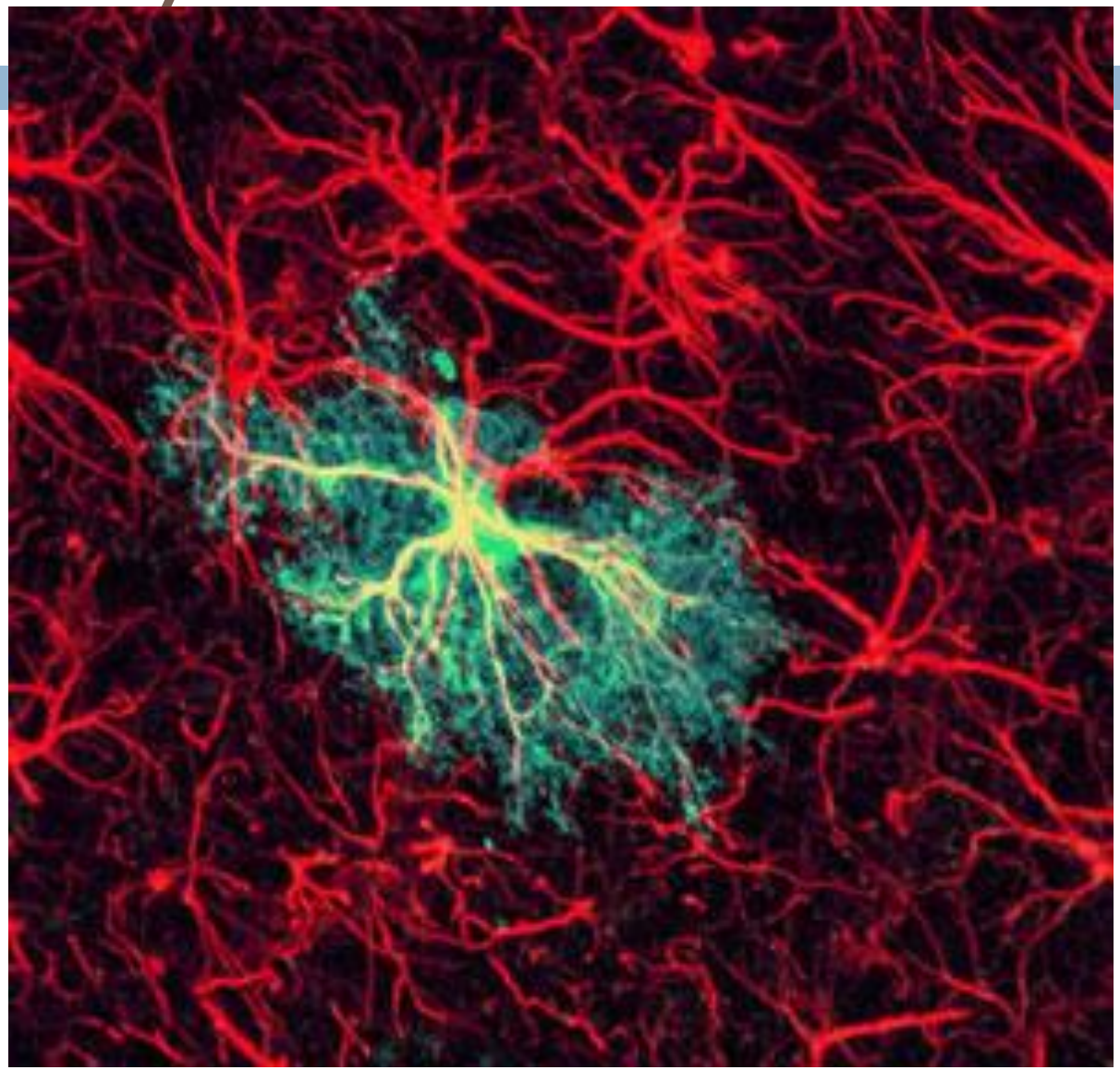
Scheme of Astrocyte with Neuron



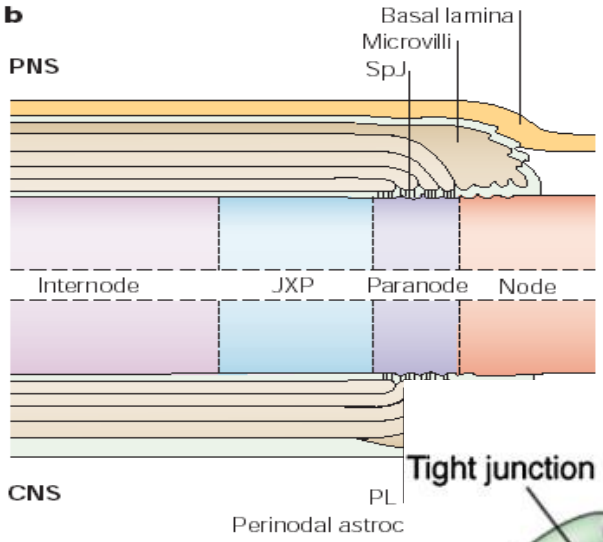
Motor Neuron in Cell Culture

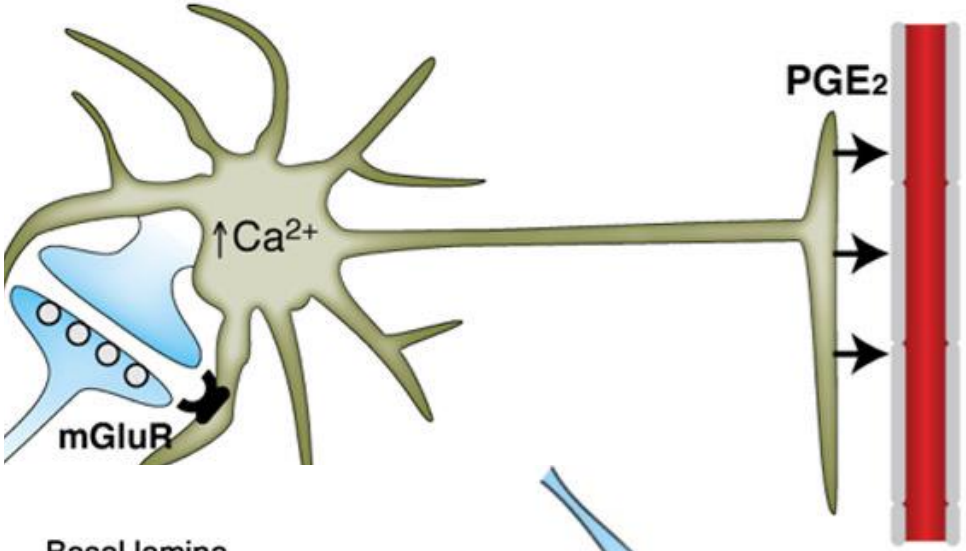


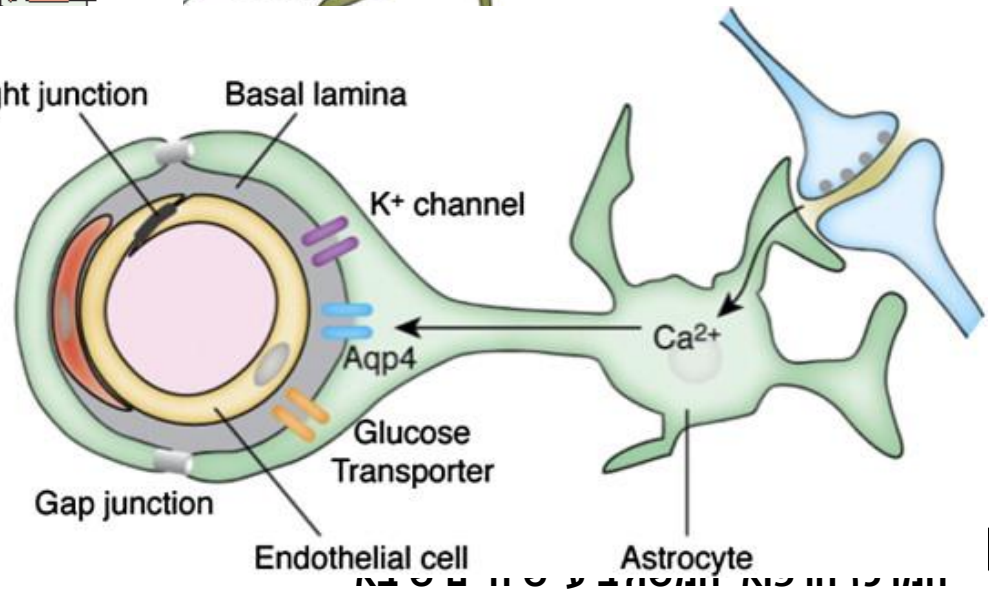
Astrocyte in Brain with Neurons



Glial endfeet at synapse and node of Ranvier

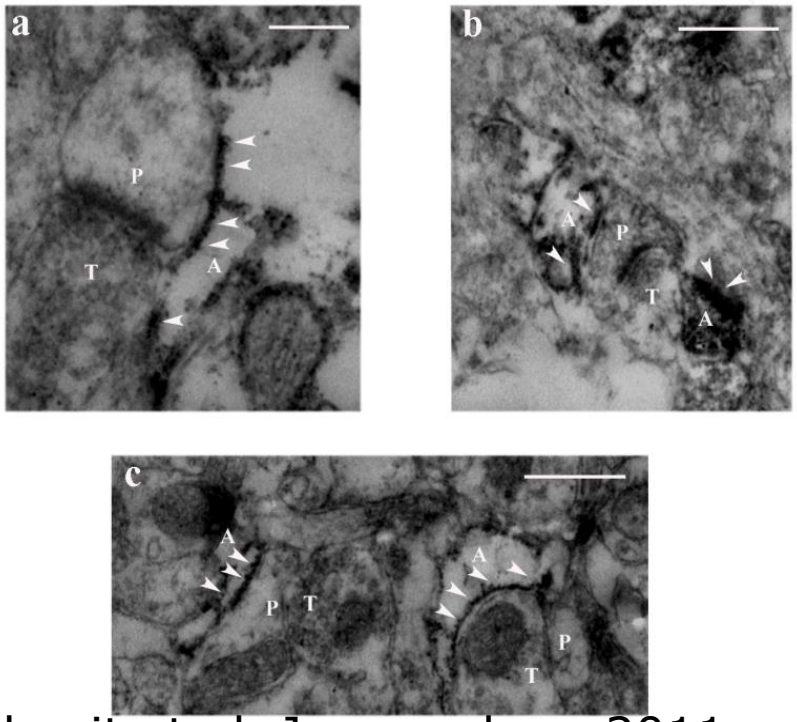




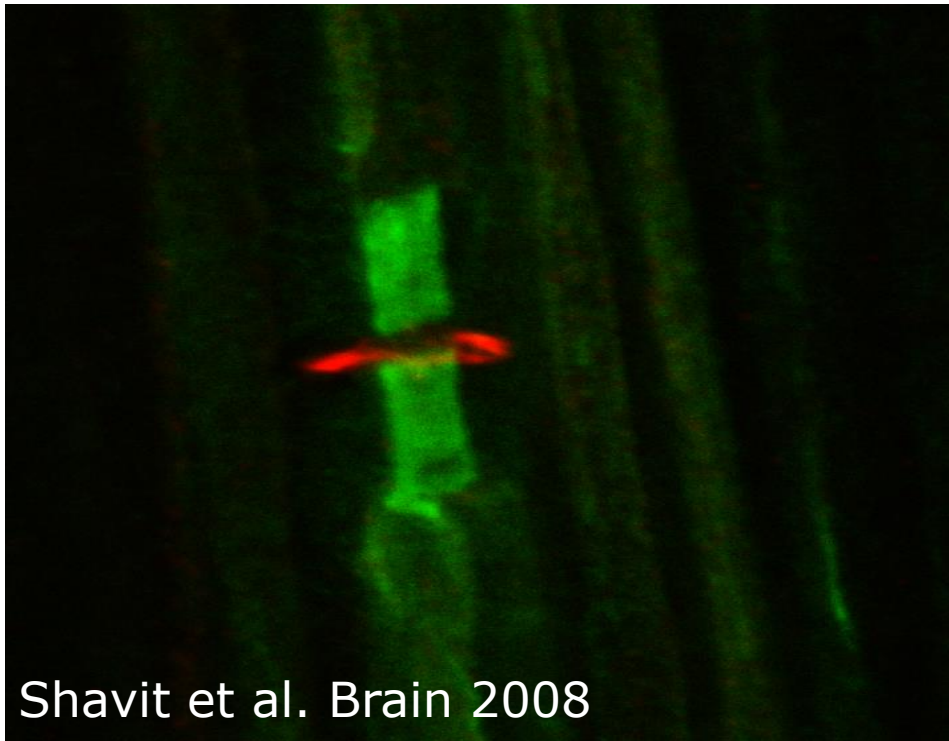


Development of novel PAR-1-based therapeutic compounds for neuro-inflammatory and malignant diseases: diabetic neuropathy and glio-blastoma multiforme

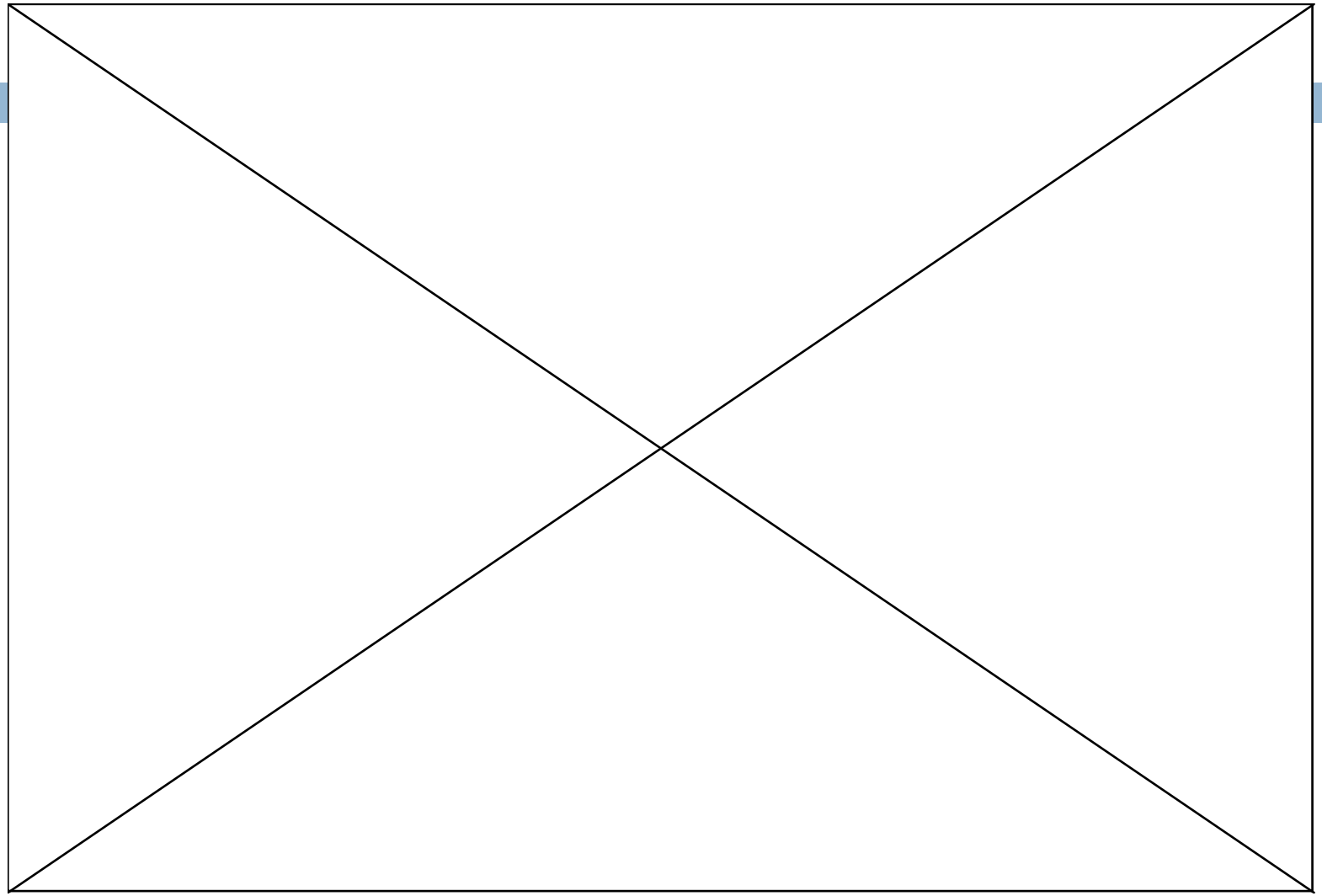
- The thrombin receptor PAR-1 (protease activated receptor) is found mainly on astrocytes
- We have found PAR-1 both at the synapse and node of Ranvier



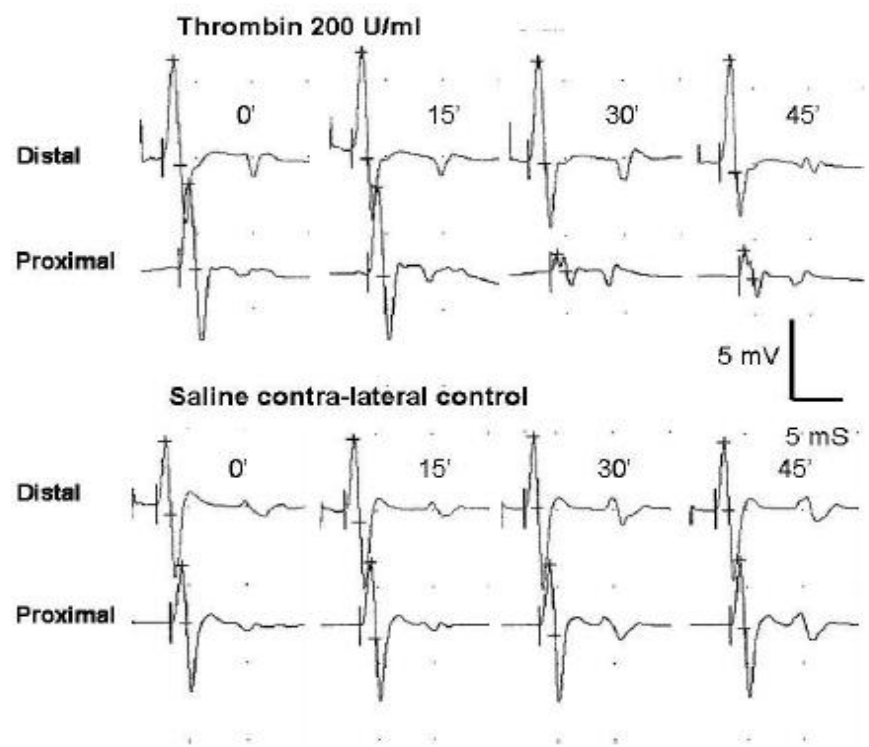
Shavit et al. J neurochem 2011

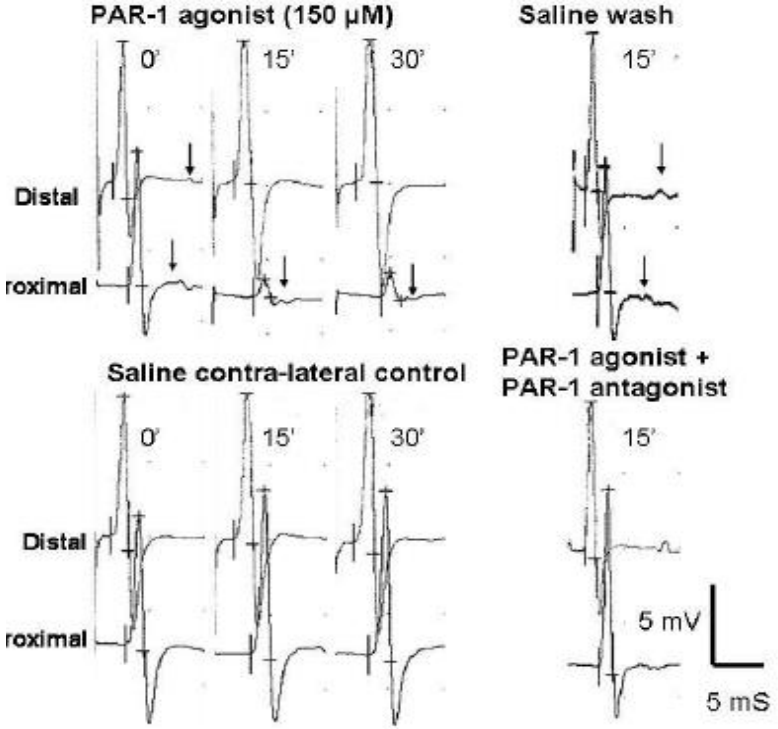


Shavit et al. Brain 2008



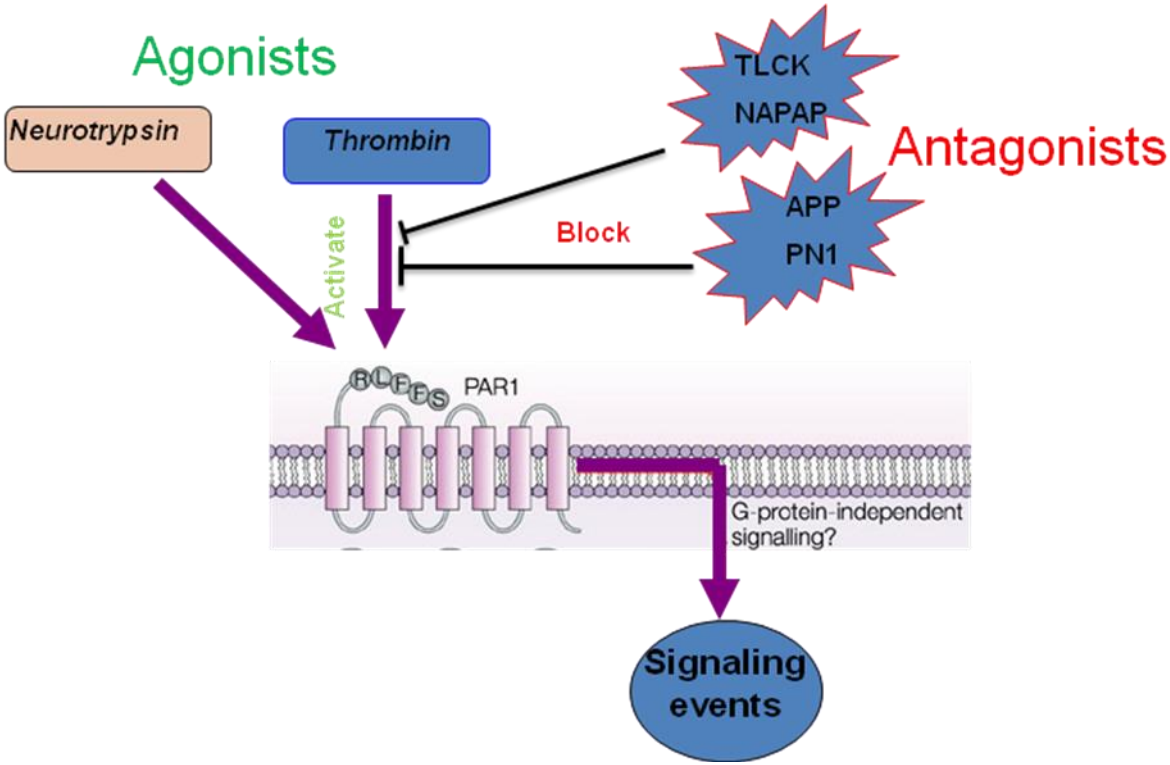
Thrombin receptor PAR-1 on myelin at the node of Ranvier: a new anatomy and physiology of conduction block



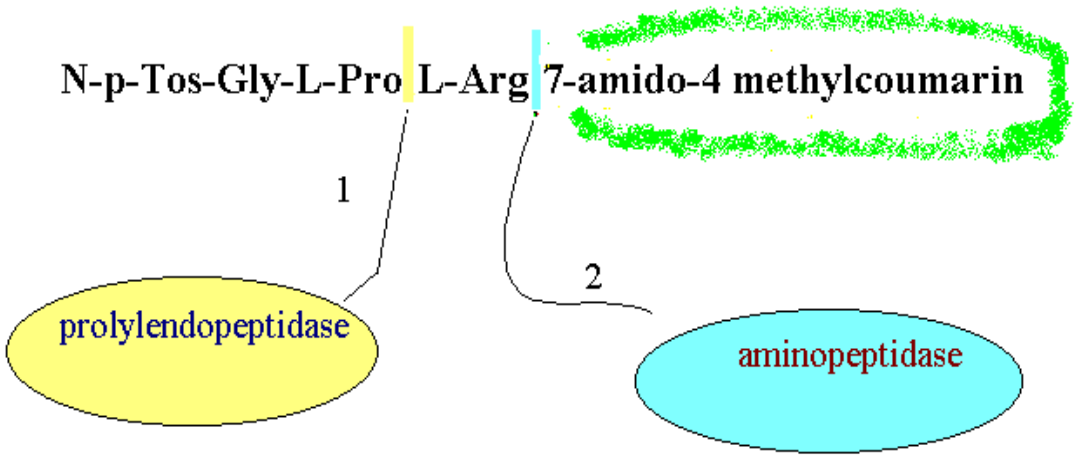


Shavit et al. *Brain.* 2008

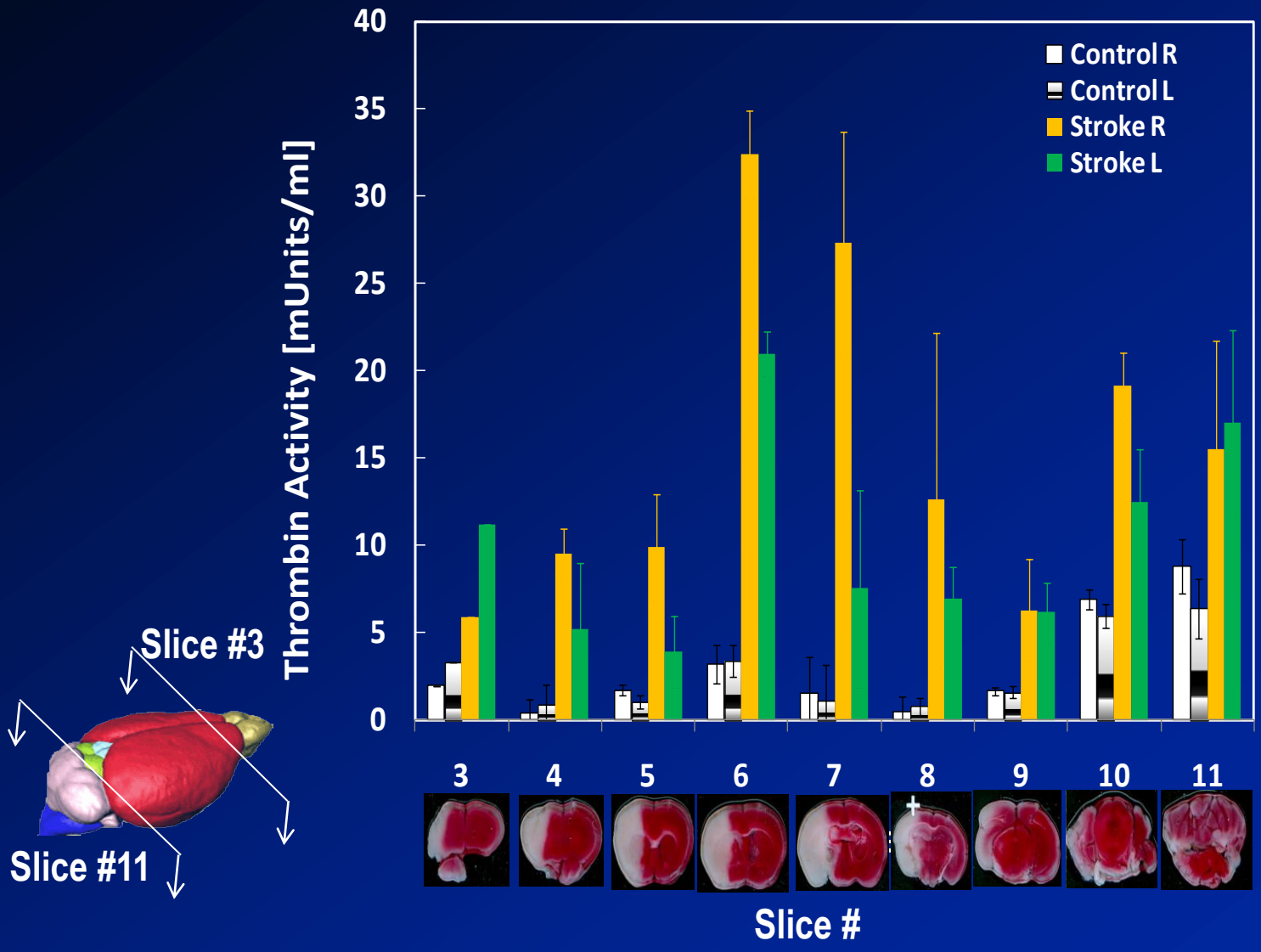
PAR1 activation by independent proteases
PAR-1 physiological/pathophysiological role



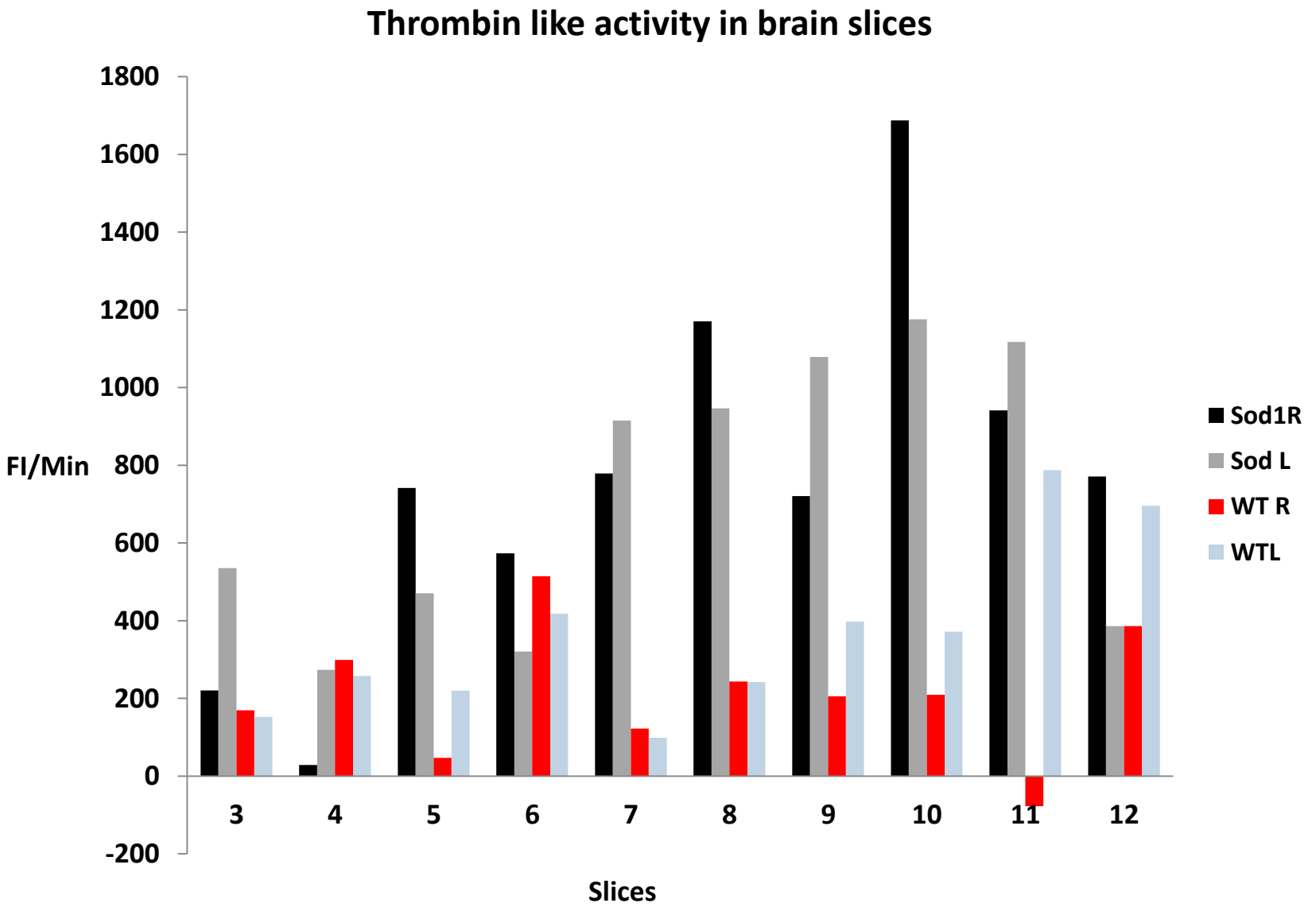
Thrombin-like activity in brain



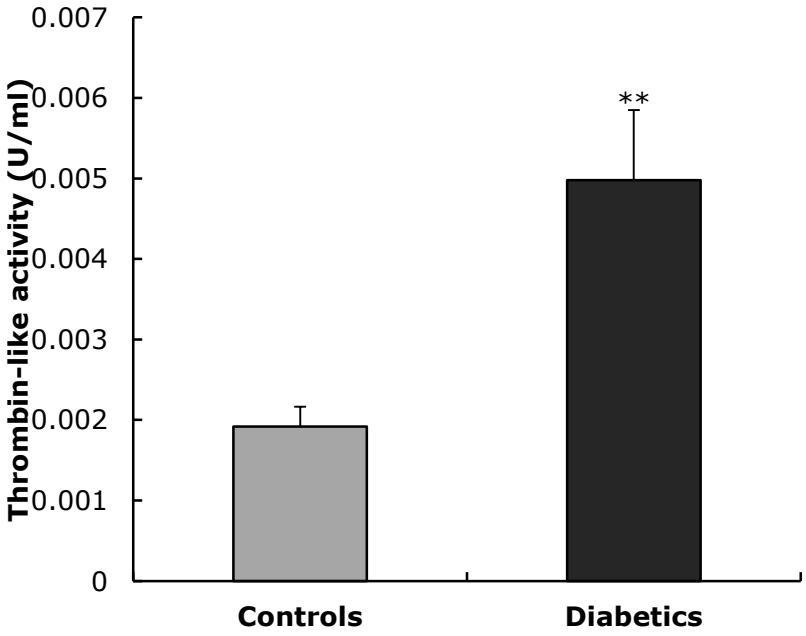
Thrombin Activity in Ischemic vs. Healthy Brains

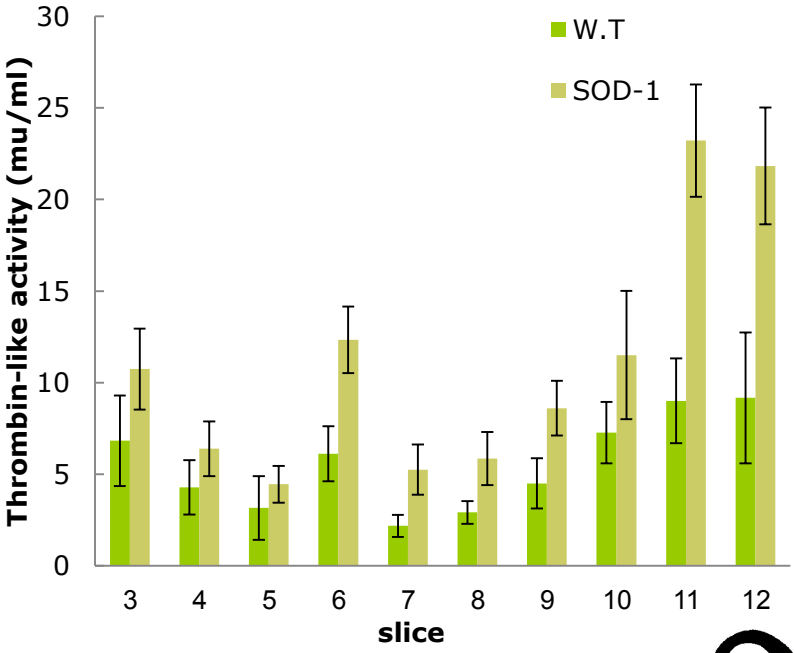


- 24h following MCA
- Each group n=6

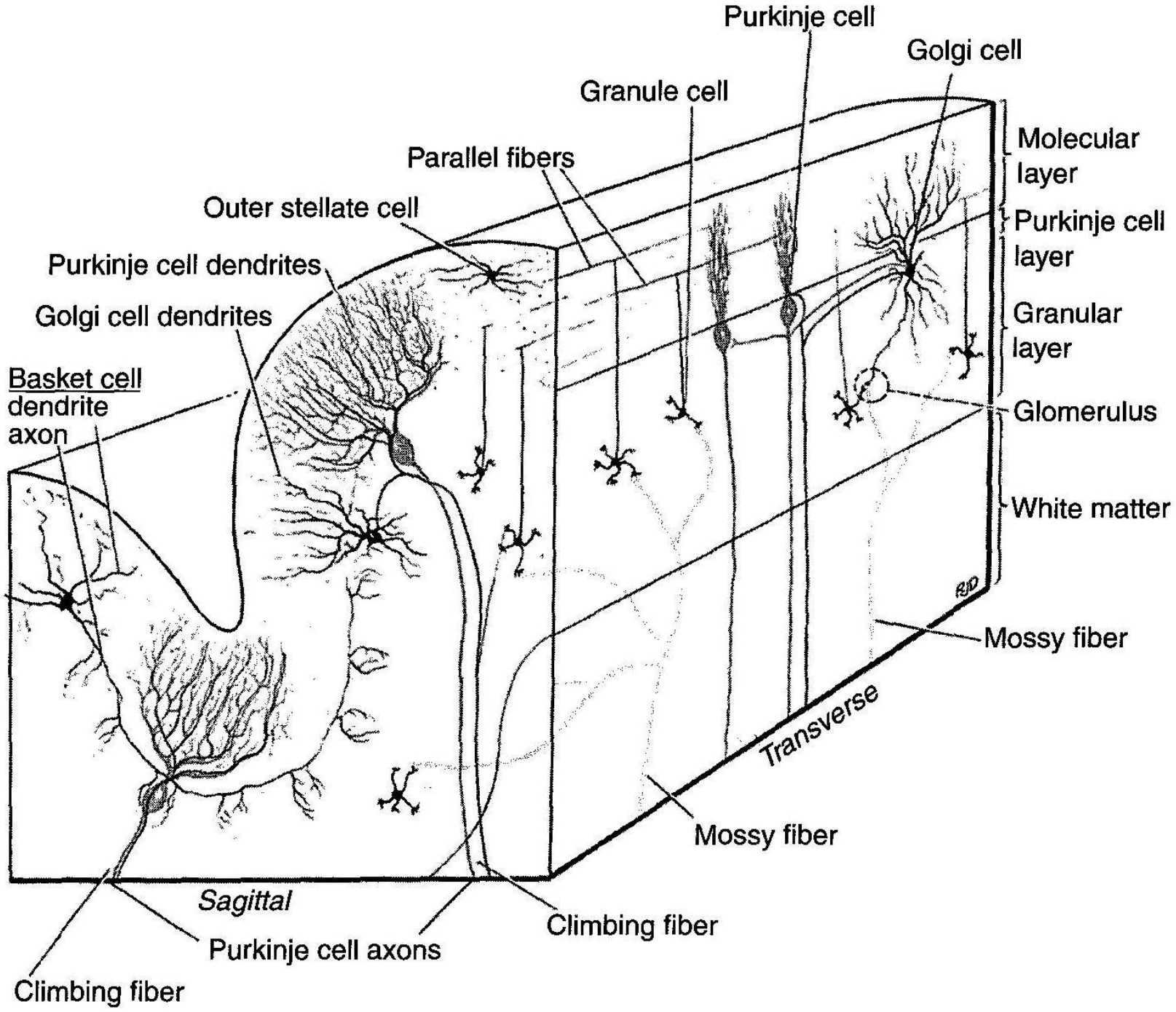

Thrombin like activity in brain slices
1800
1600
1400
1200
1000
800
600
400
200
0
-200
FI/Min
3
4
5
6
7
8
9
10
11
12
Slices
Sod1R
Sod L
WT R
WTL

Significant elevated thrombin-like activity in disease models involving the peripheral nervous system

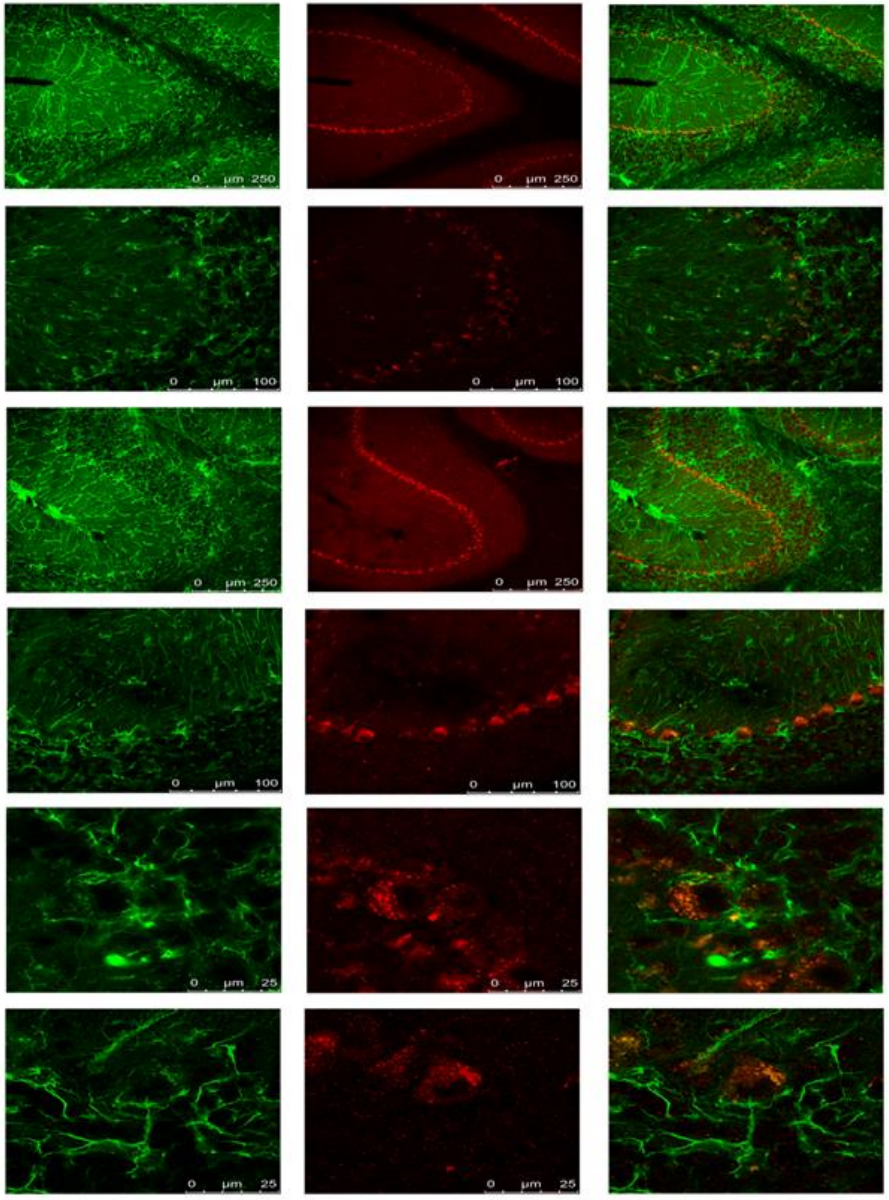


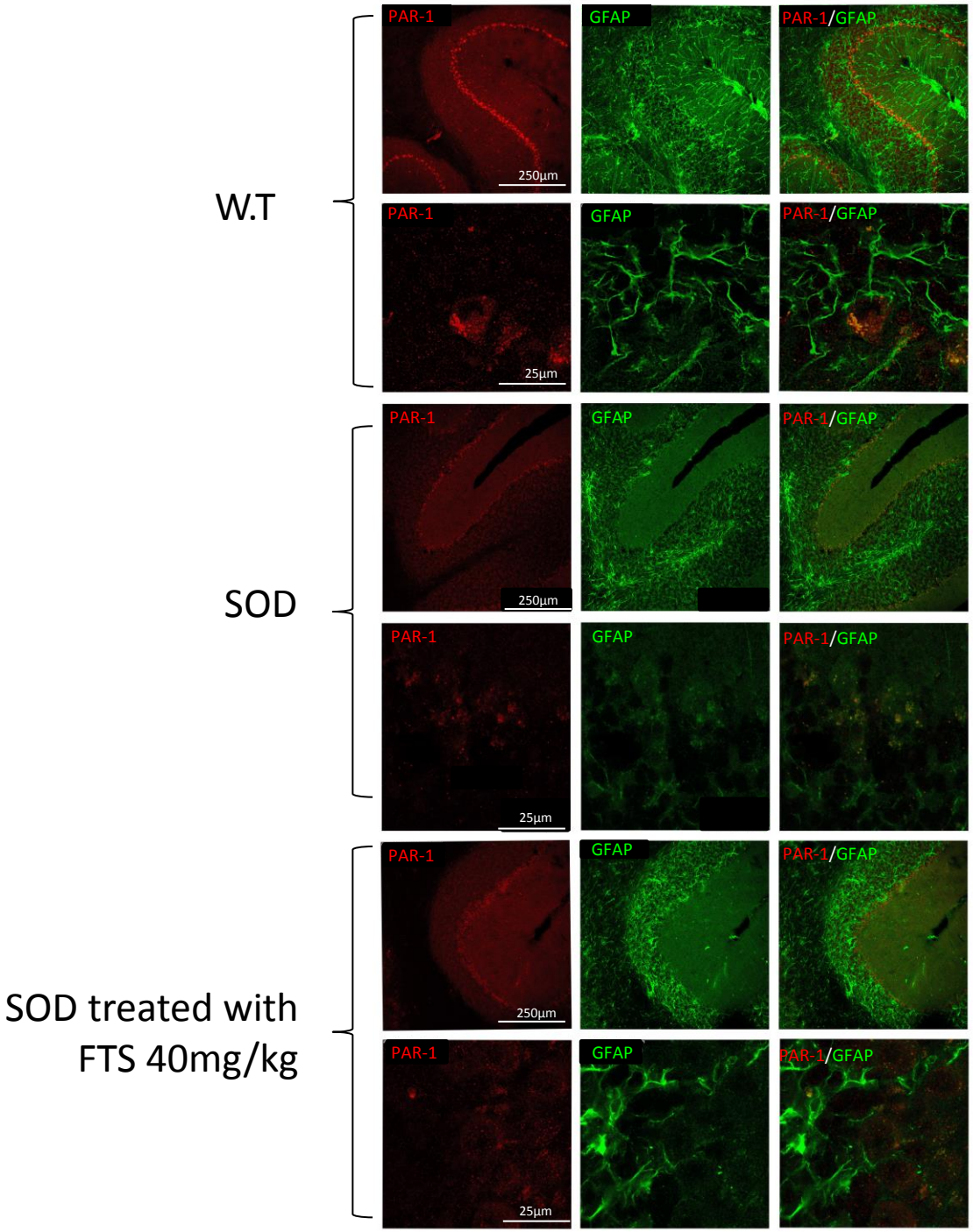


Aronovich et al. (submitted) Abu Rahal et al. (submitted)

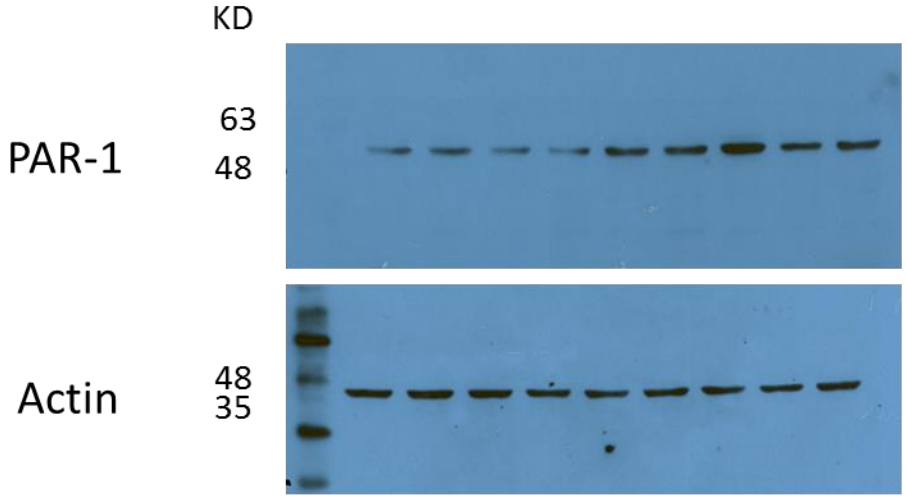

Purkinje cell
Golgi cell
Granule cell
Parallel fibers
Outer stellate cell
Purkinje cell dendrites
Golgi cell dendrites
Basket cell
dendrite
axon
Molecular layer
Purkinje cell layer
Granular layer
Glomerulus
White matter
Mossy fiber
Transverse
Mossy fiber
Sagittal
Climbing fiber
Purkinje cell axons
Climbing fiber

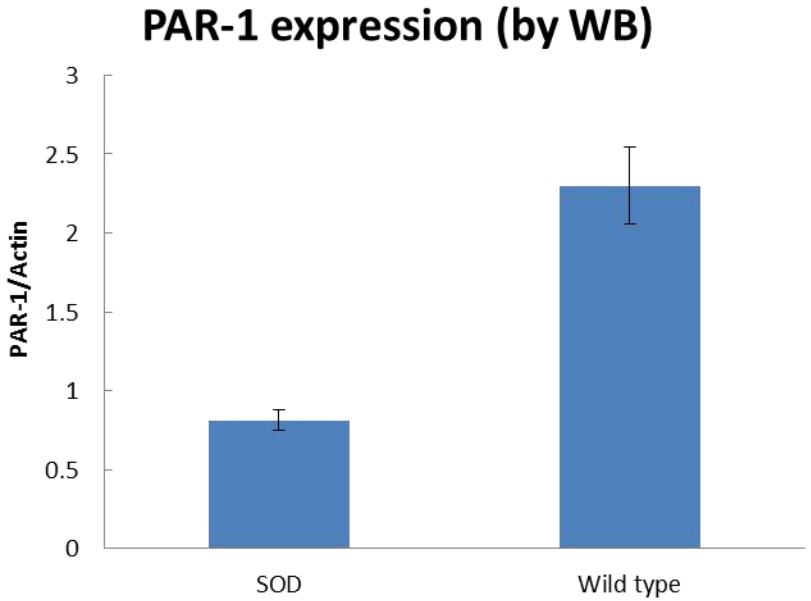
PAR-1 (red) Astrocytes (green) in cerebellum of Wild Type Mice



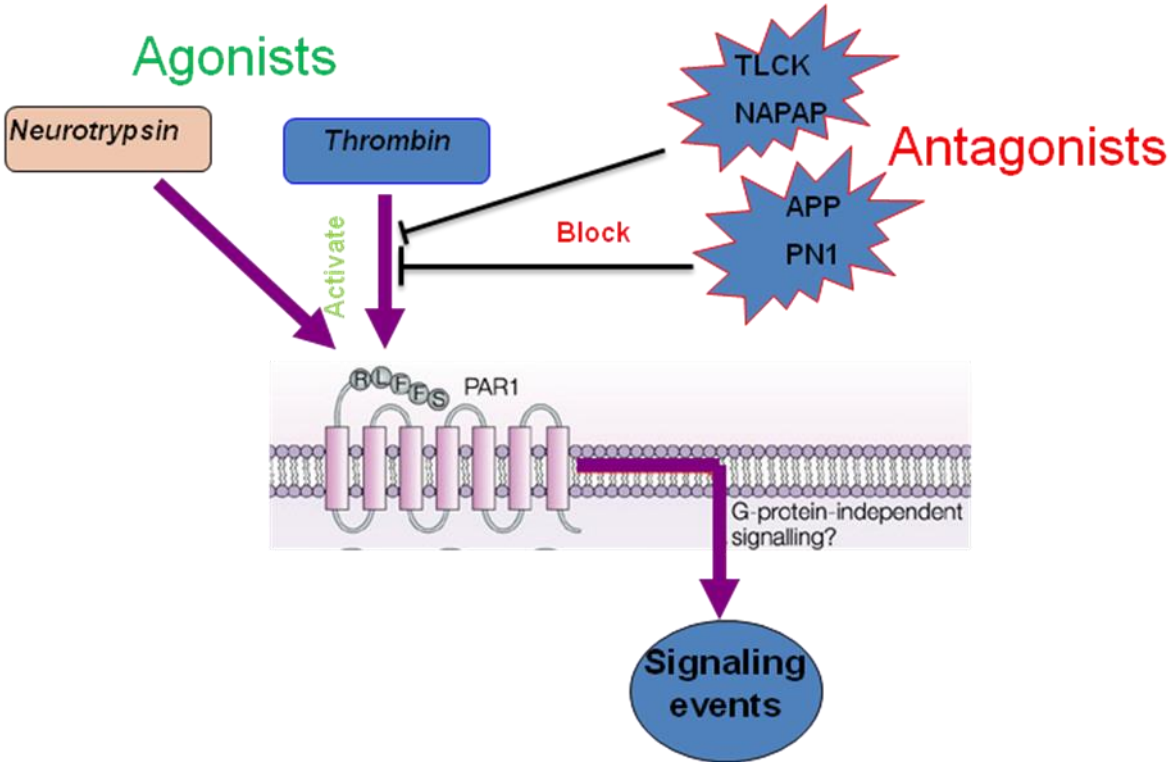

W.T
SOD
SOD treated with
FTS 40mg/kg
PAR-1
GFAP
PAR-1/GFAP
250µm
25µm

PAR-1 in Frontal Brain of SOD ALS Mouse Model

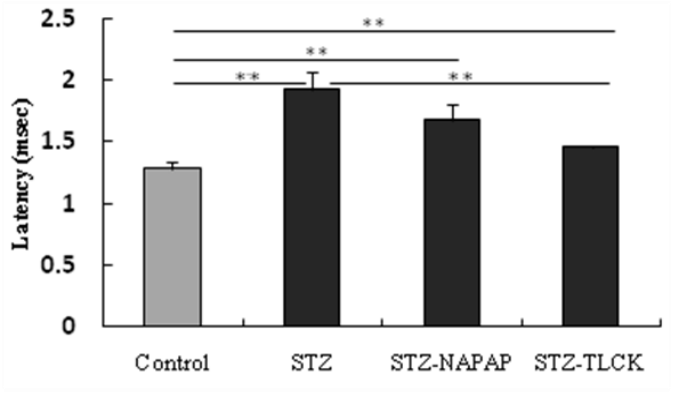


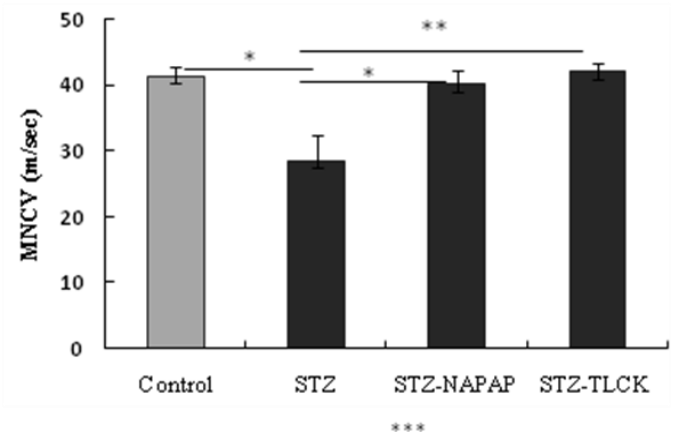


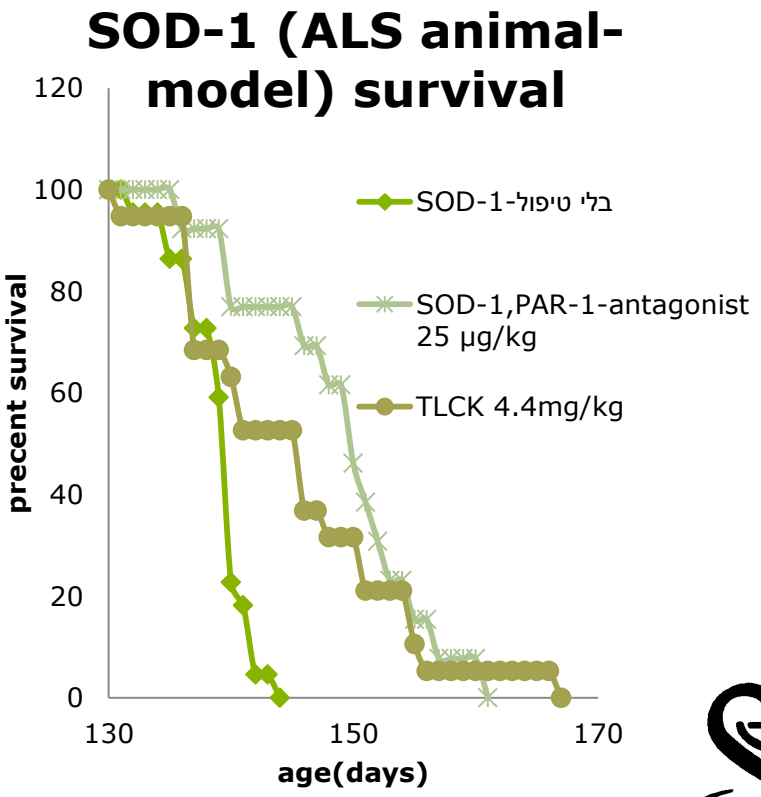
PAR1 activation by independent proteases
PAR-1 physiological/pathophysiological role



Thrombin inhibition (specific and general) significantly improves nerve-conduction and survival in neuroinflammation-involved diseases



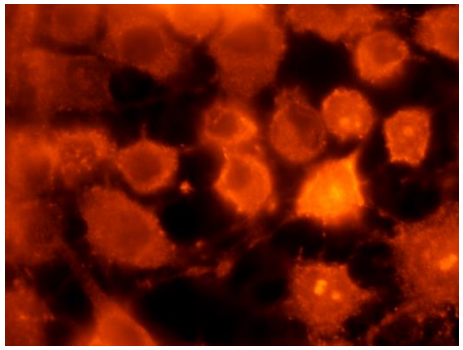


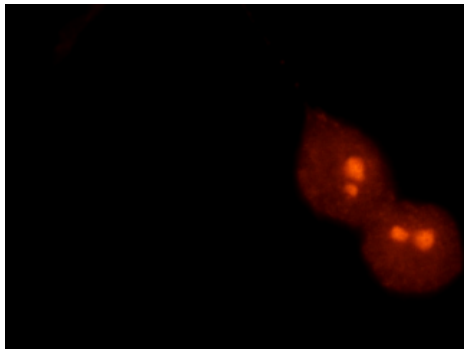


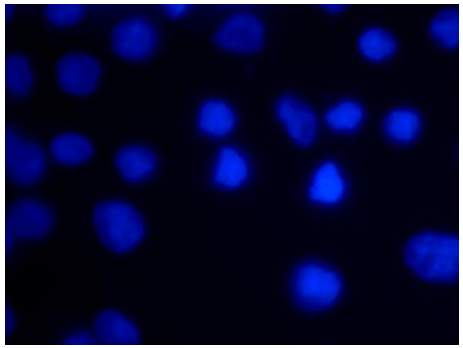
Increased PAR-1 level in glioma cells

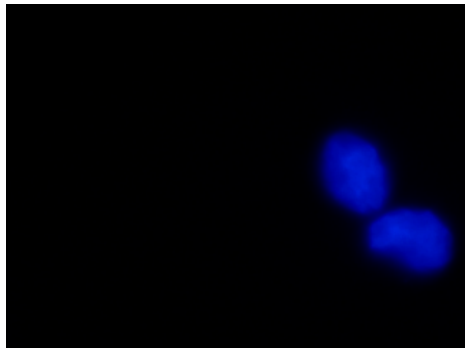
FCS free

FCS









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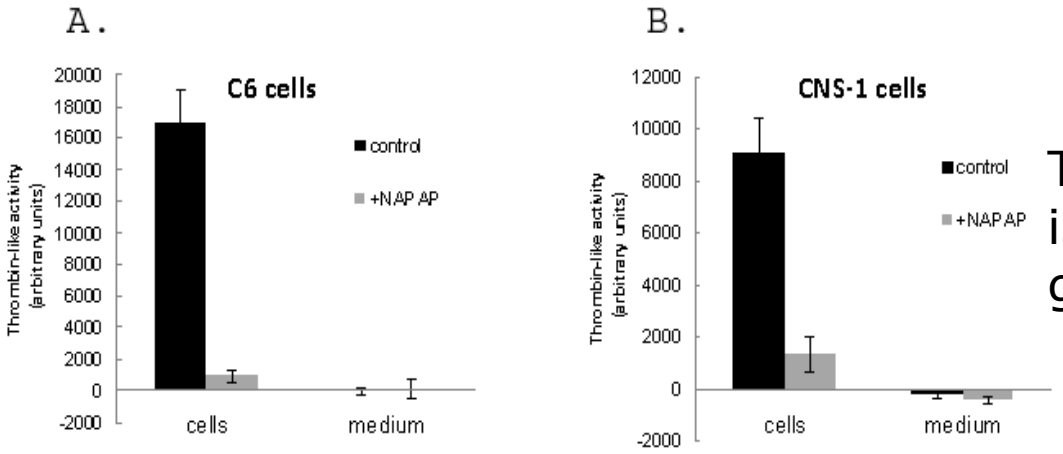
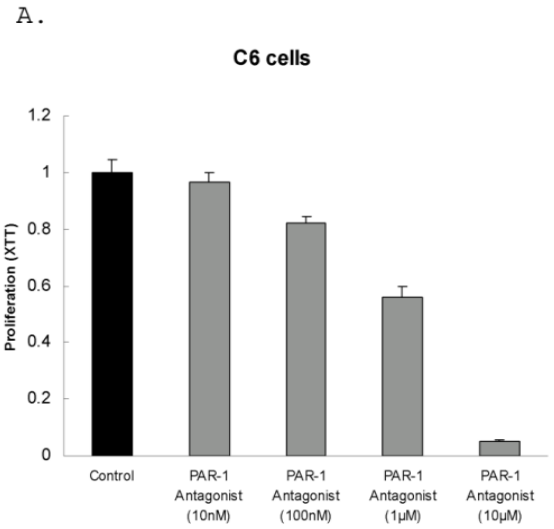
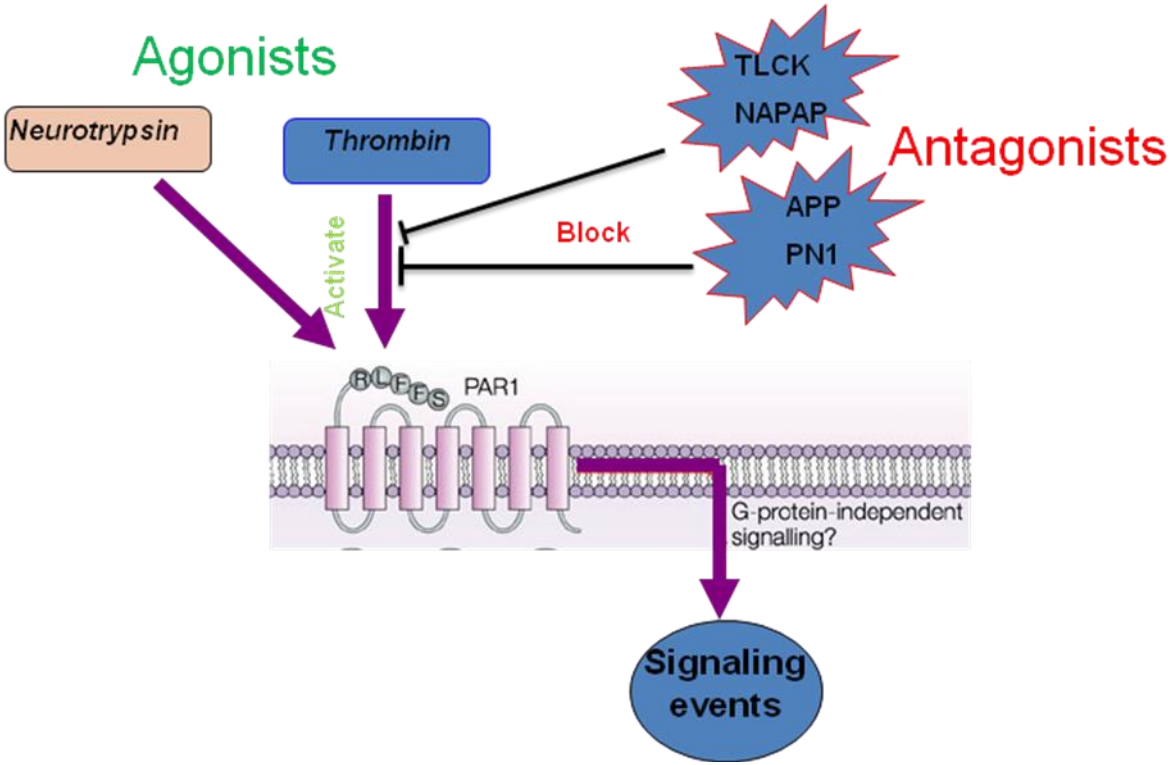


Thrombin-like activity is generated by glioma cell-lines



PAR-1 pathway modulation inhibits glioma-cells proliferation

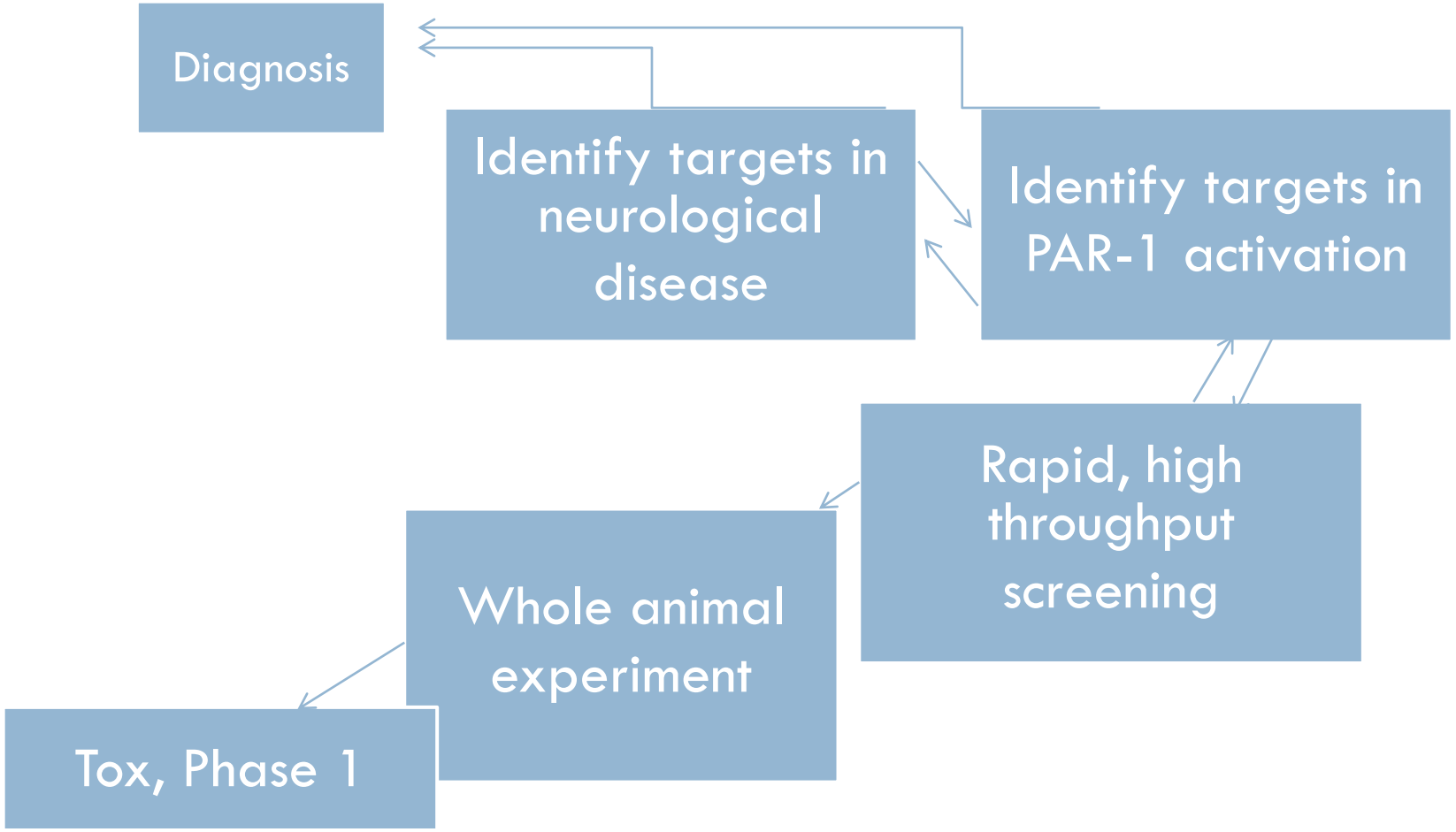
PAR1 activation by independent proteases
PAR-1 physiological/pathophysiological role



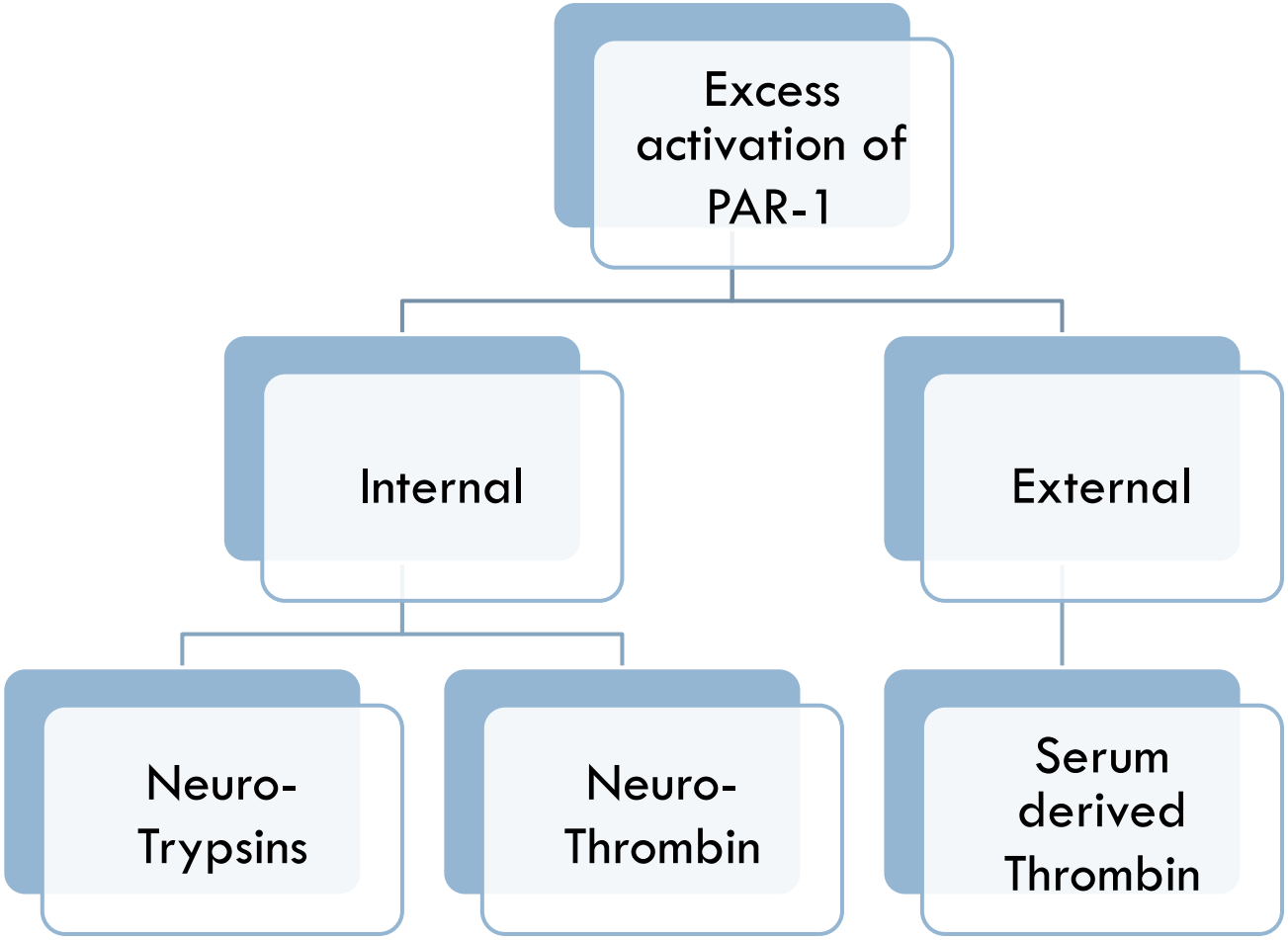
Existing drugs may not be ideal

- Do not cross blood brain barrier
- Toxic to normal brain function
- Intrinsically inhibit coagulation
- Block one pathway only (e.g. PAR-1/Par-4)
- May miss specific brain disease targets
- Pose complex IP issues

General Strategy Outline



Disease targets: Outline



Targeting central and peripheral nervous system diseases

Internal

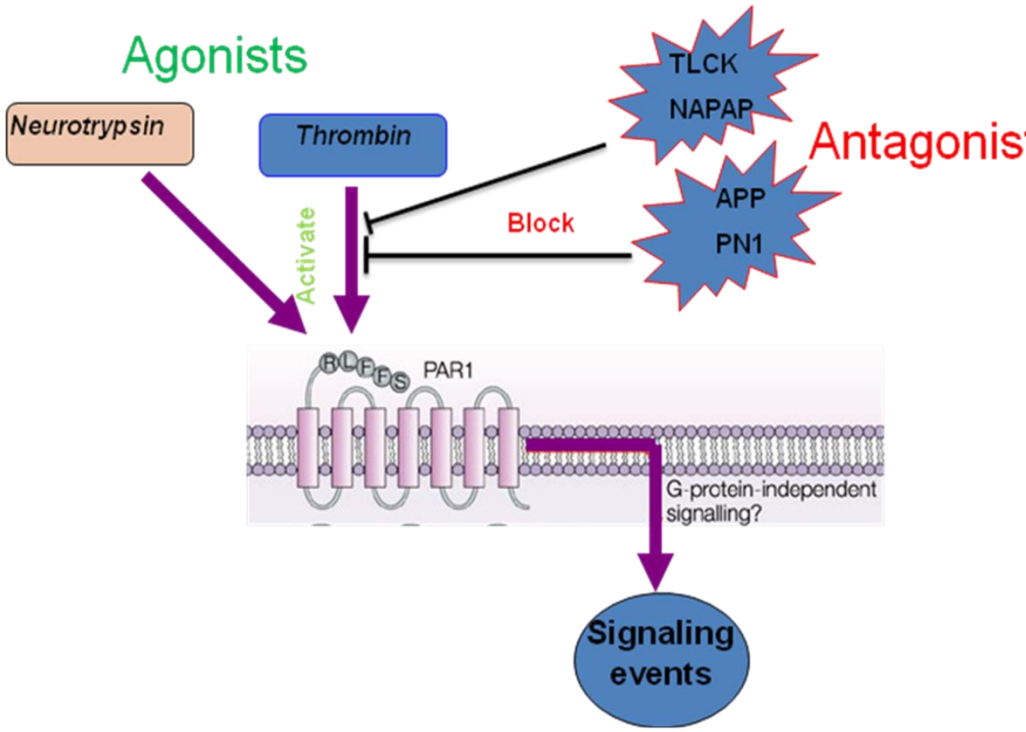
- Autoimmune (GBS)
- Neoplastic (GBM)
- Neurodegenerative (ALS)
- Diabetic neuropathy
- Epilepsy (Generation)

External

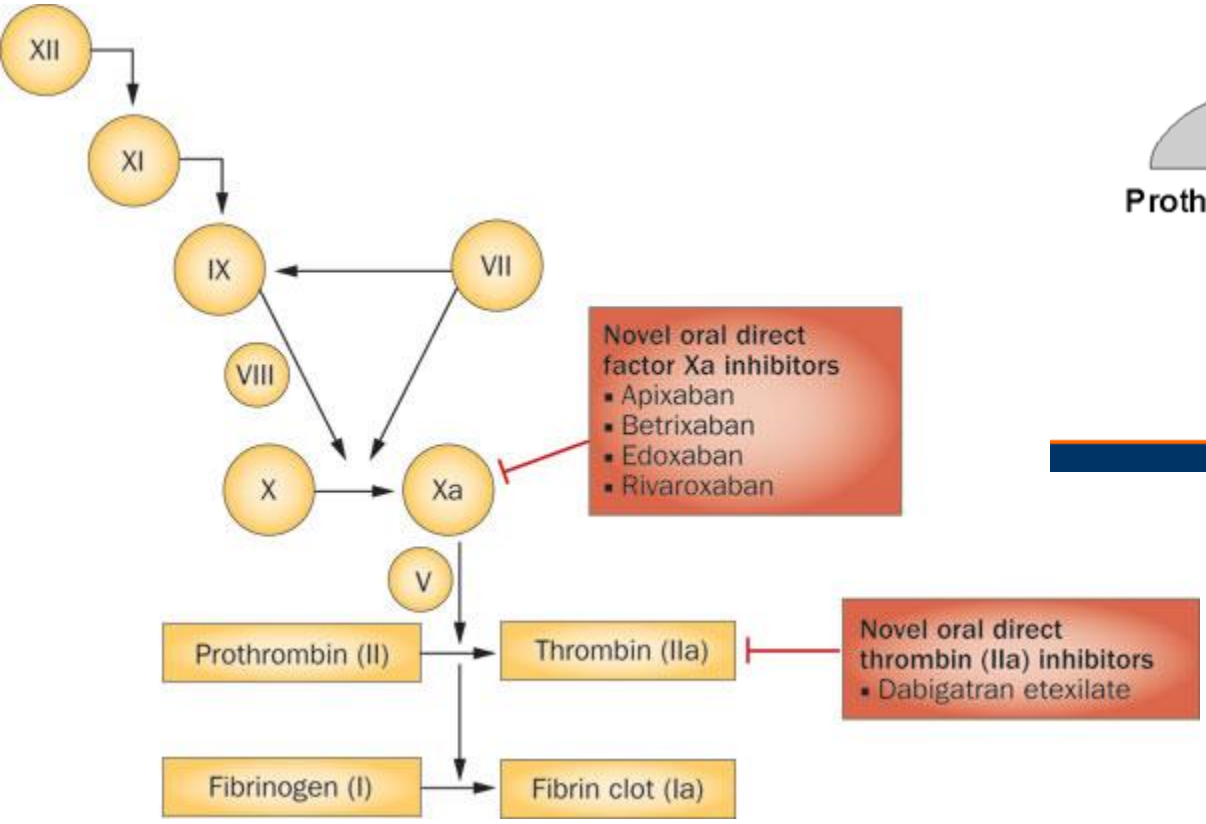
- ICH/Stroke
- Trauma
- Infection
- Metabolic (Diabetes)
- Epilepsy (Symptom)

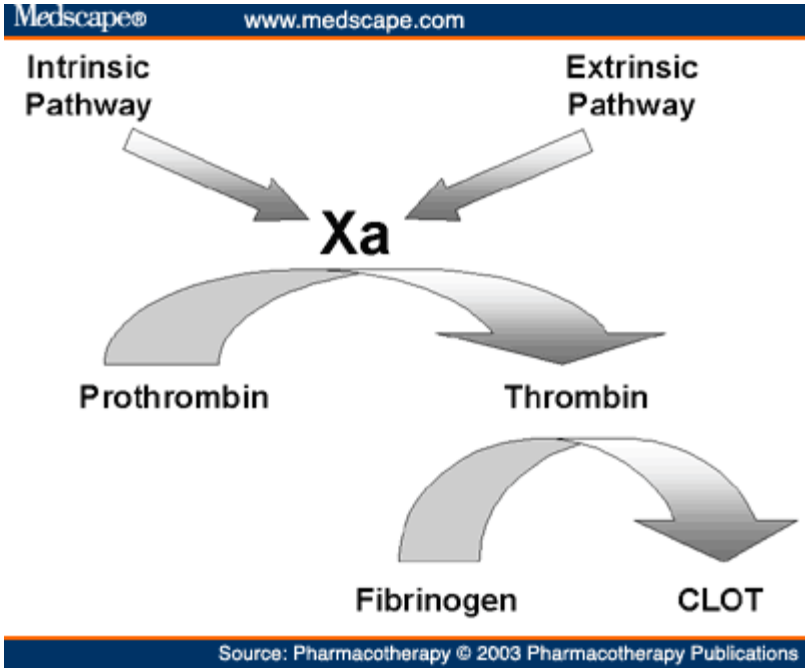
Targets in the PAR-1 activation pathway: protease based approaches

- Identity of protease (thrombin/trypsin)
- Synthesis (Vit K)
- Release
- Activation (cascade)
- Endogenous inhibitors
- Docking at PAR-1



שרשרת הקרישה
מה שמקובל





The group