Thrombin and Protease Activated Receptor-1 (PAR-1) in Minimal Traumatic Brain Injury (mTBI) in Mice

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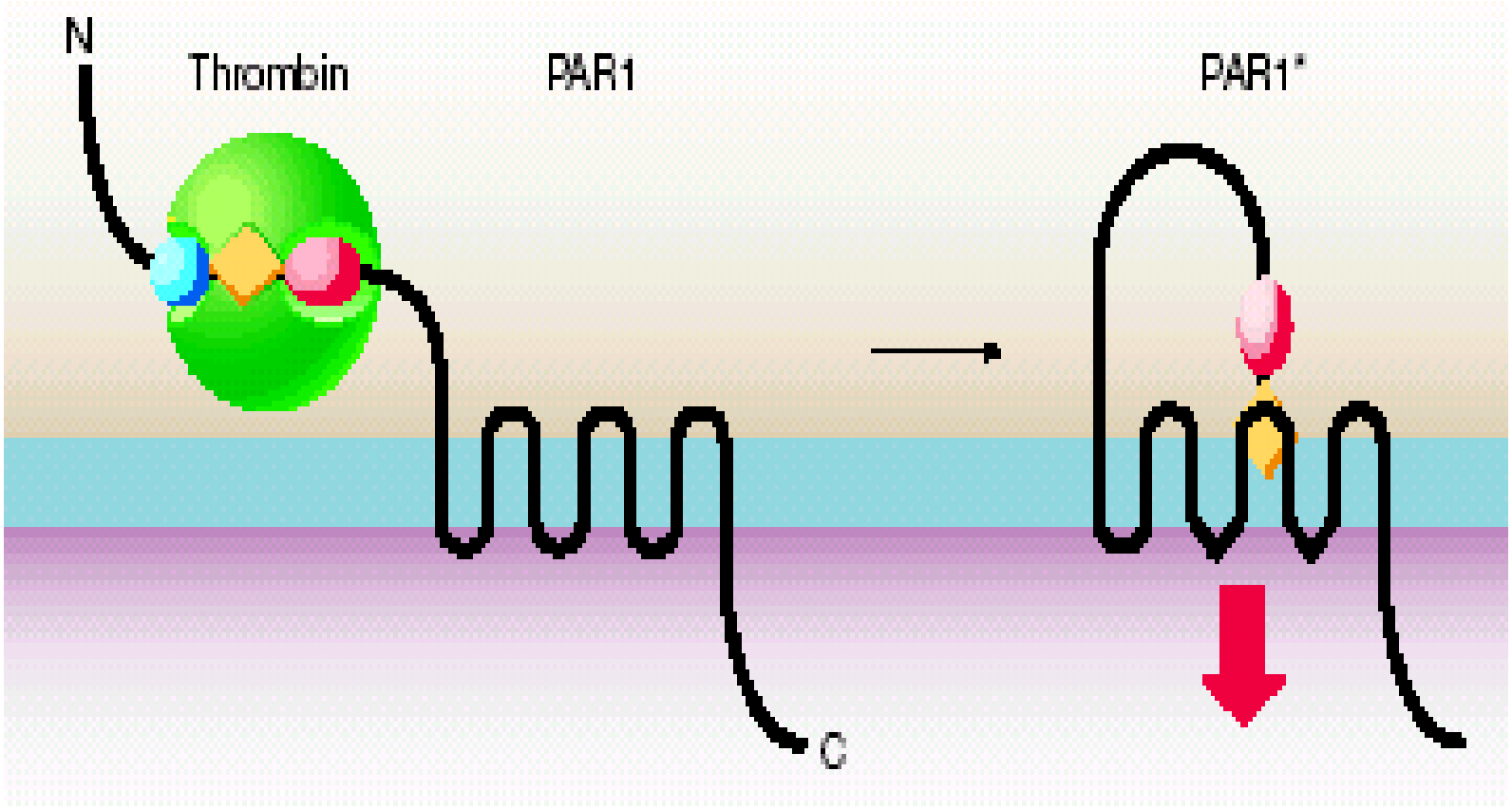
Why mTBI ?

- Affects millions every year
- Associated with cognitive dysfunction and epilepsy
- Definite biochemical markers are still sparse
- Pathophysiology is yet to be determined conclusively
- Simple and well established model

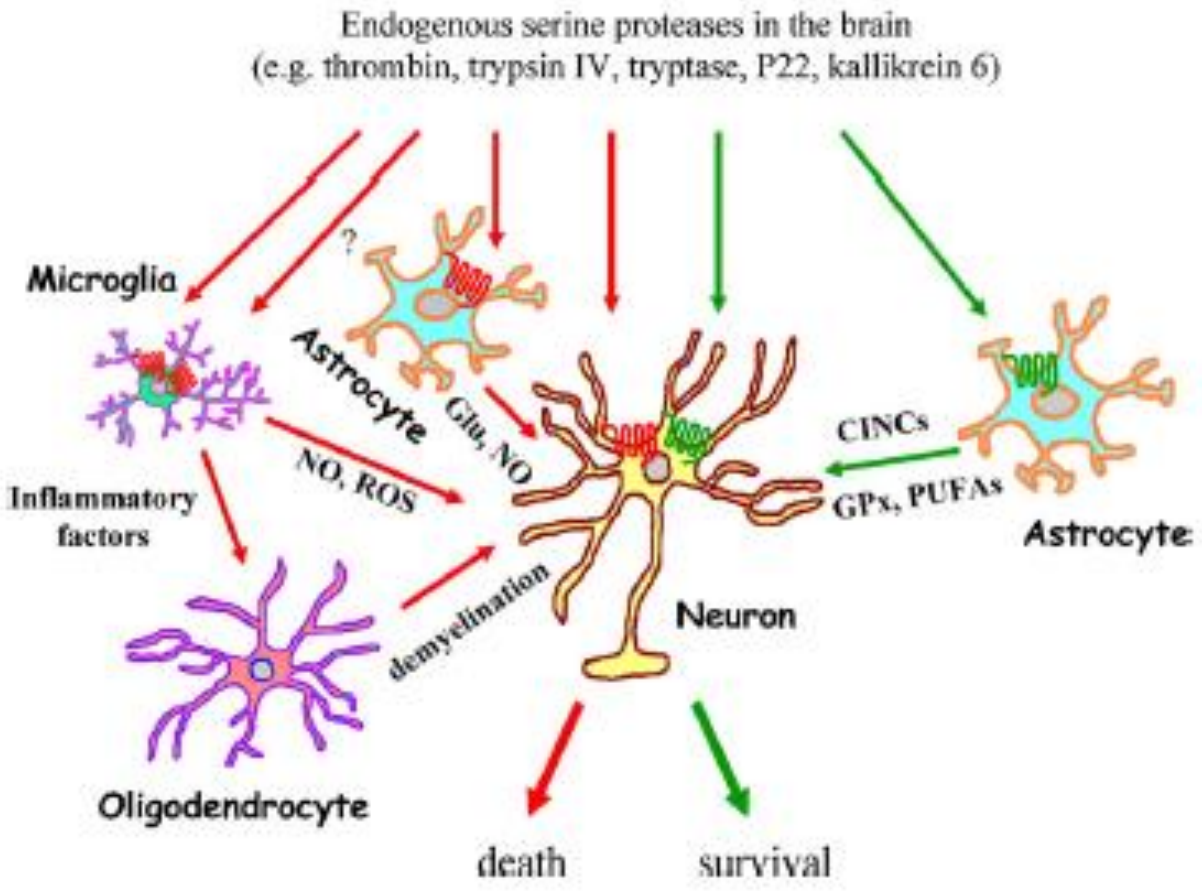
Why PAR-1 (and Thrombin) ?

- Present in the brain – neurons and glia, activated by serum and CNS proteases



Why PAR-1 (and Thrombin) ?

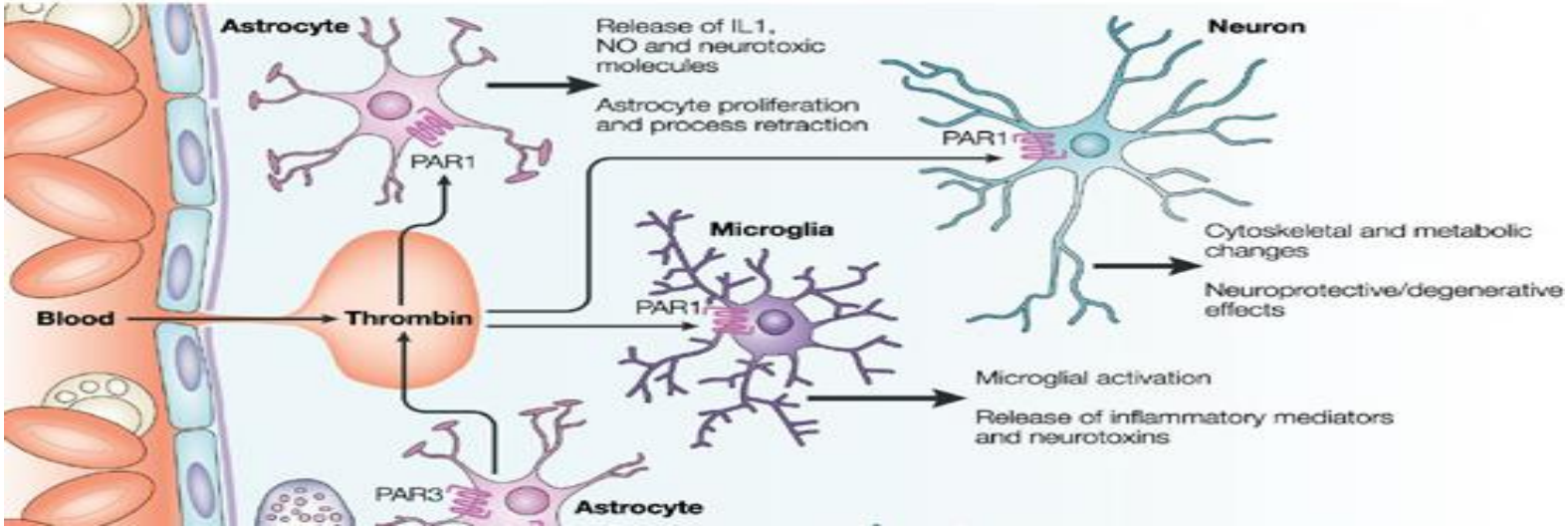
- Involved in neural damage, neuro-inflammation and neuro-protection



Starting Hypothesis A

Proteases in the brain

- Traumatic BBB disruption can result in serum proteases such as thrombin leakage to brain tissue

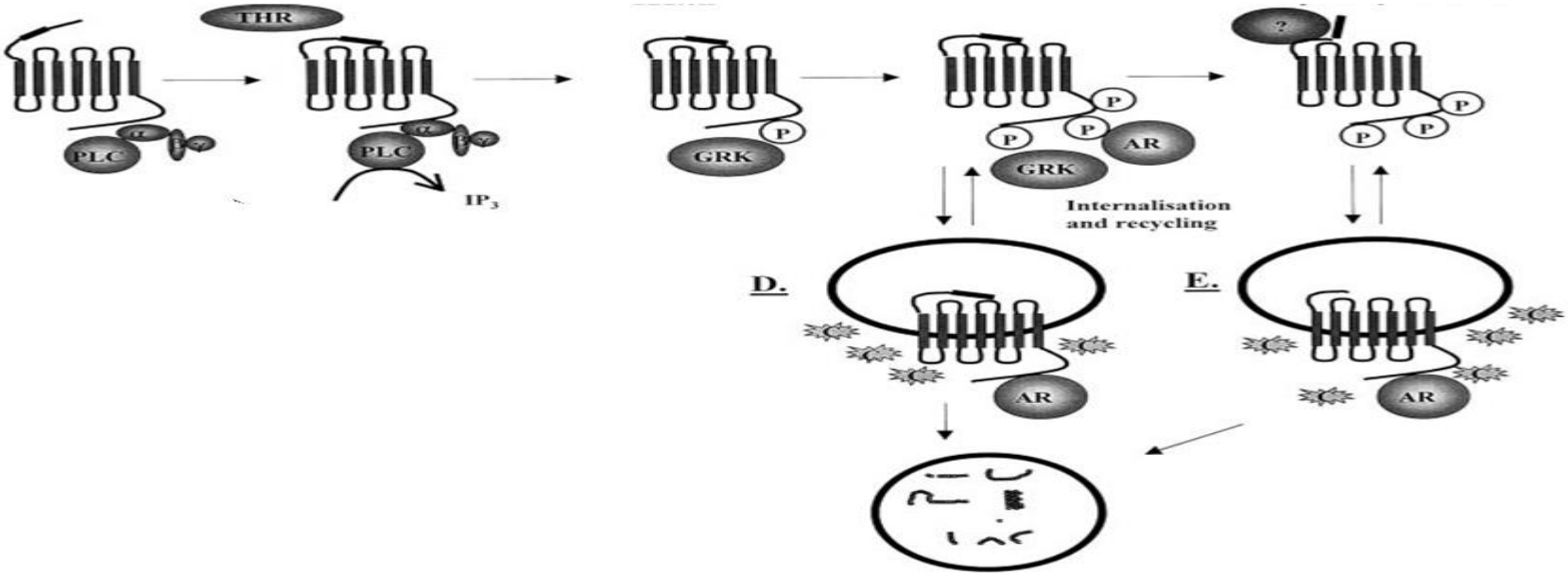


- Therefore traumatized brain is expected to exhibit elevation in thrombin or thrombin-like activity

Starting hypothesis - B

PAR-1

- Proteolytic activation is known to downregulate PAR-1 by internalization and/or degradation



- Thus PAR-1 levels are expected to decline following proteases elevation in mTBI

Starting hypothesis - C

Protease inhibitors

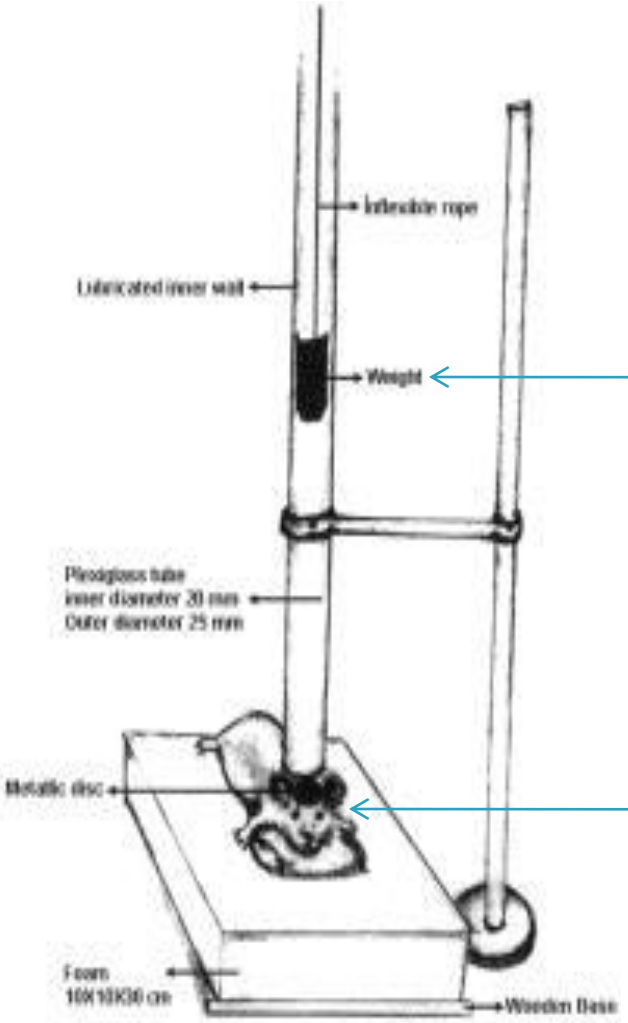
- Elevation of proteolytic or inflammatory activity is expected to draw a contra-regulatory response.

- Such a response in the CNS has been linked to PN-1,KPI-APP and increased thrombin activity inhibition in CNS inflammation (Beilin et al., 2005)

  - Thus levels of these proteins are expected to increase following mTBI and subsequent peak of proteolytic activity

Experimental model

Trauma induction in mice (male)

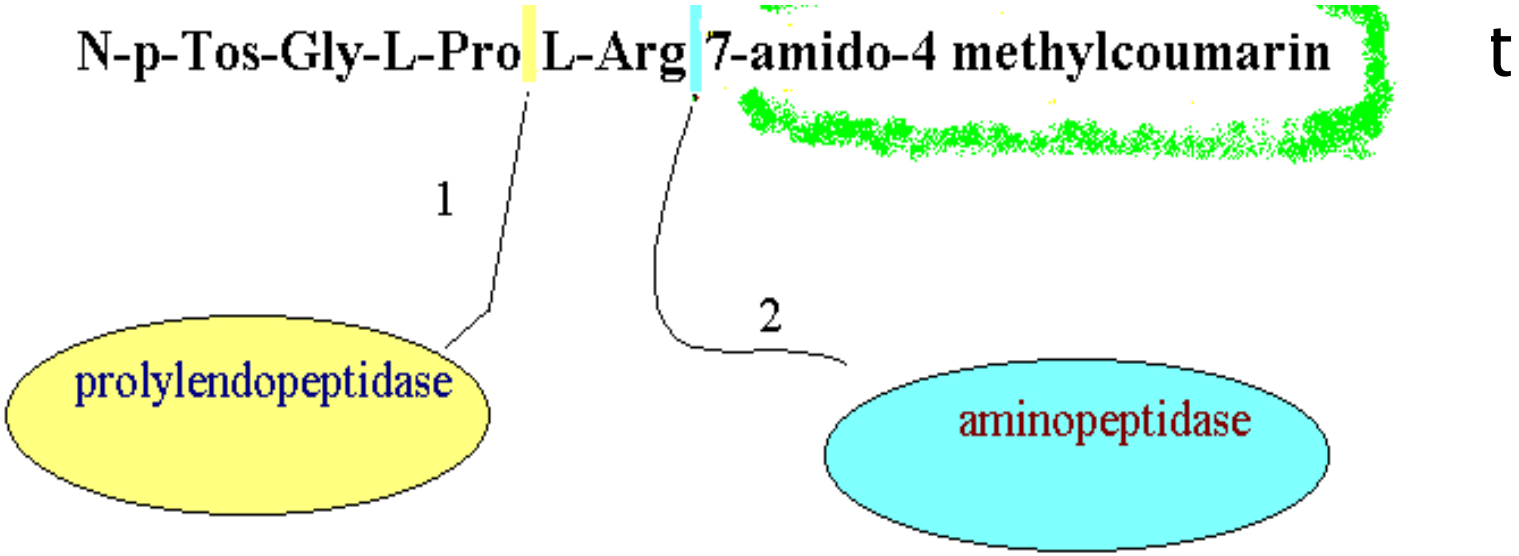


Experimental model

Assays

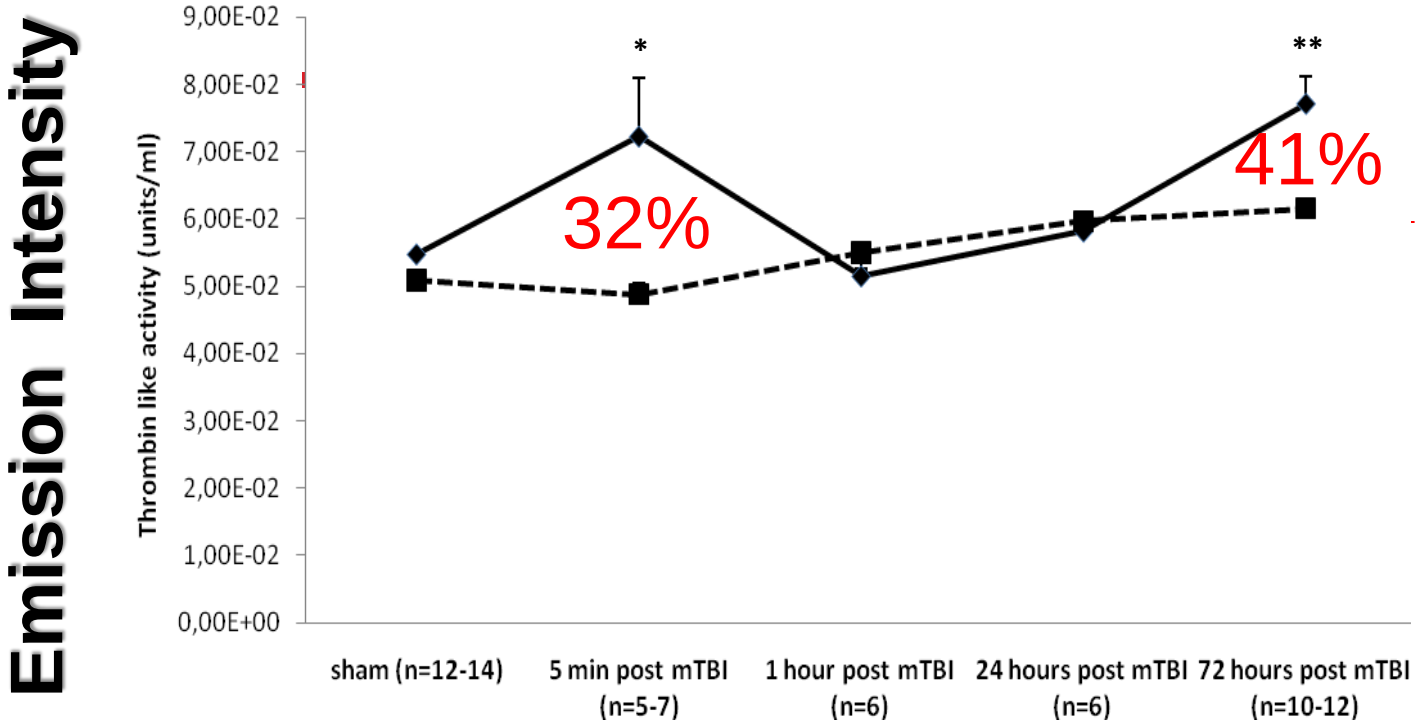
In Vitro

- Fluorometric enzymatic activity of brain slices (=cleavage of flourogenic thrombin substrate)



Results

Thrombin like activity in mTBI brains rises <u>acutely and chronically</u>



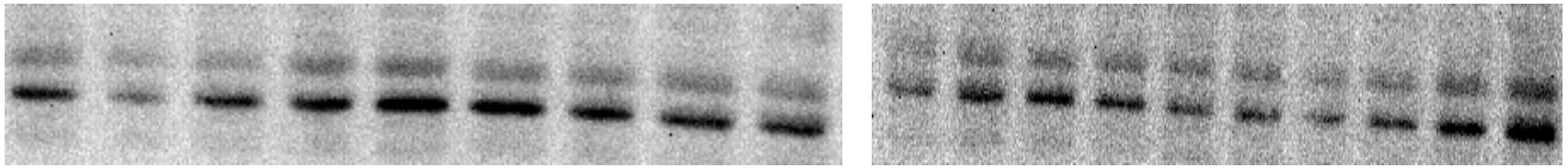
And is reduced to baseline by thrombin inhibitor

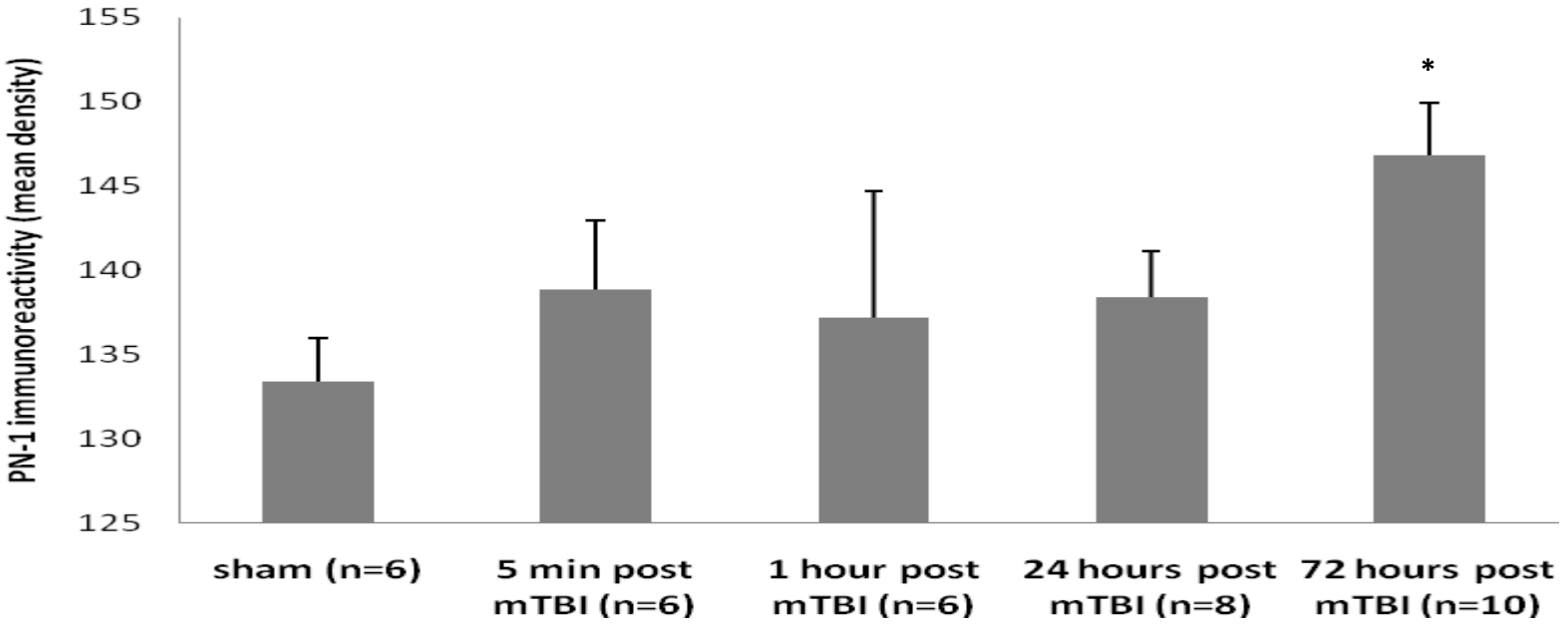
Results

Immunoblot

PN-1 levels rise

Intact | 5 min | 1 hour | 24 hours | 72 hours

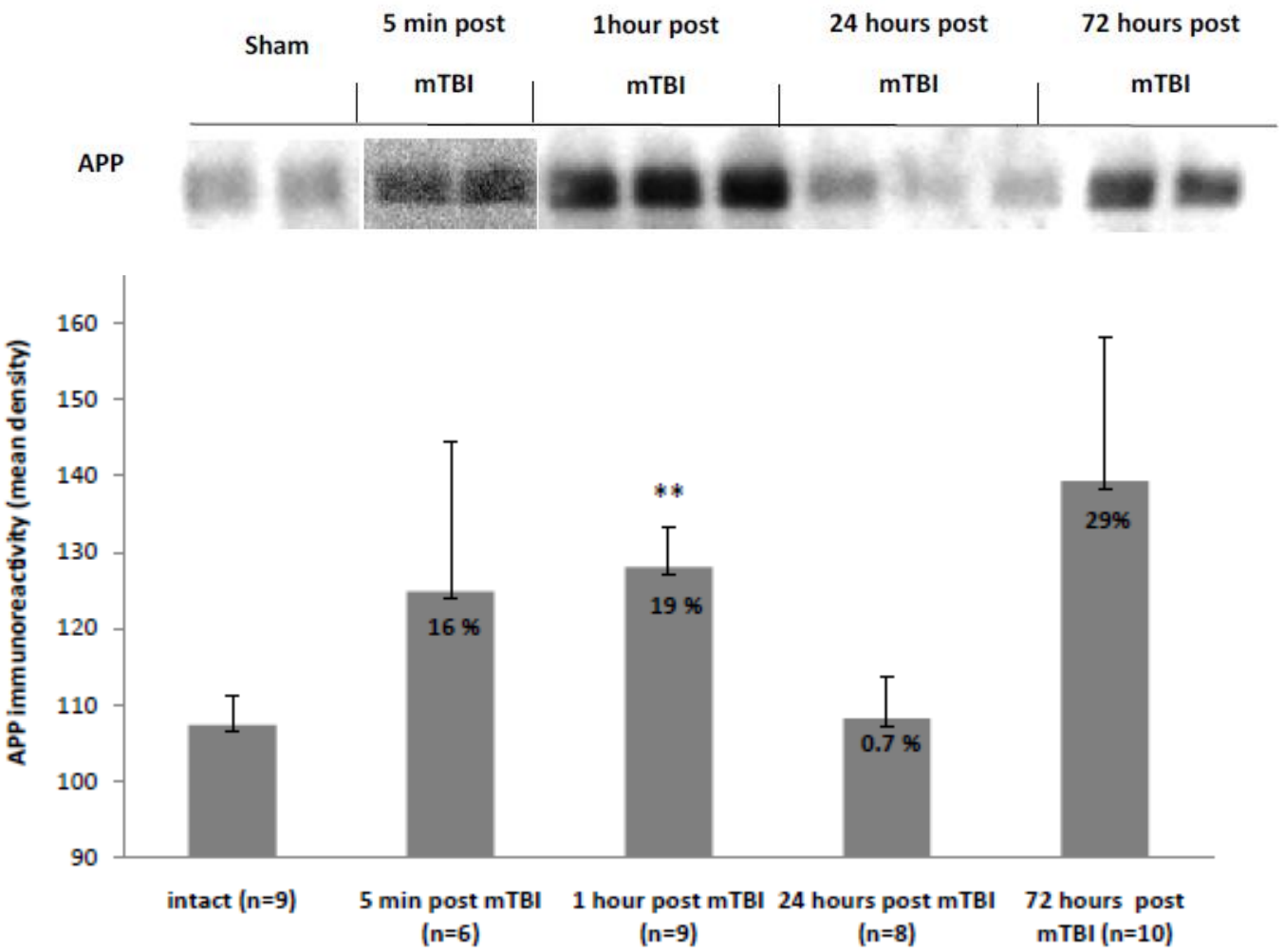




Results

Immunoblot

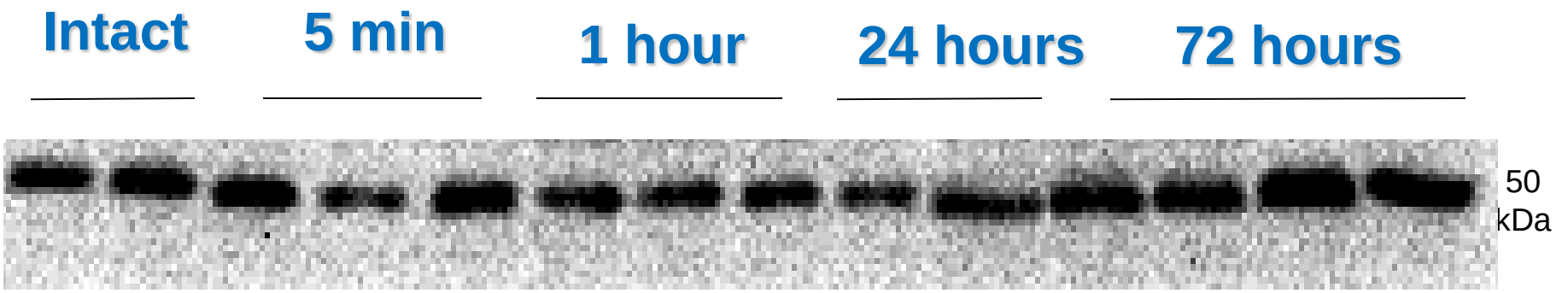
KPI-APP levels rise

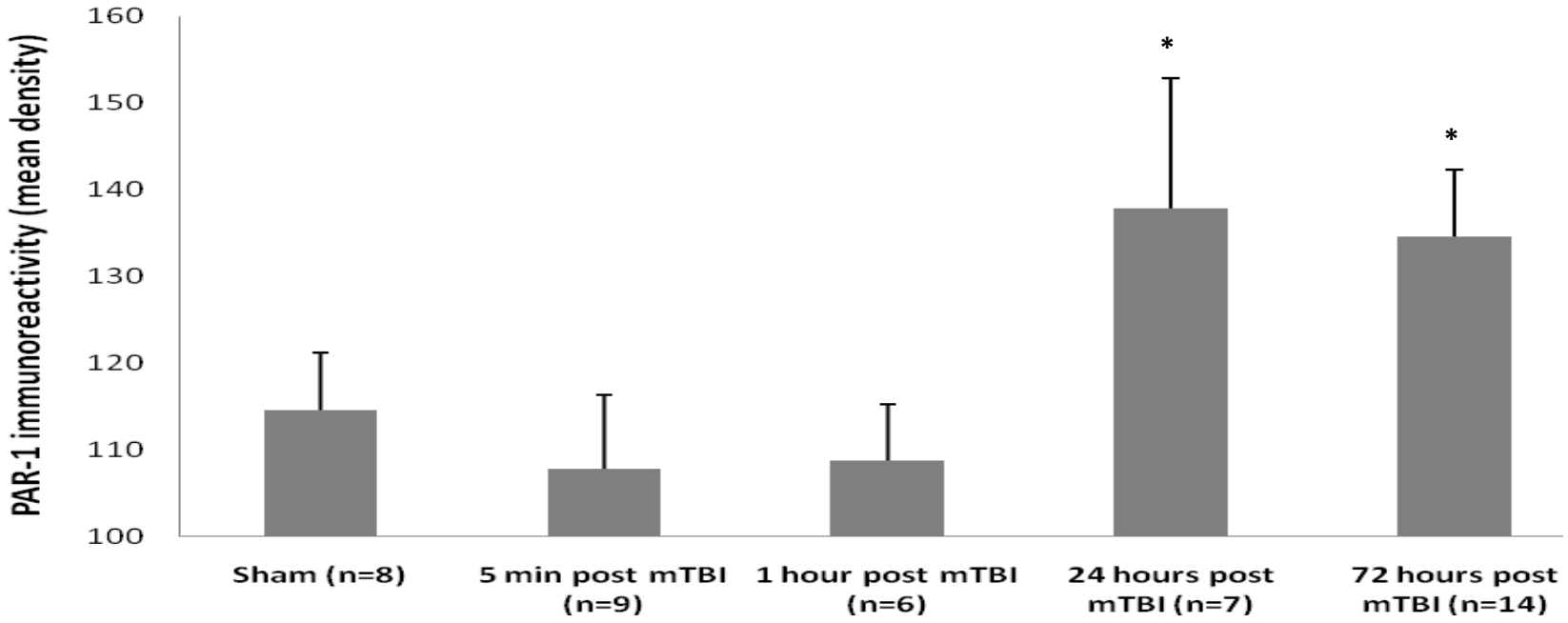


Results

Immunoblot

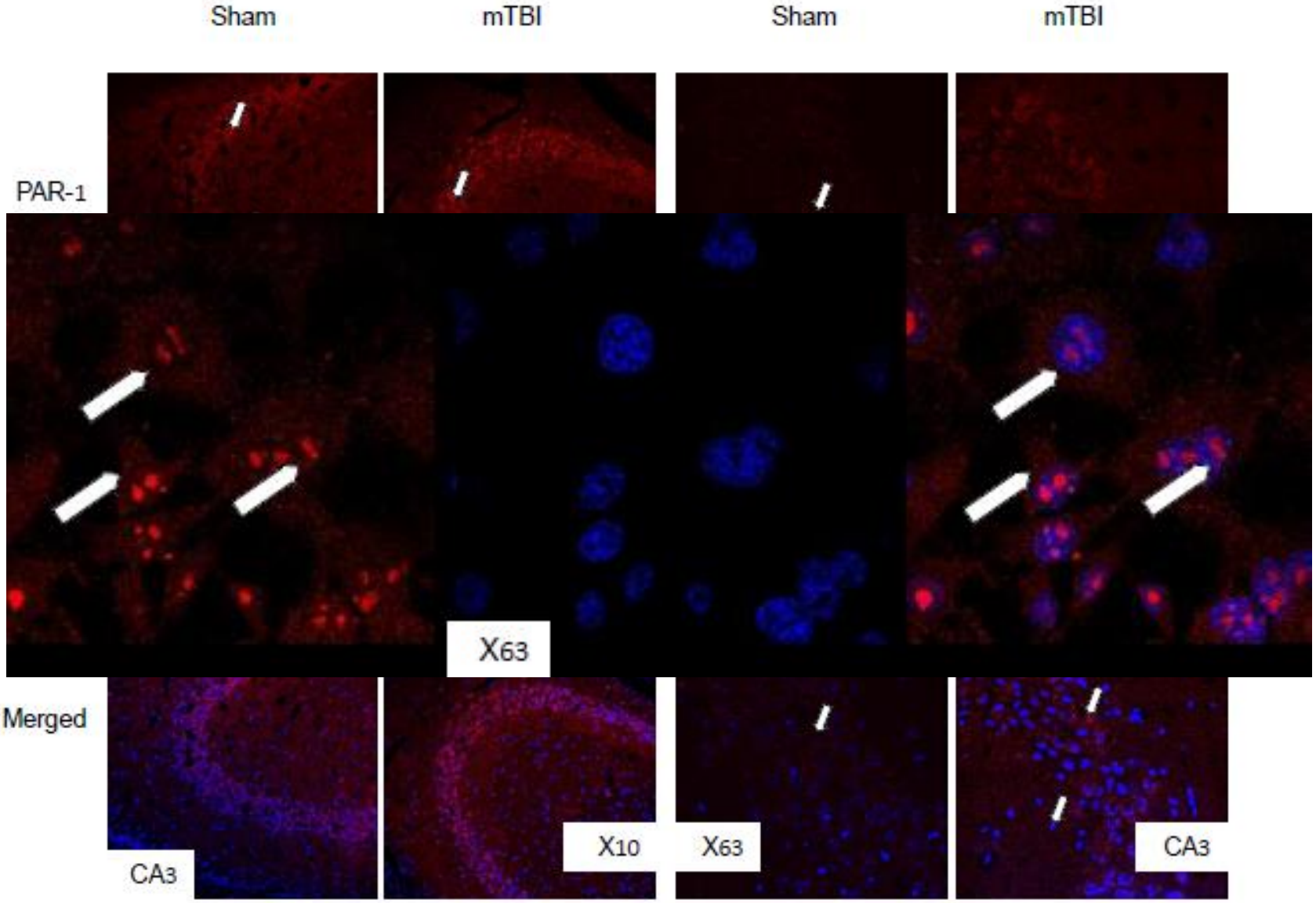
PAR-1 levels rise





Results

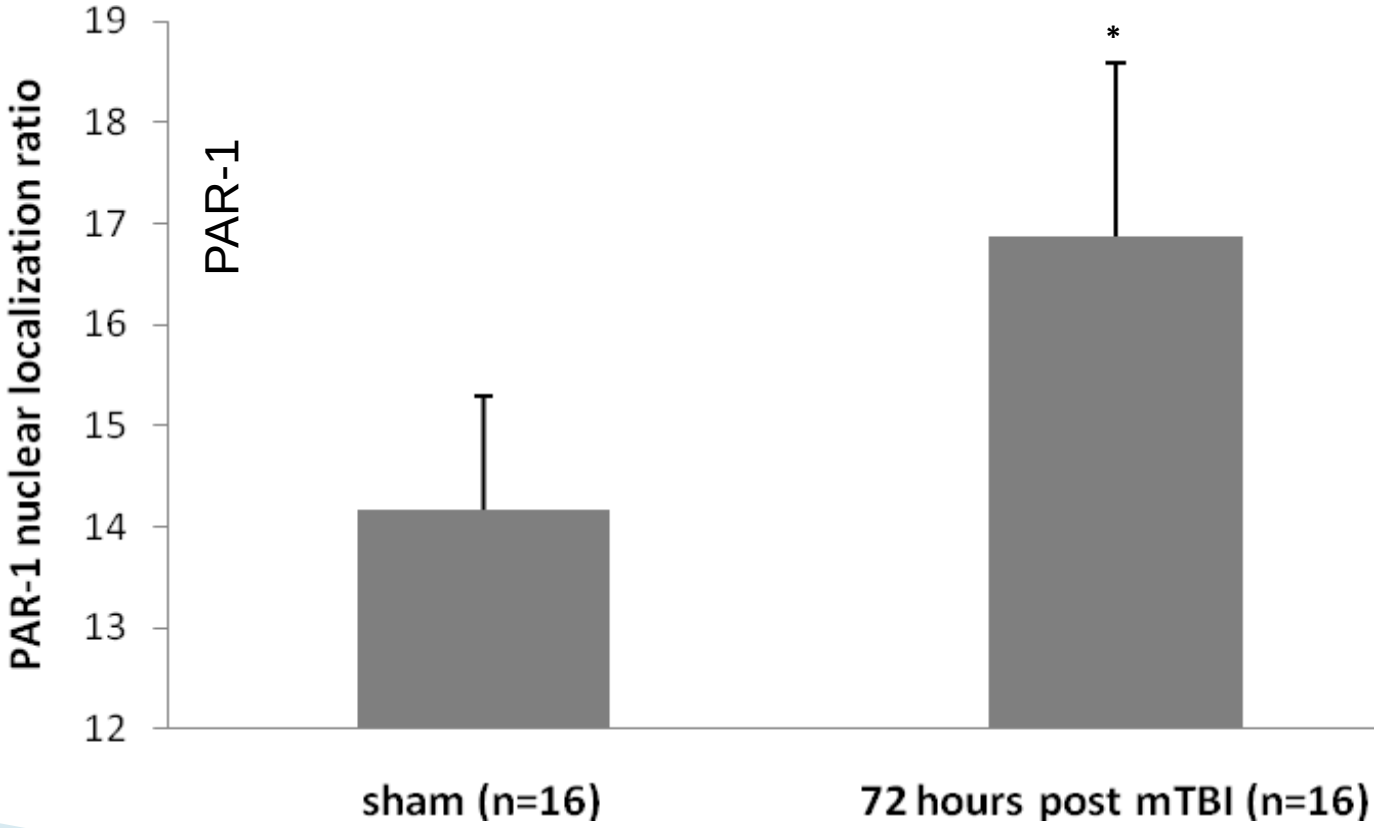
Immunofluorescence of the brain



Results

Immunofluorescence

PAR-1 migrates to the nuclei in CA3

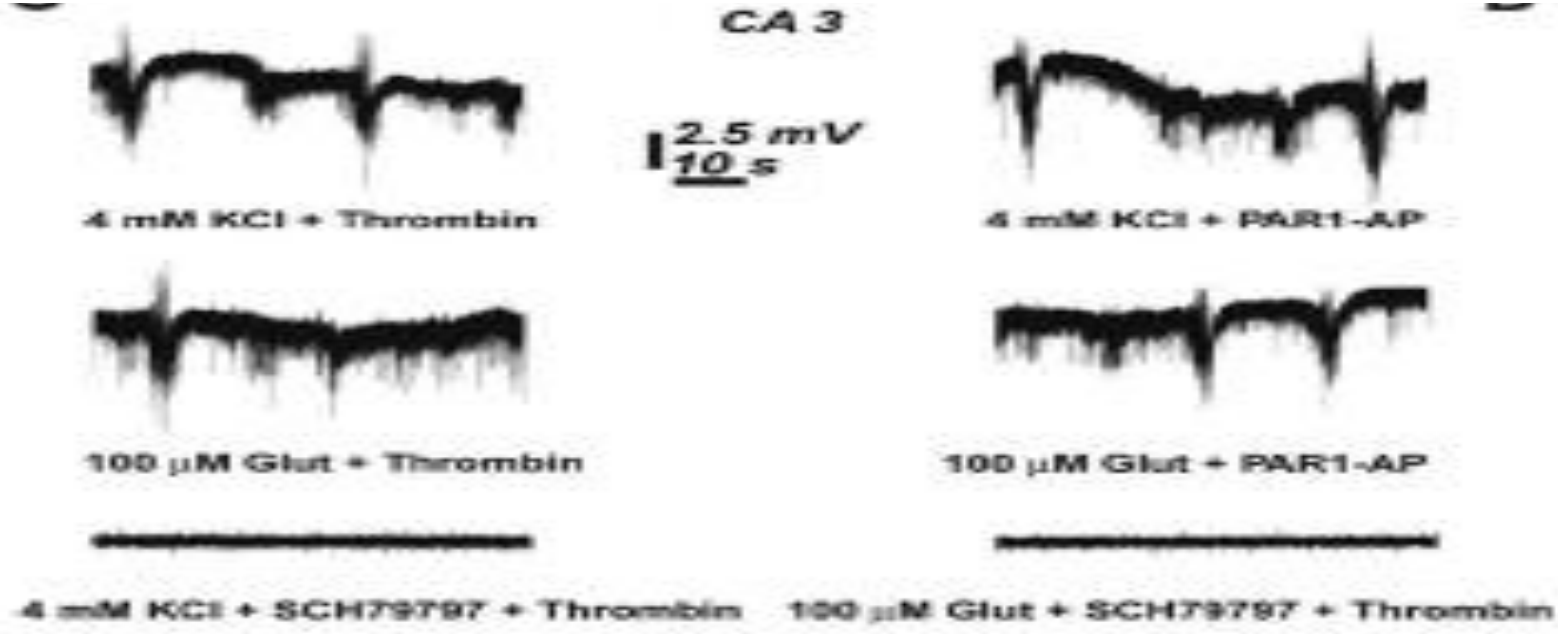


Interim summary 1

	Expected	Observed
Brain thrombin or like activity	 immediate	 late
Brain protease inhibition		
Brain PAR-1 levels		 late
Brain PAR-1 localization	?	Nuclear

Emerging hypothesis B

Behavioral effect of PAR-1 elevation



- Thus, mice with elevated PAR-1(=mTBI) are likely to exhibit more epileptic like activity following thrombin exposure

Maggio N., et al., J.Neuroscience 2008

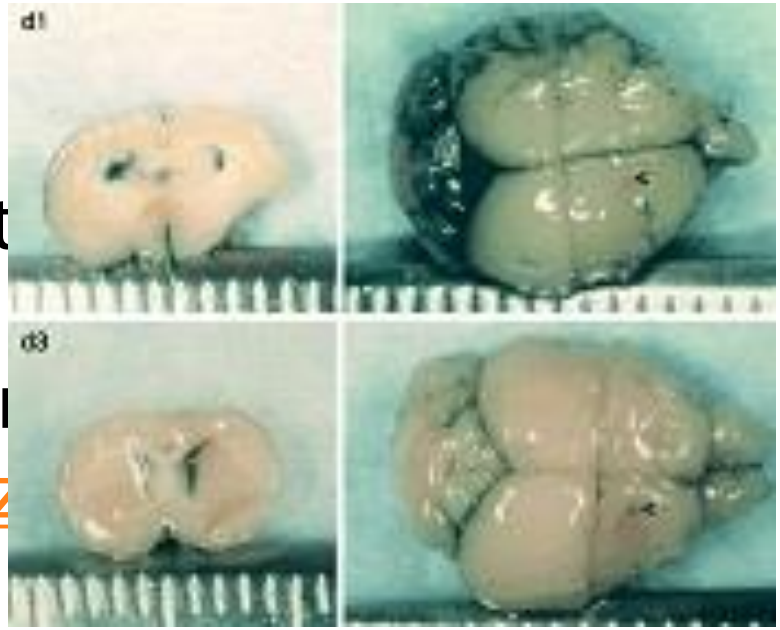
Experimental model

Assays

In vivo

- Intracerebroventricular
- ost
- ezi

r seiz
the first 20 minutes post

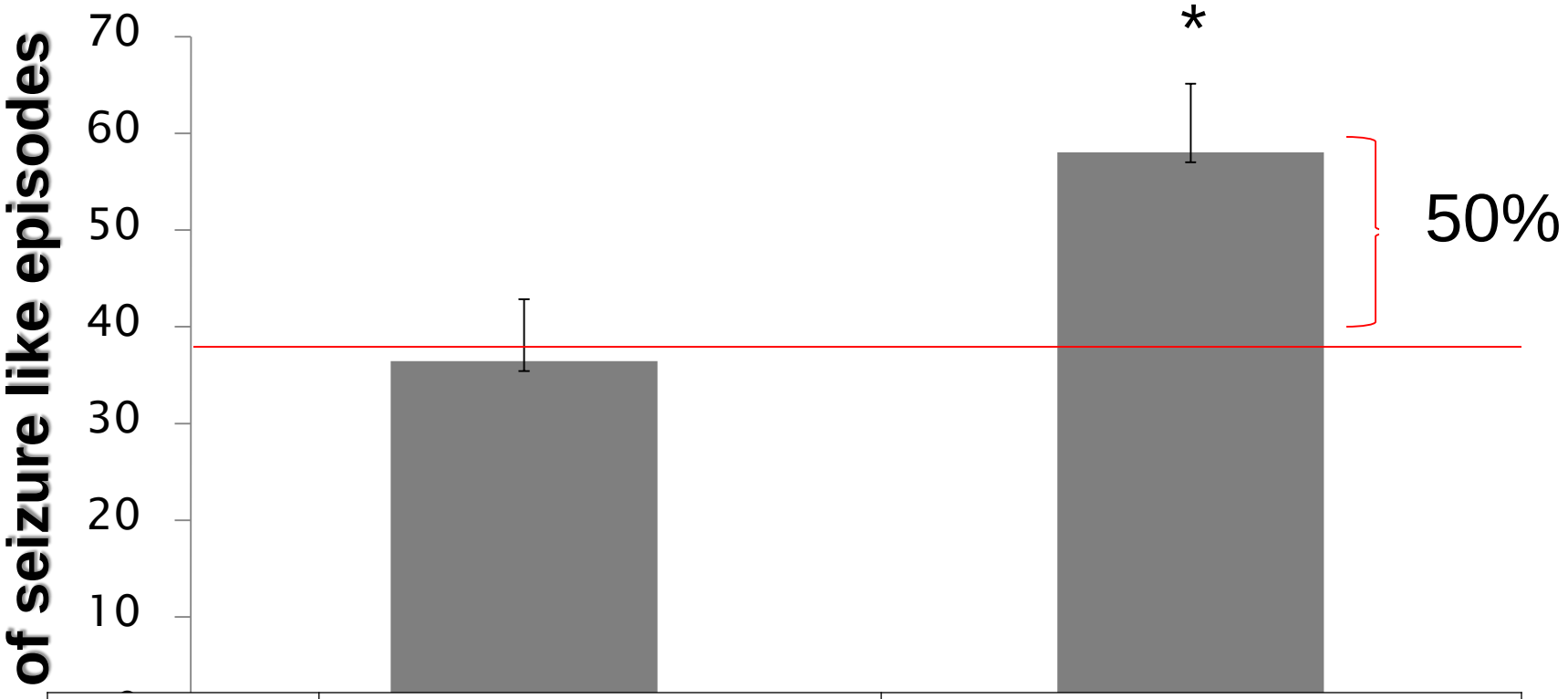


- EEG validation of the seizure response

Sundaresan et al., J.Virol. 2000

Results
Behavior

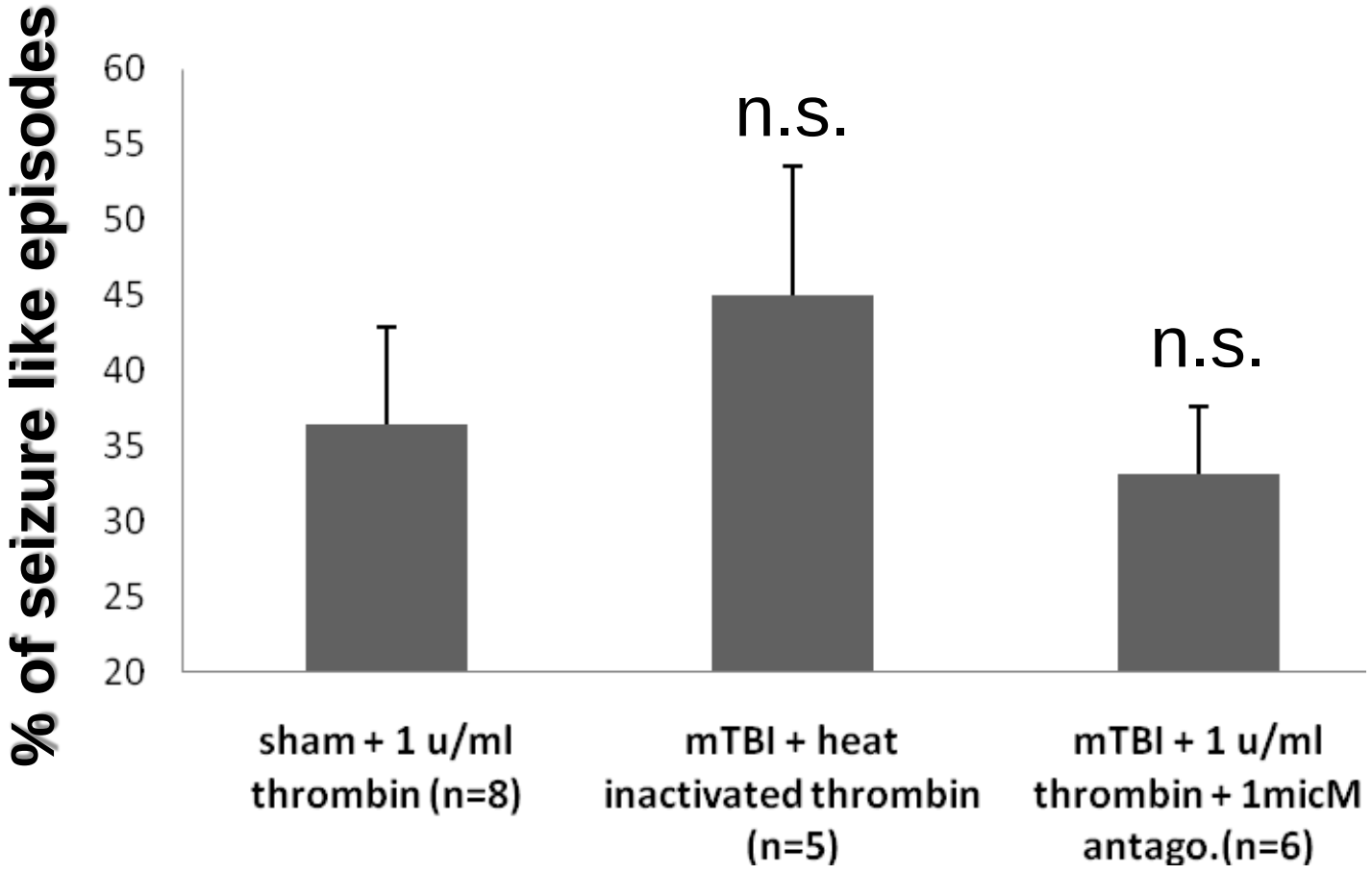
Proportion of thrombin induced seizure episodes rises



mTBI	intact	72 hours post mTBI
Injection	thrombin (n=8)	thrombin (n=9)

Results
Behavior

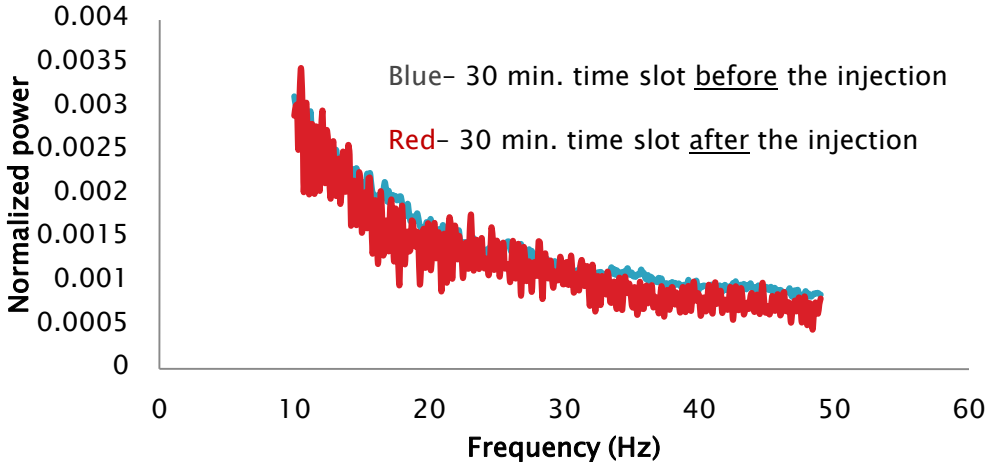
And PAR-1 antagonism abolishes epileptic like response



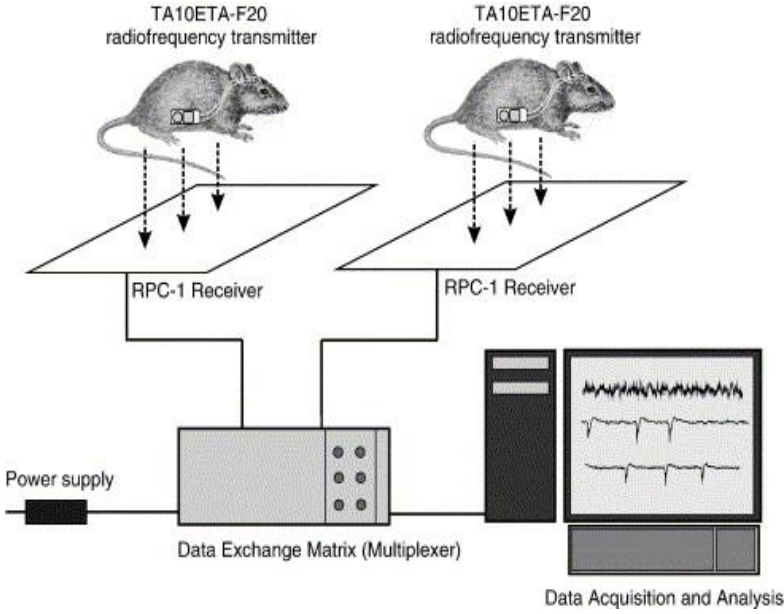
Results

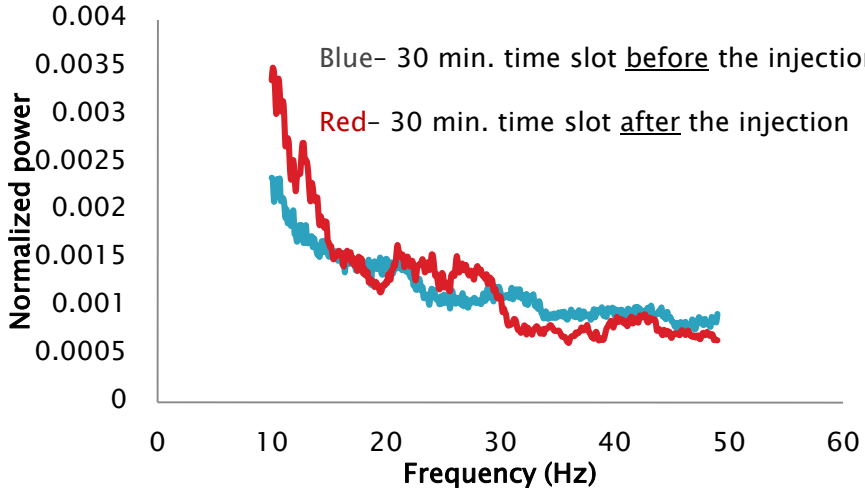
EEG

Epileptiform EEG activity of thrombin injected mice



Saline injection
(n=3)





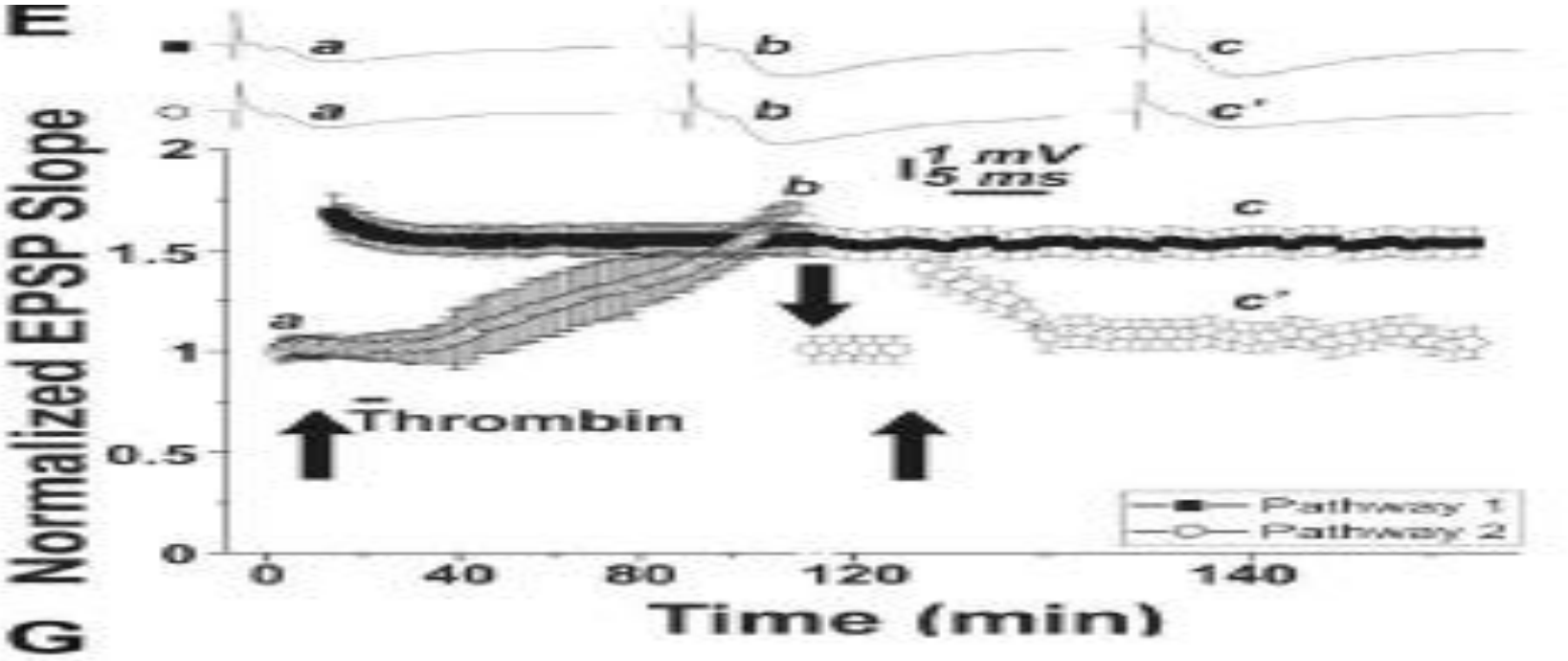
Thrombin injection
(n=2)

Interim summary 2

	Expected	Observed
Thrombin responsiveness in mTBI animals	⇧	⇧
Electrophysiological pattern of the behavioral response	**epileptiform**	**epileptiform**

Emerging hypothesis C

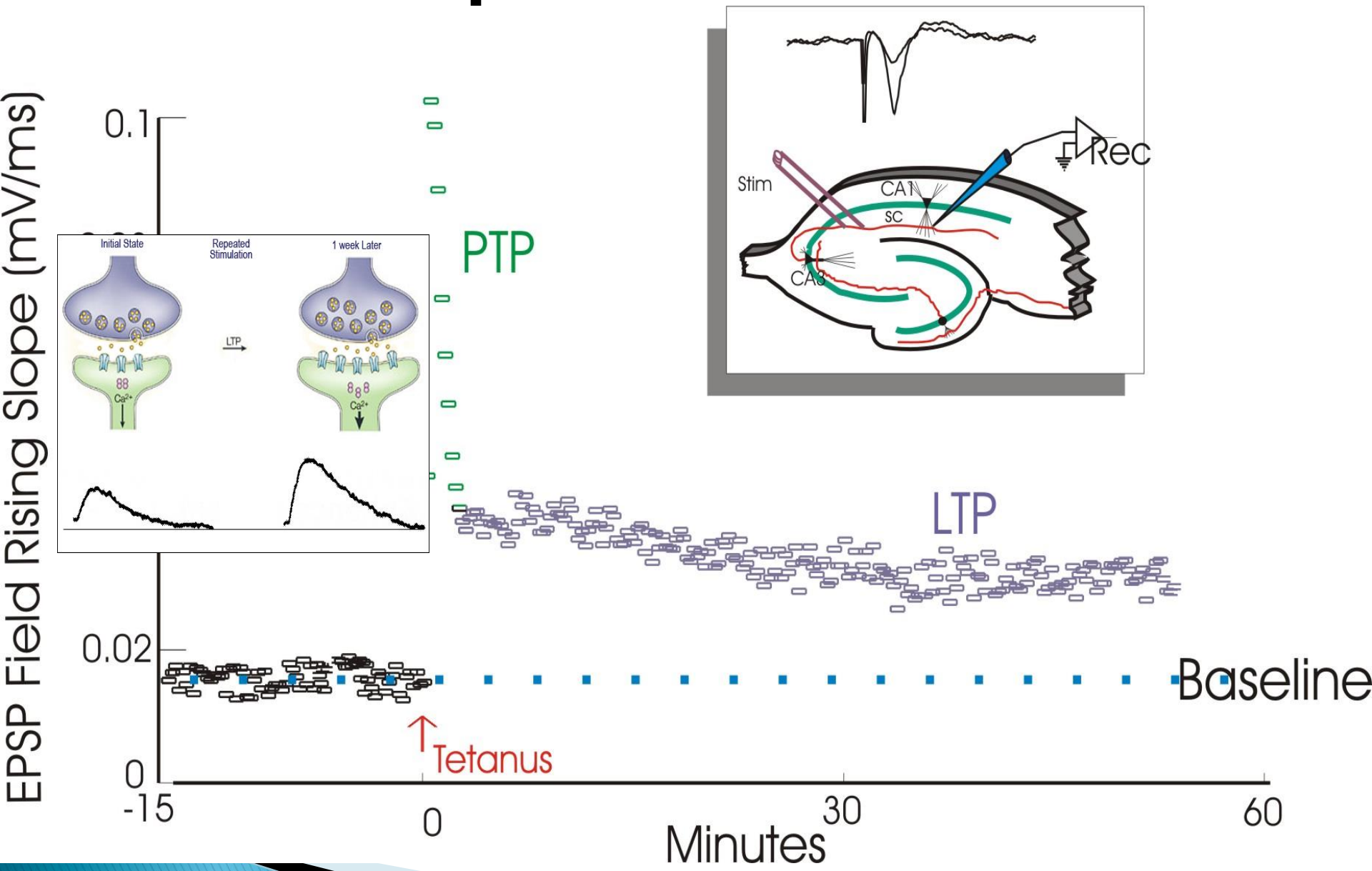
Behavioral effect of elevated thrombin activity



- Thus, elevation of thrombin in brain tissue (via mTBI or direct injection) may result in memory formation impairment.

Maggio N., et al., J.Neuroscience 2008

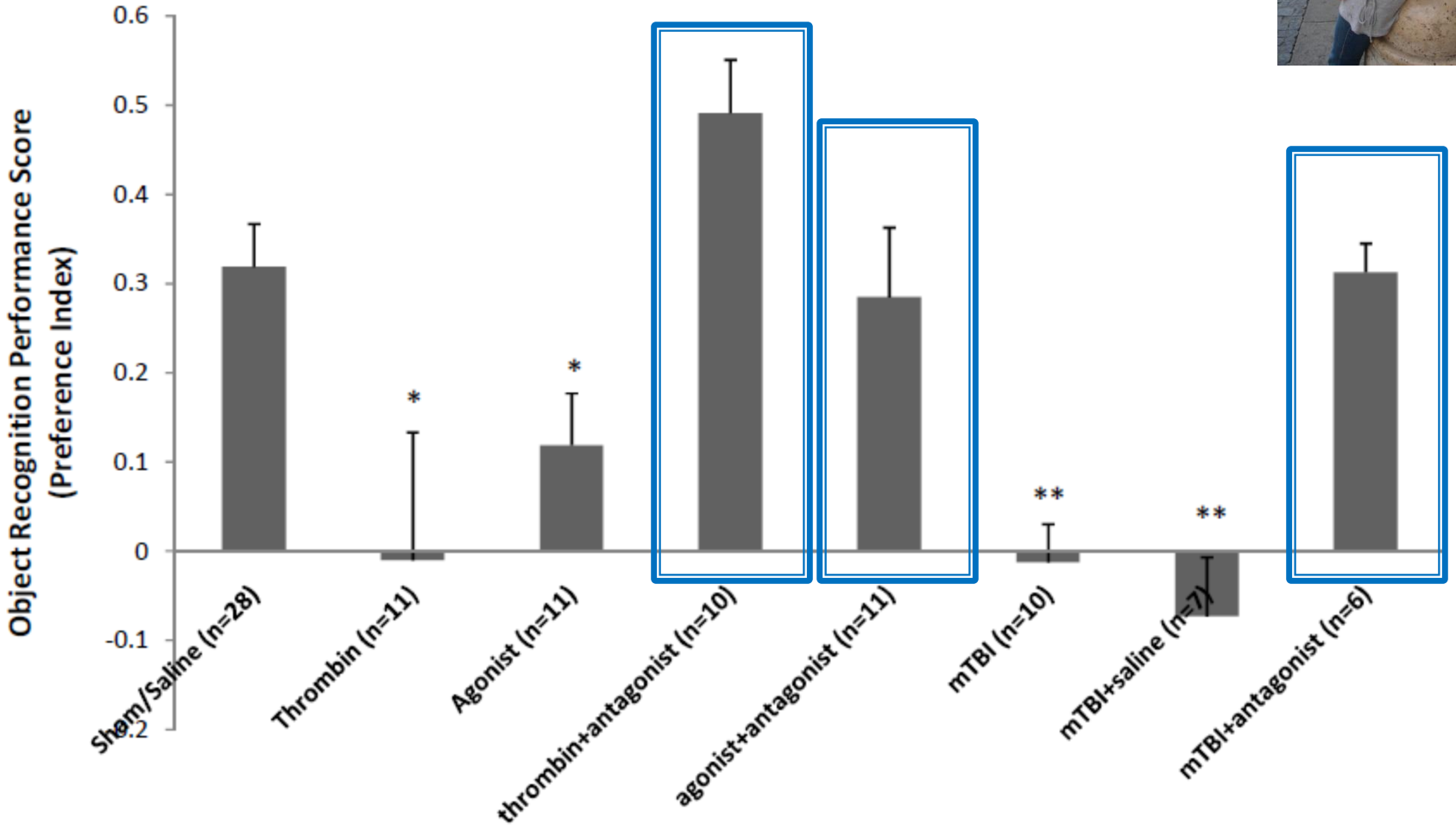
Experimental model



Results

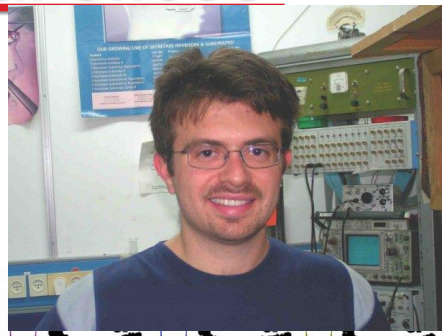
Recognition memory of mice is impaired following mTBI or thrombin exposure

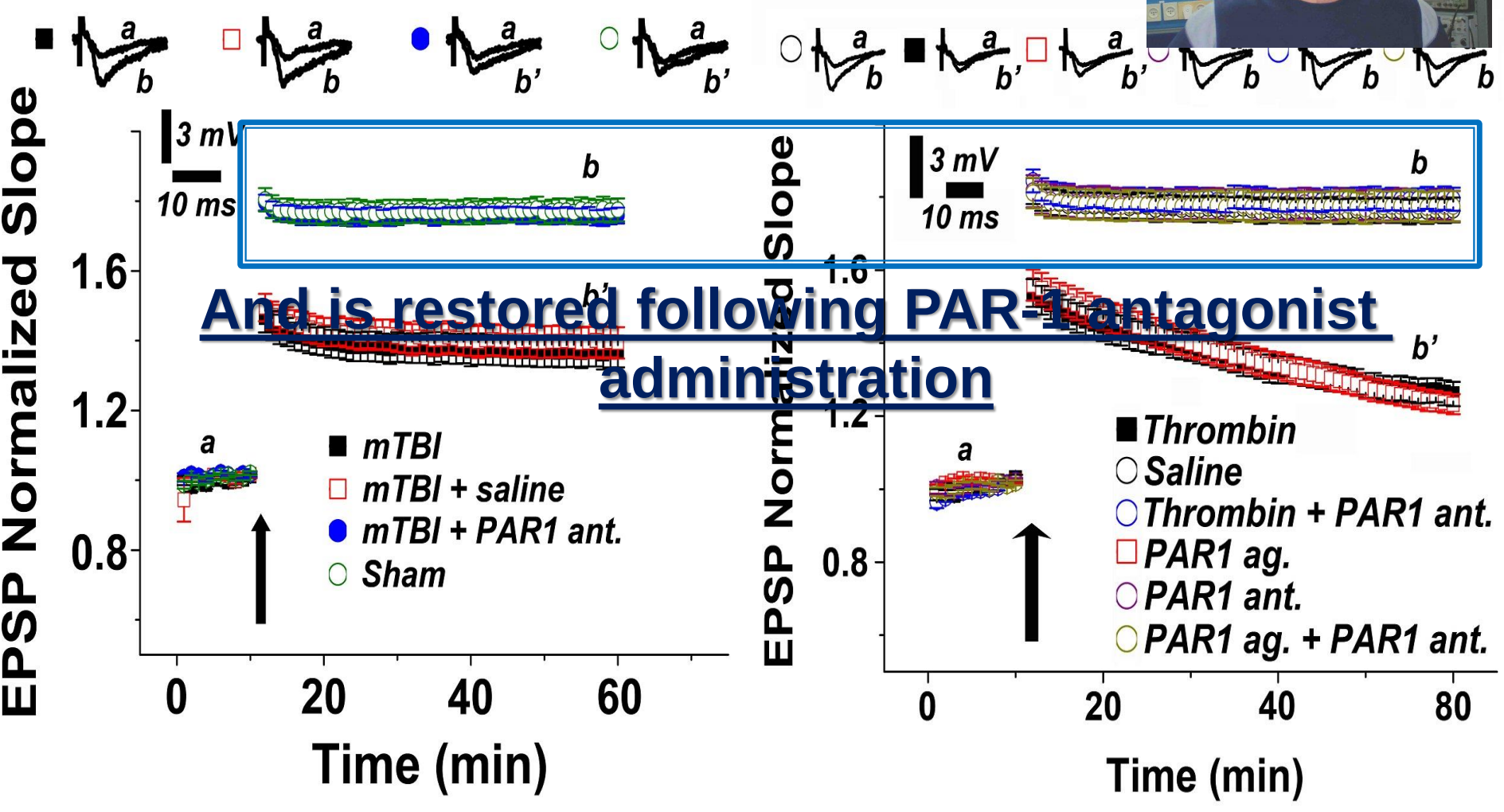
And is restored following PAR-1 antagonist administration



Results

Long term LTP formation in hippocampal slices is impaired following mTBI or thrombin ICV injection





And is restored following PAR-1 antagonist administration

Interim summary 3

	Expected	Observed
mTBI and thrombin effect on cognitive performance	negative	negative
mTBI and thrombin effect on LTP formation	negative	negative

Conclusions

- **mTBI results in increased thrombin like activity in the brain – acute and prolonged**

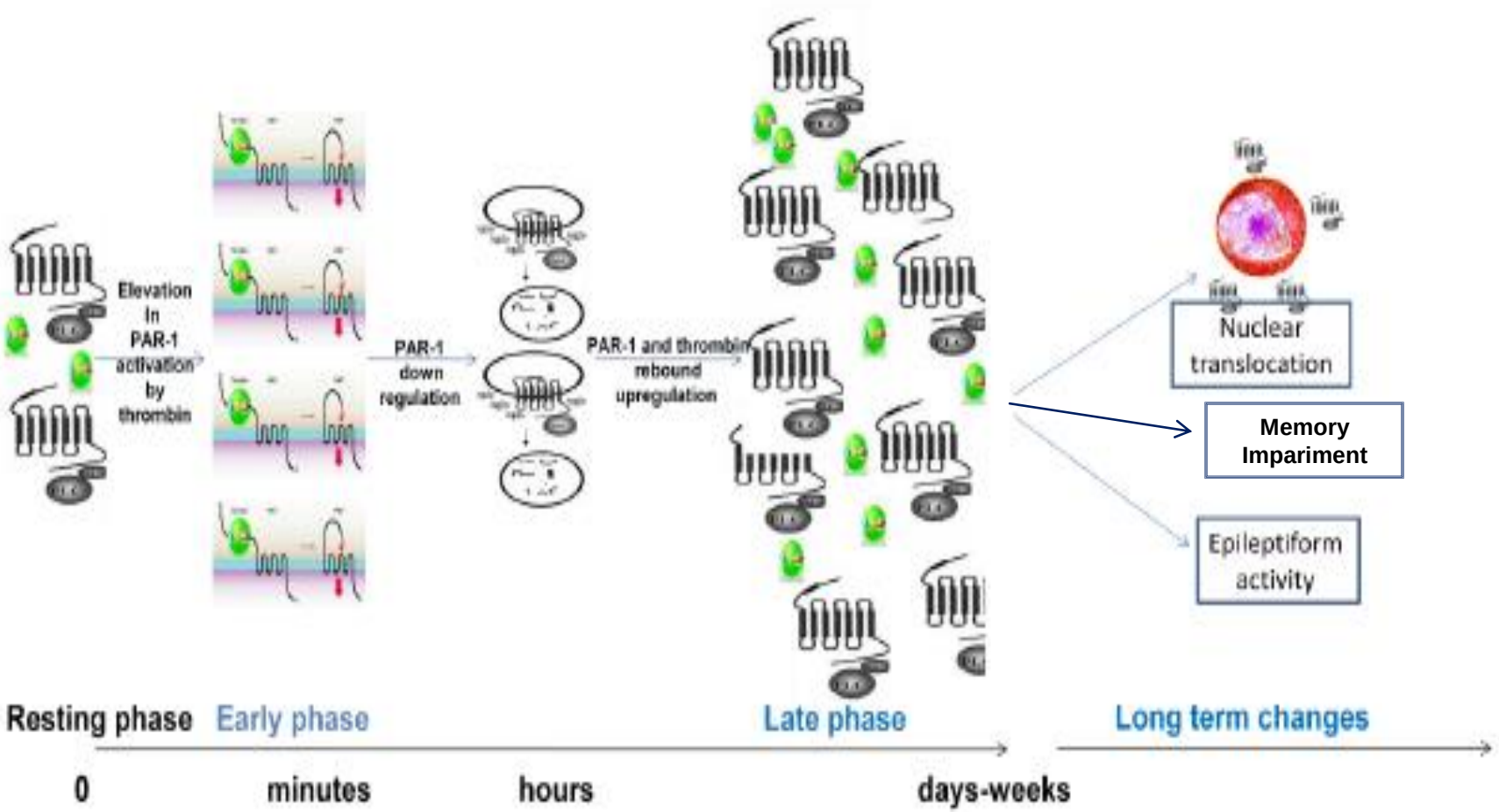
- mTBI results in upregulation of CNS protease inhibitors

- **mTBI results in upregulation of PAR-1**

- mTBI results in nuclear relocalization (possibly by trafficking) of PAR-1 in the hippocampus

- **These changes lead to neurophysiological and cognitive deficits – which are reversed by PAR-1 blockade**

Proposed model



Thank you !!!

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- Dr.Aviva Katzav

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Prof. Alon Friedman
Itai Weissberg
(BGU)

Conclusions

- Thrombin exposure leads to PAR-1 upregulation in glial cells, a prominent player in tri-partide synapse

- These changes lead to increased sensitivity to thrombin-induced epiletiform seizures

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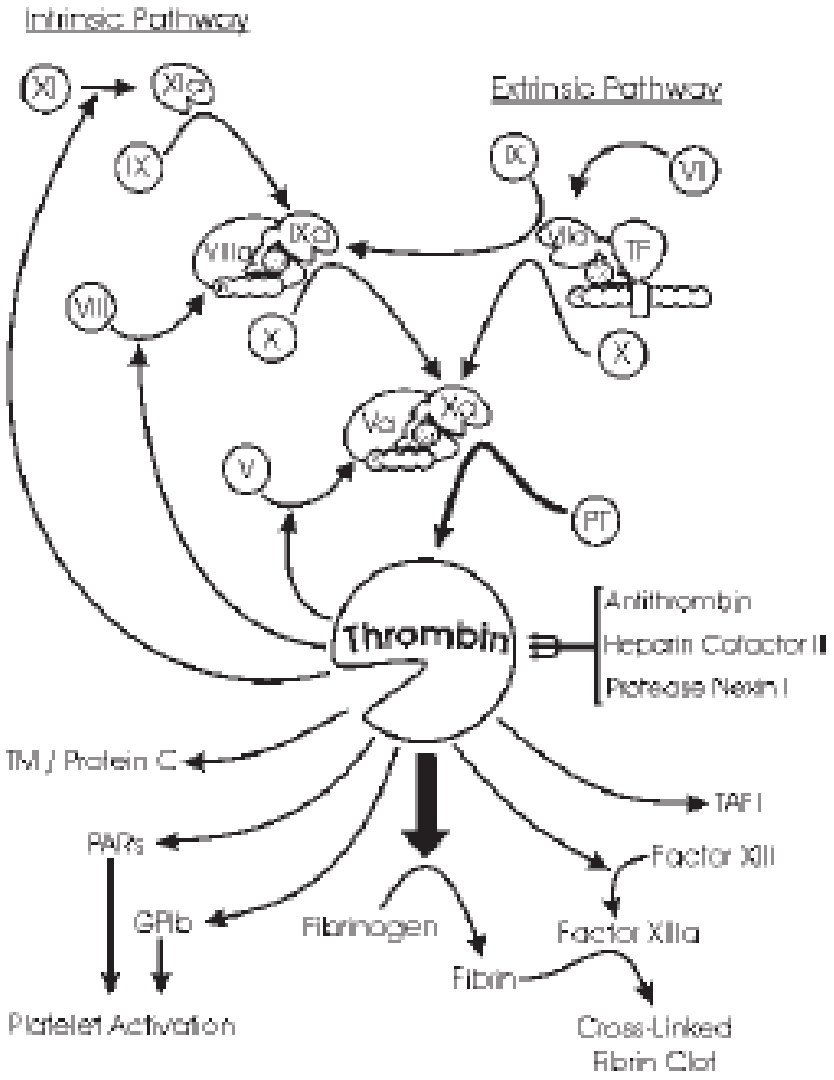
Intrinsic Pathway
Extrinsic Pathway
XI
XIa
IX
IXa
VIIIa
VIII
X
VII
TF
VIIa
Va
Xa
V
PT
Thrombin
Antithrombin
Heparin Cofactor II
Protease Nexin I
TM / Protein C
PARs
GPIb
Fibrinogen
Fibrin
TAFI
Factor XIII
Factor XIIIa
Platelet Activation
Cross-Linked
Fibrin Clot

Table 1 – Protease-activated receptors: subtypes, agonists, and antagonists

	PAR-1	PAR-2	PAR-3	PAR-4
Cleavage sites	$R^{41}\downarrow S^{42}$FLLRN (h) $R^{41}\downarrow S^{42}$FFLRN (r, m)	$R^{36}\downarrow S^{37}$LIGKV (h) $R^{36}\downarrow S^{37}$LIGRL (r) $R^{34}\downarrow S^{35}$LIGRL (m)	$K^{38}\downarrow T^{39}$FRGAP (h) $K^{37}\downarrow S^{38}$FNGGP (m)	$R^{47}\downarrow G^{48}$YPGQV (h) $R^{58}\downarrow G^{59}$FPGKP (r) $R^{59}\downarrow G^{60}$YPGKF (m)
Protease agonists	Thrombin Trypsin Mesotrypsin/Trypsin IV (?) FVIIa FXa APC Granzyme A Arginine-specific gingipains-R	Trypsin Tryptase Mesotrypsin/Trypsin IV (?) P22 Kallikrein-5, -6, -14 FVIIa and FXa MT-SP1 Proteinase-3 Acrosien Der P3 and P9 Arginine-specific gingipains-R	Thrombin	Thrombin Trypsin Mesotrypsin/Trypsin IV (?) Kallikrein-14 Cathepsin G FVIIa FXa Arginine-specific gingipains-R
Peptide agonists	SFLLR-NH_2 TFLLR-NH_2[a] TRag[b] TFRIFD	SLIGKV-NH_2 SLIGRL-NH_2[a] SFLLR-NH_2 *Trans*-cinnamoyl-LIGRLO-NH_2 2-furoyl-LIGRLO-NH_2 [c]	None	GYPGQV-NH_2 GFPGKP-NH_2 GYPGKF-NH_2 AYPGKF-NH_2[a]
Antagonists	3-mercapto-propionyl-F-Cha-Cha-RKPNDK-amide *Trans*-cinnamoyl-parafluoro-F-paraguanidino-FLRR-amide N-palmitoyl-RCLSSSAVANRS-amide RPPGF-OH RWJ56110 RWJ58259 SCH79797 FR171113 Merck isoxazole 1	N^1-3-methylbutyryl-N^4-6-aminohexanoyl-piperazine	None	*Trans*-cinnamoyl-YPGKF-amide *Trans*-cinnamoyl-APGKF-amide N-palmitoyl-SGRRYGHALR-amide YD-3

Note. a: standard PAR activating peptide. c: most potent and selective PAR-2 peptide agonist.
b: putative and selective PAR-1 peptide agonist. h, human; r, rat; m, mouse; ↓, cleavage site.
?: It is still open which PARs are activated by mesotrypsin/trypsin IV in the brain.