

INFLAMMATION RESEARCH NETWORK

Protease-Activated Receptors and Pain

Nathalie Vergnolle

Inserm

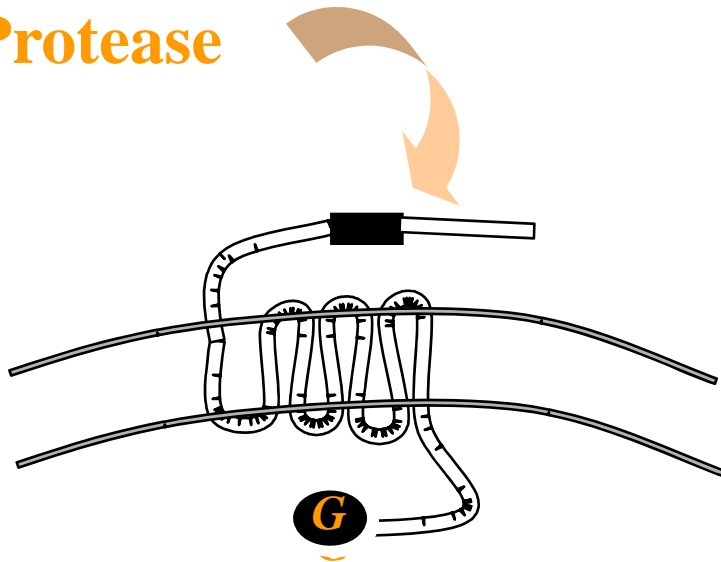
Institut national
de la santé et de la recherche médicale

U 563 Toulouse, France



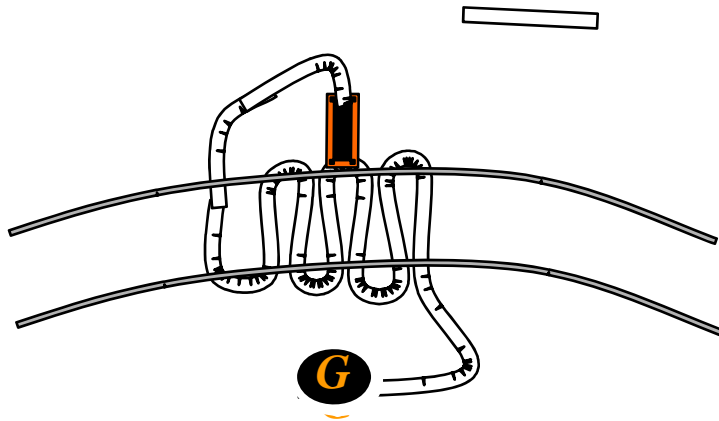
- G-protein coupled receptors
- activated by proteases

Protease



Protease-mediated cell signaling: PAR activation

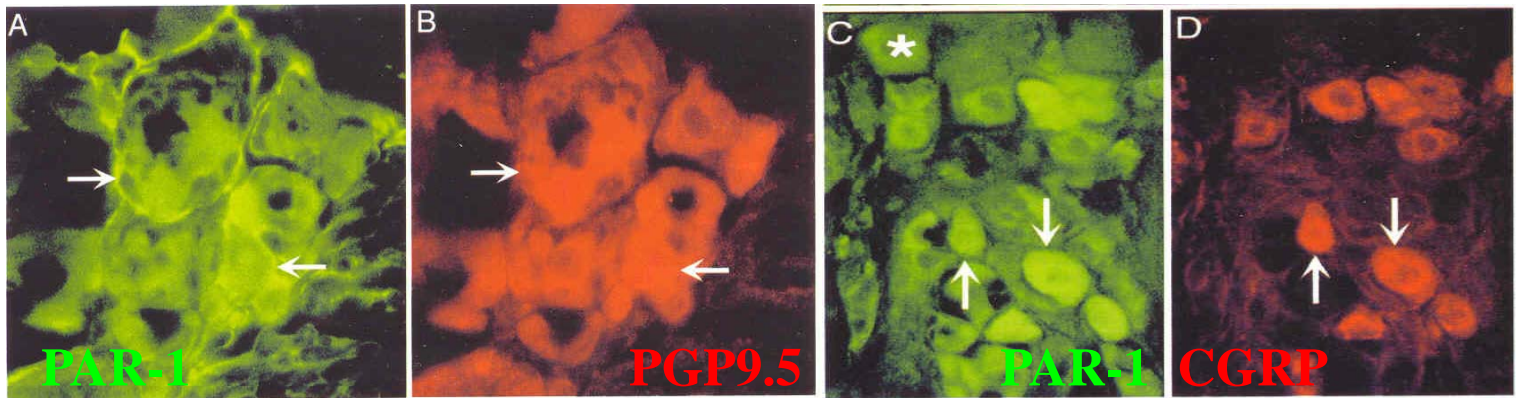
- G-protein coupled receptors
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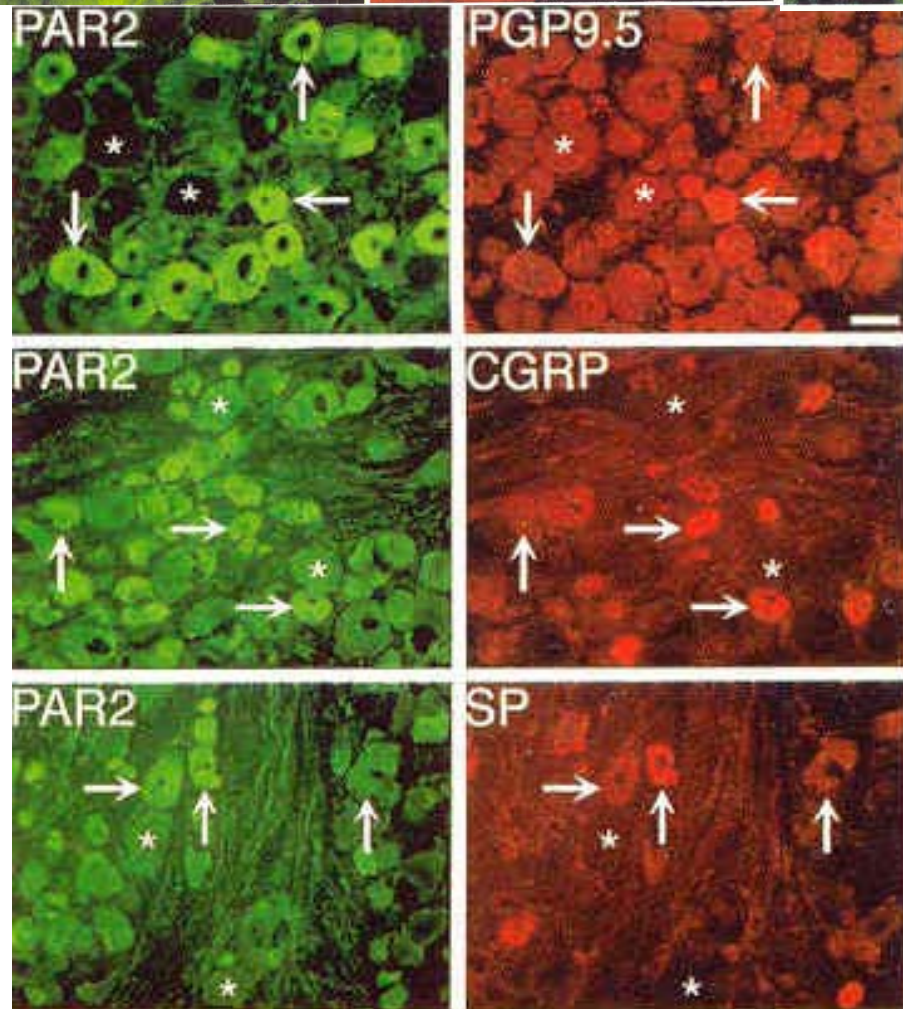
Protease-mediated cell signaling: PAR activation

PAR activation

	Serine proteases	Cleavage domain	Activating peptide
PAR ₁	Thrombin FXa Granzyme A Gingipain-R...	LDPR ⁴¹ ↓ S ⁴² FLLRN	SFLLRN TFLLRN
PAR ₂	Trypsin Tryptase FVIIa FXa Gingipain-R...	SKGR ³⁴ ↓ S ³⁵ LIGKV	SLIGKV (human) SLIGRL (mouse)
PAR ₃	Thrombin	LPIK ³⁸ ↓ T ³⁹ FRGAP	
PAR ₄	Trypsin Thrombin Cathepsin G Gingipain-R	PAPR ⁴⁷ ↓ G ⁴⁸ YPGQV	GYPGQV (human) GYPGKF (mouse) AYPGKF



De Garavilla et al. Br. J. Pharmacol. 2001



**Extrinsic
sensory
nerves**

**Dorsal root
ganglia neurons**

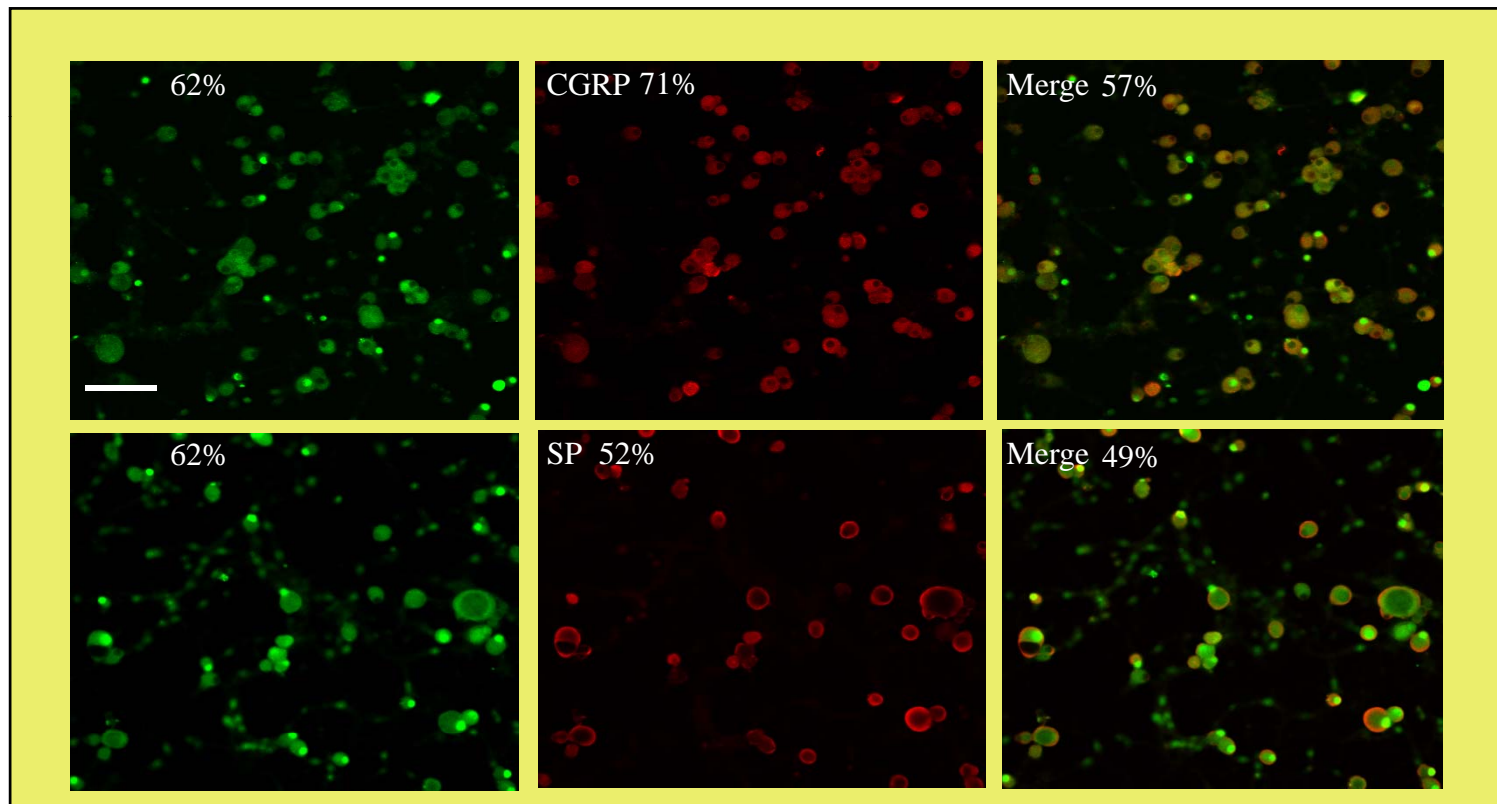
Steinhoff et al. Nat. Med. 2000

PAR-4 on sensory neurons (mouse DRGs neurons)

— : 50 μ m

PAR-4

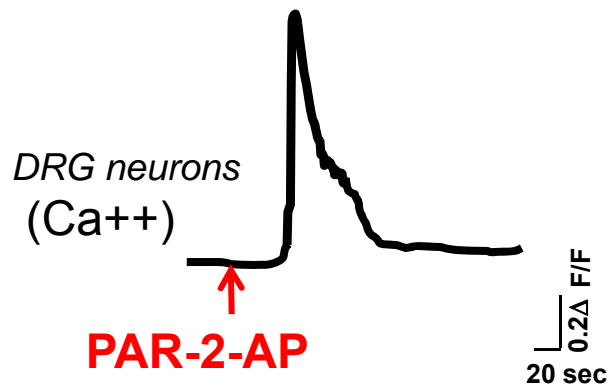
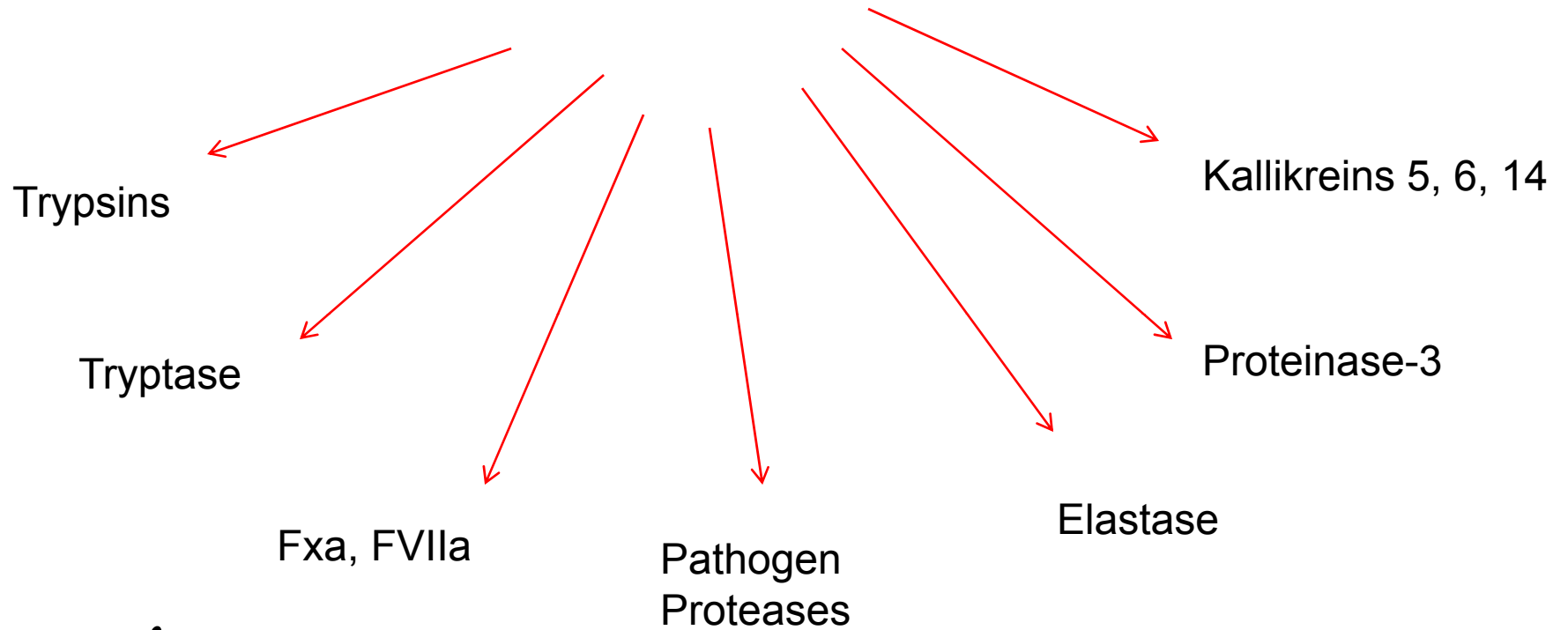
CGRP



PAR-4

SP

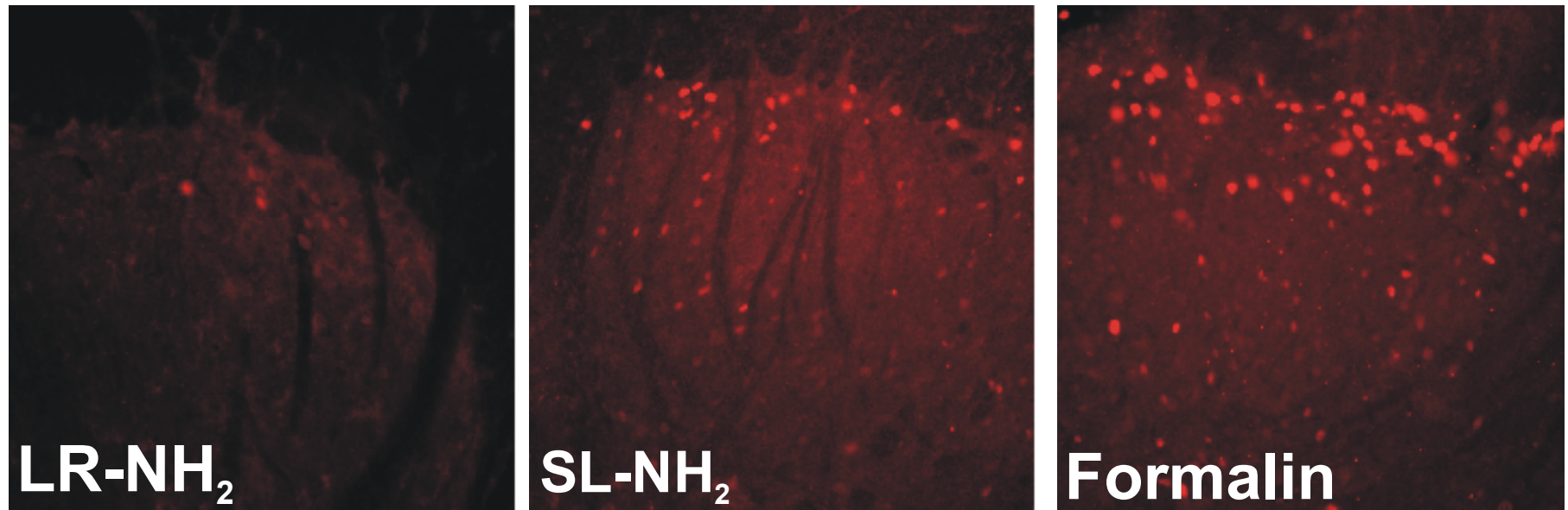
PAR-2



Long-term hyper-excitability

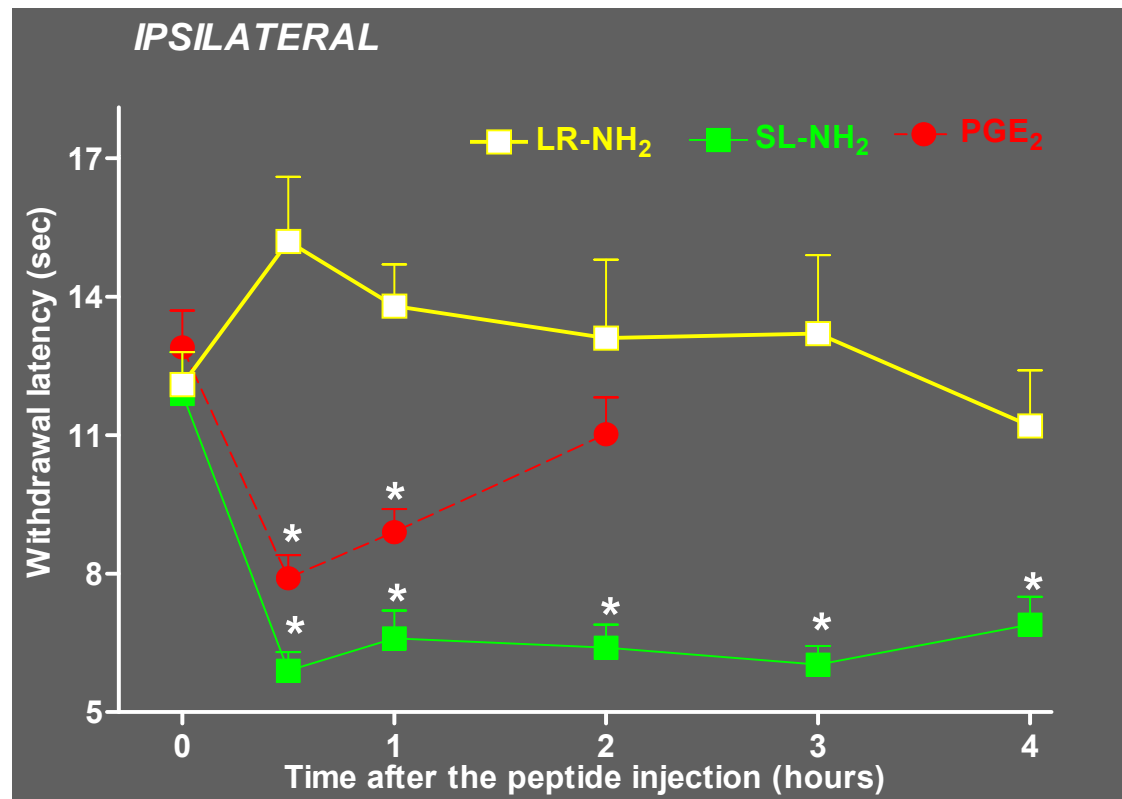
Reed et al. et al. J. Physiol. 2003
Kirkup et al. J. Physiol. 2003

PAR-2 Activation and Formalin Induce Fos Expression in the Dorsal Horn of the Spinal Cord



PAR-2-AP intraplantar injection induced spinal activation of nociceptors

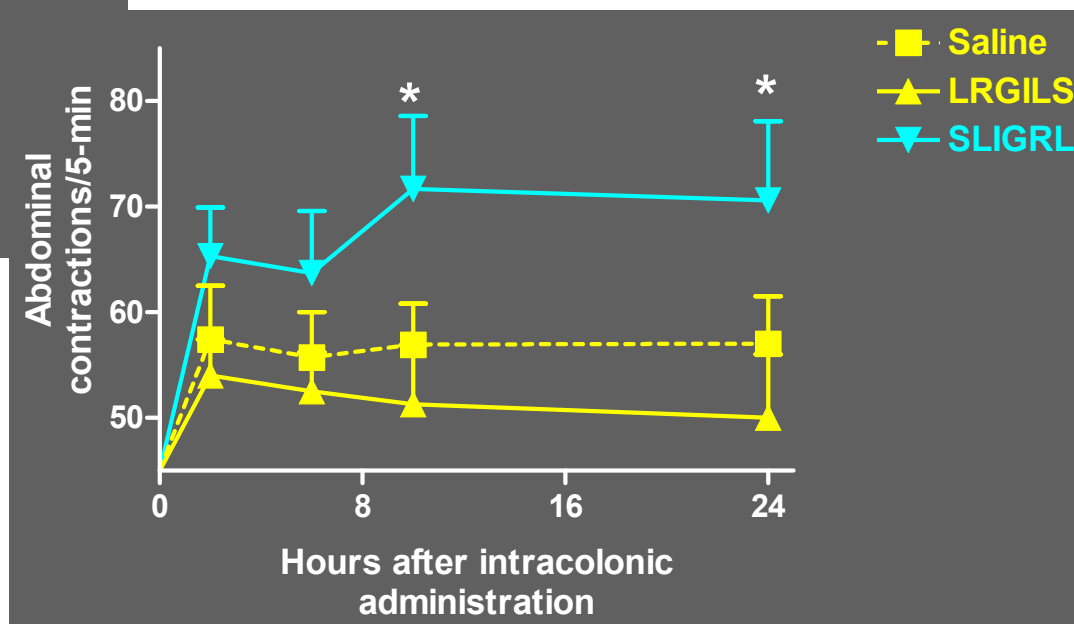
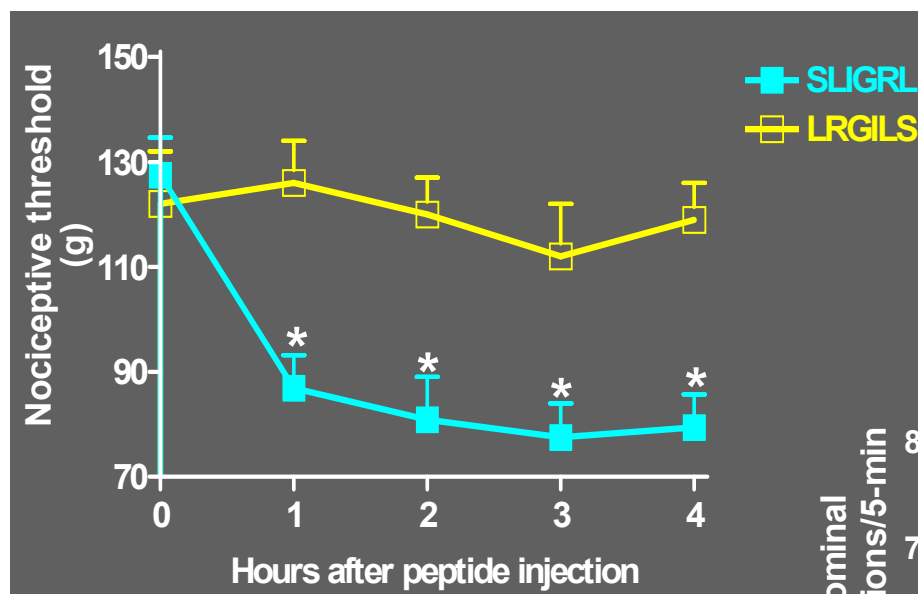
Thermal hyperalgesia after PAR-2 agonist injection



All peptides, 10 µg/paw

Vergnolle et al. Nat. Med. 2001

Mechanical hyperalgesia after PAR-2 agonist injection



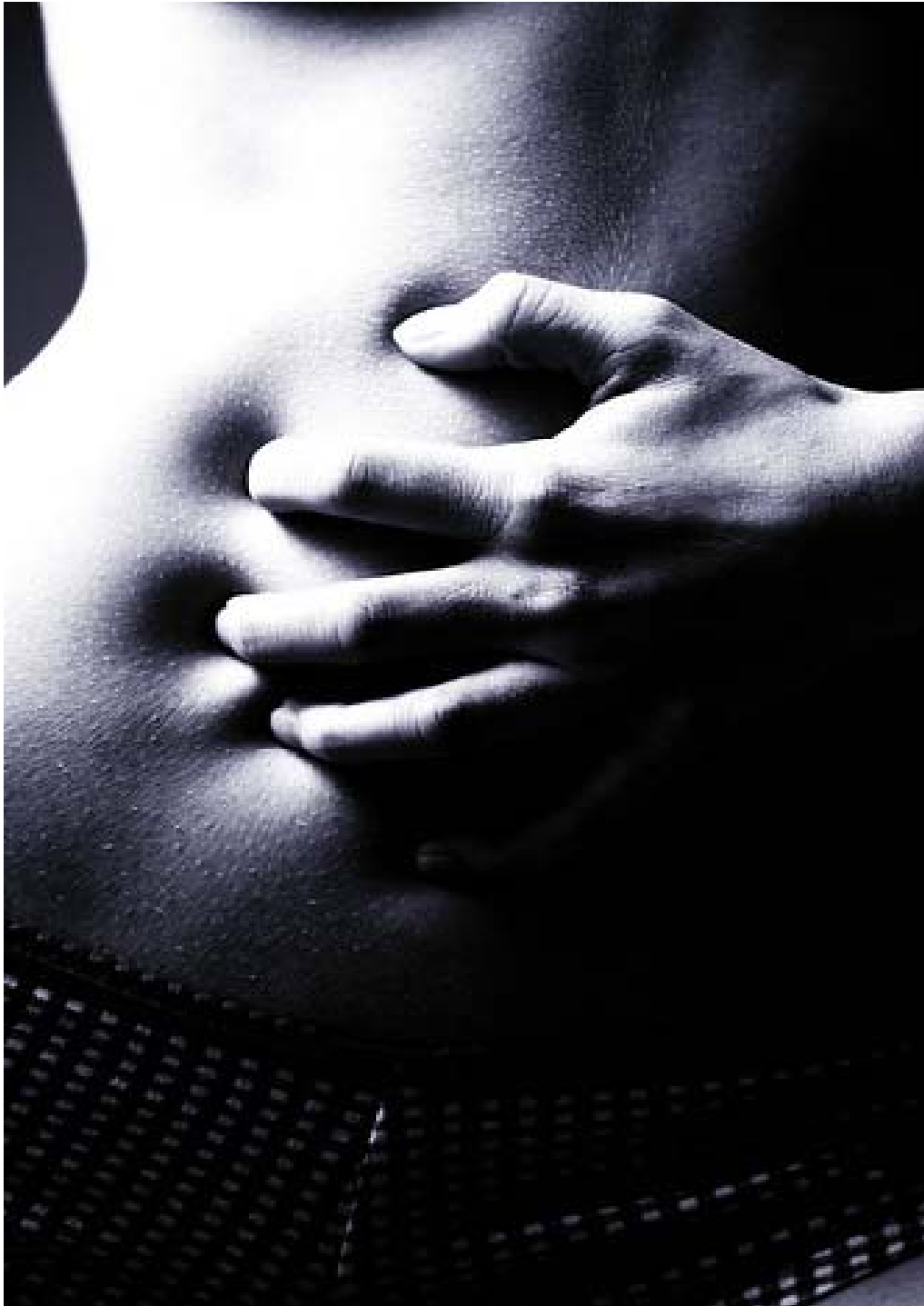
All peptides, 10 μ g/paw

Vergnolle et al. Nat. Med. 2001

Involvement of PAR-2 in models of pain

PAR-2-deficient mice: No hyperalgesia in response to compound 48/80 (mast cell degranulator)

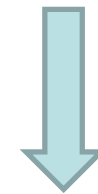
Decreased hyperalgesia in response to formalin



Role for PAR-2 in human diseases associated with pain symptoms?

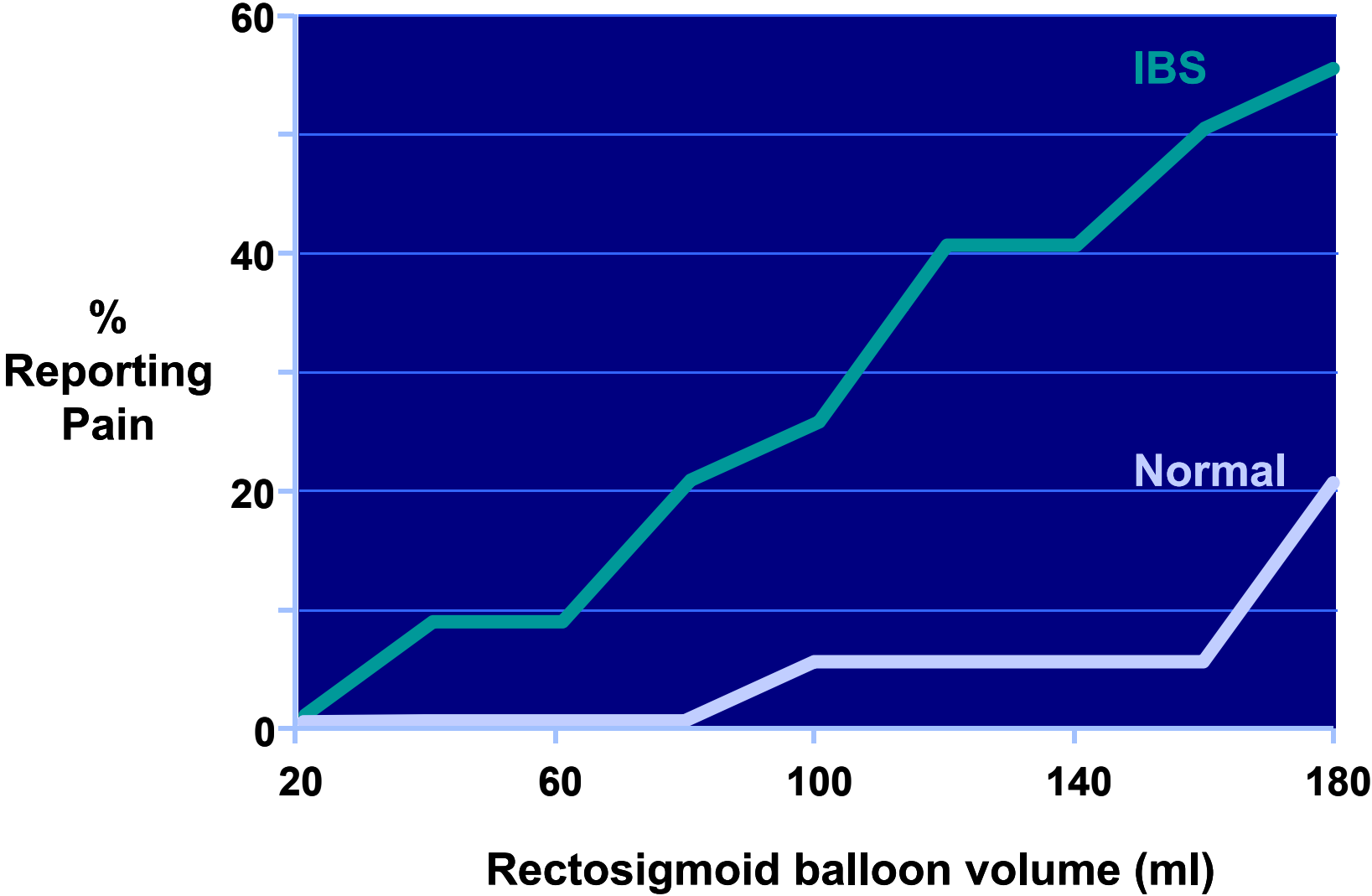
Irritable Bowel Syndrome

Chronic medical condition, characterized by abdominal pain, discomfort, and altered bowel functions



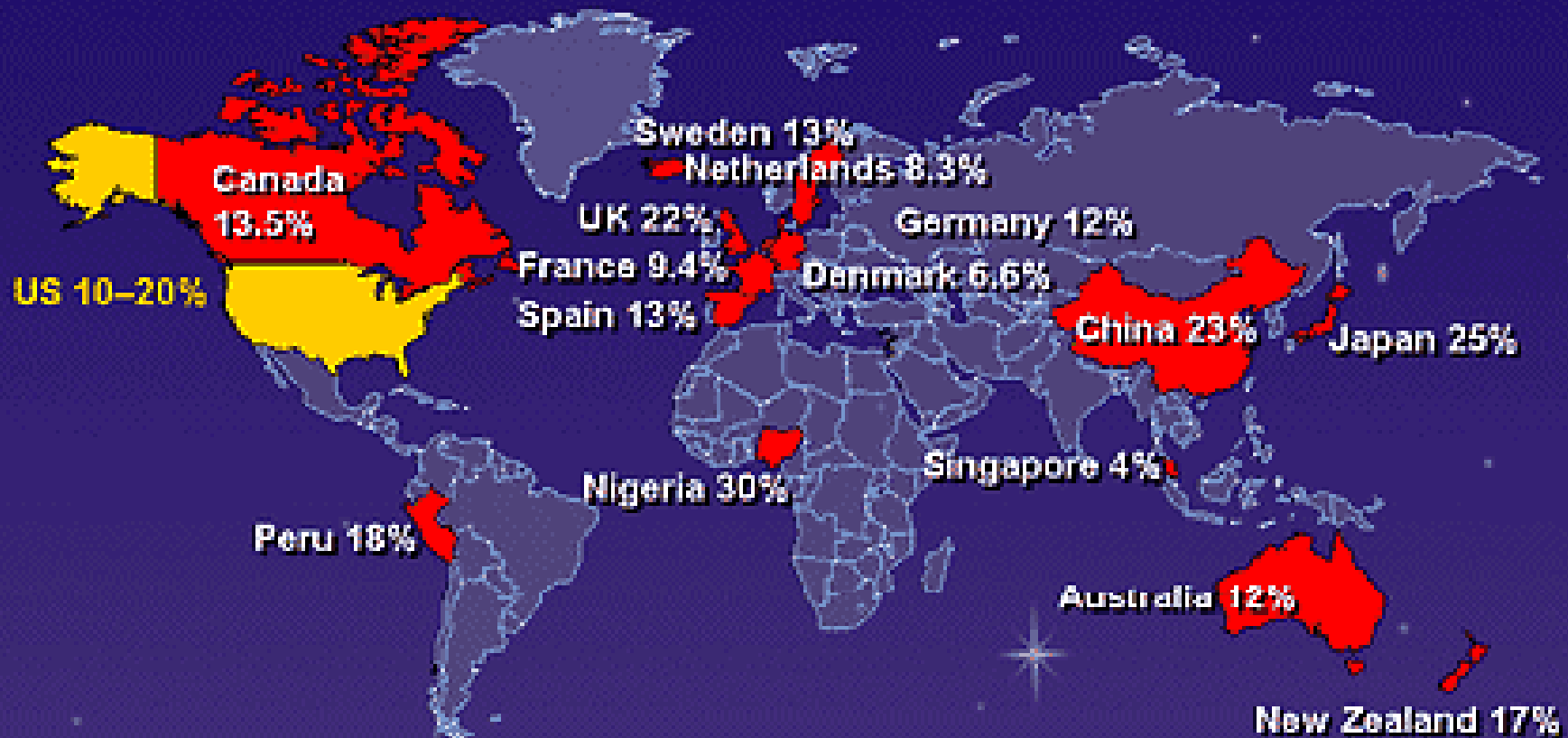
Hypersensitivity to colorectal distension

IBS - Physiology



Whitehead et al., Gastroenterology 1990;98:1187

World prevalence of IBS



*Adapted from Camilleri et al, Aliment Pharmacol Ther 1997; 11: 3
Muller-Lisner et al, Digestion 2001; 64: 200*

Causes

- Food?
- Episode of infectious colitis
- Stress

Signs and Symptoms

- Abdominal pain or cramping
- Bloating sensation
- Gas (flatulence)
- Diarrhea, constipation or both alternating
- Mucus in the stools

Treatments

- Food supplements
- Antidepressant
- Anti-diarrheal medications
- 5-HT₃ antagonist (Alosetron)

Protocol

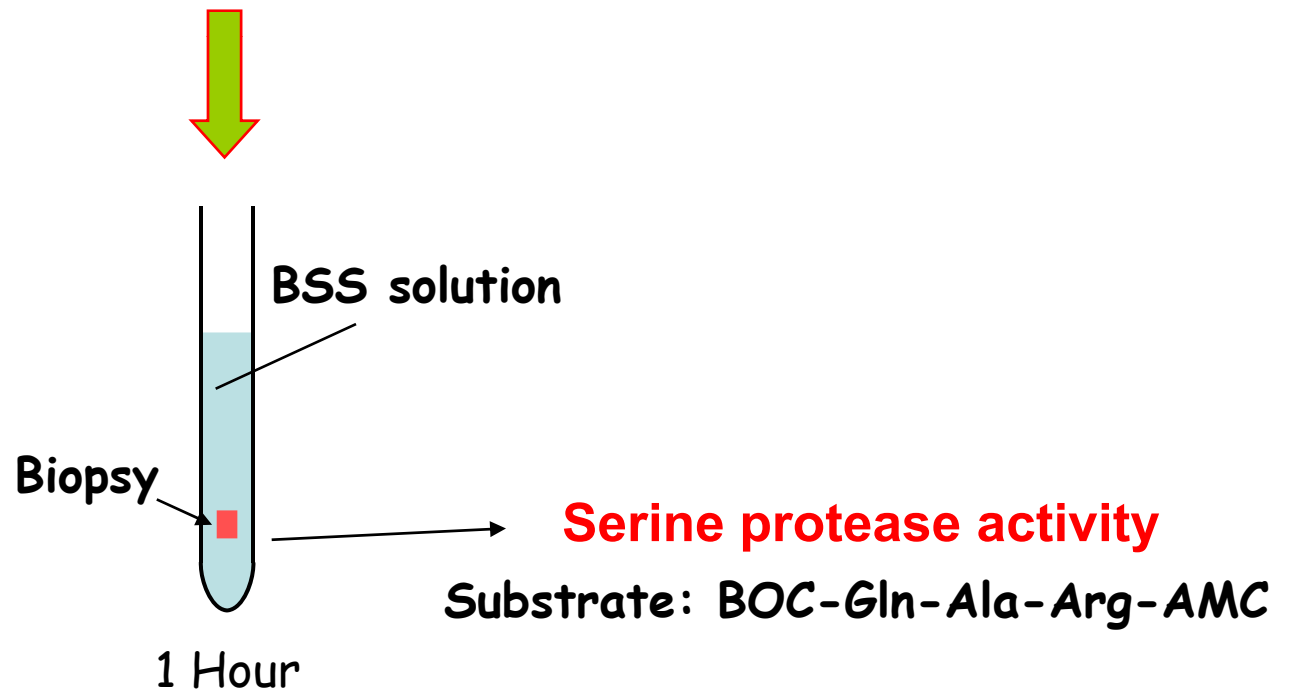
Patients: Foothills Hospital

12 Control (colon cancer screening)

Age: 52.7 ± 9.1 y **Sex ratio:** women 64%

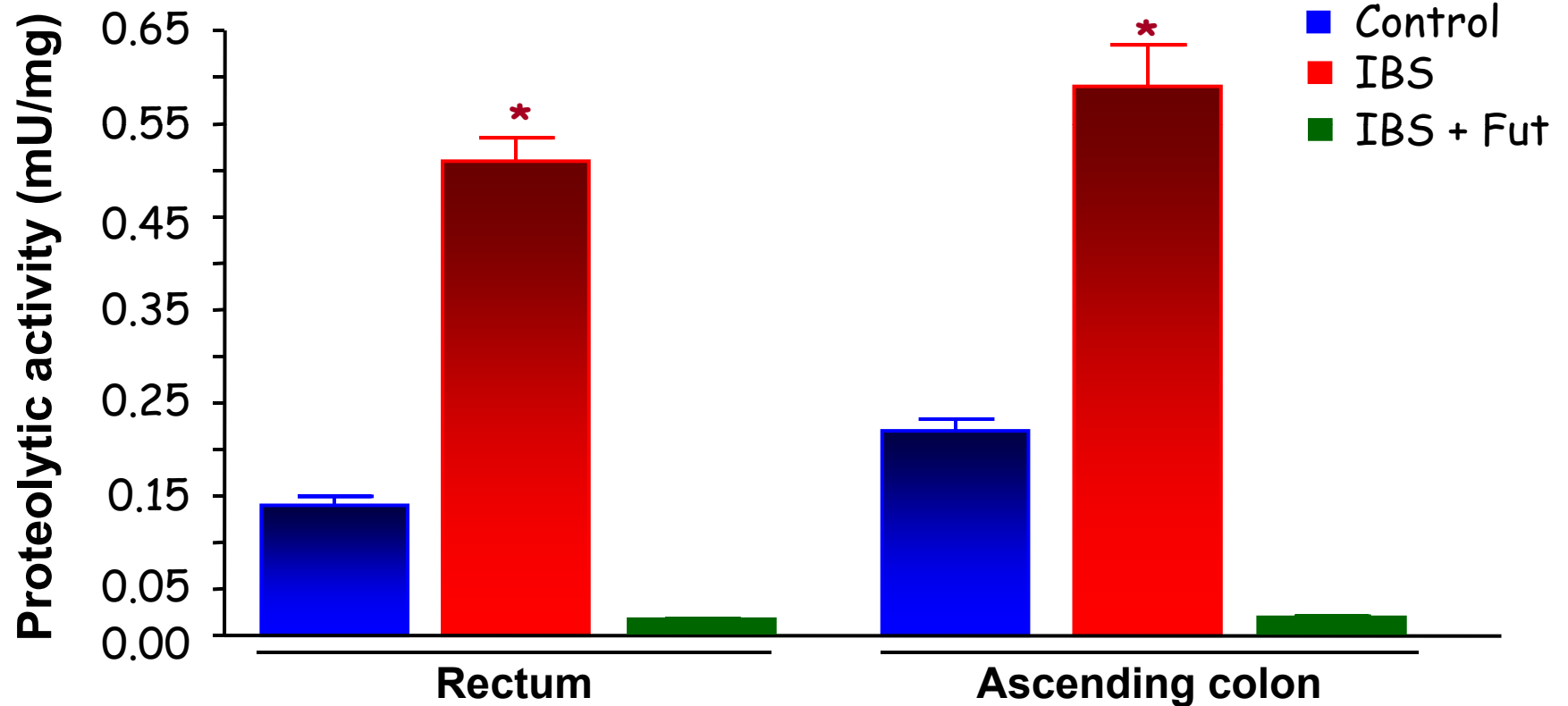
18 IBS according to Rome II criteria

Age: 44.3 ± 14.1 y **Sex ratio:** women 83.3%



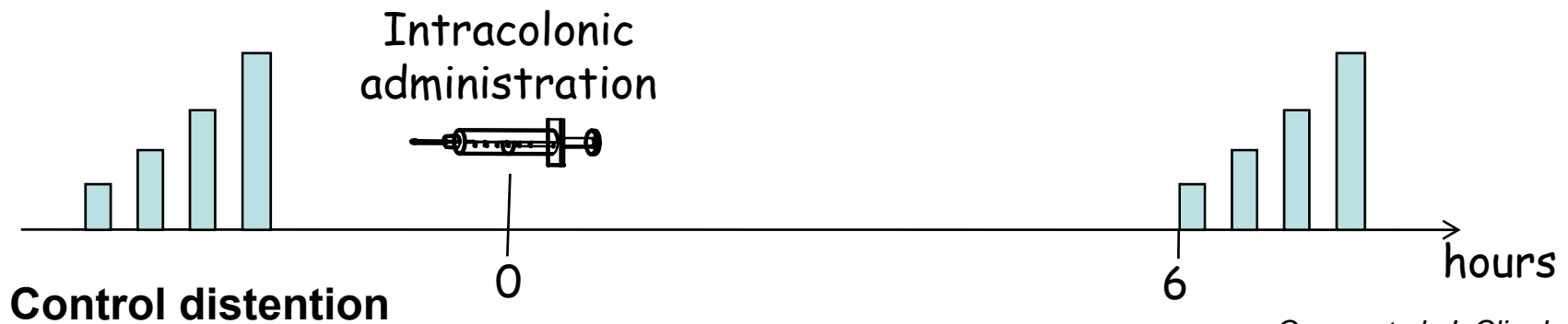
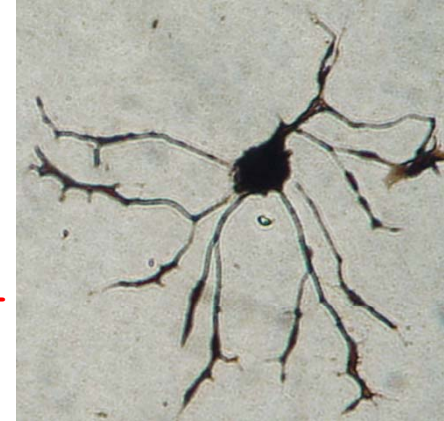
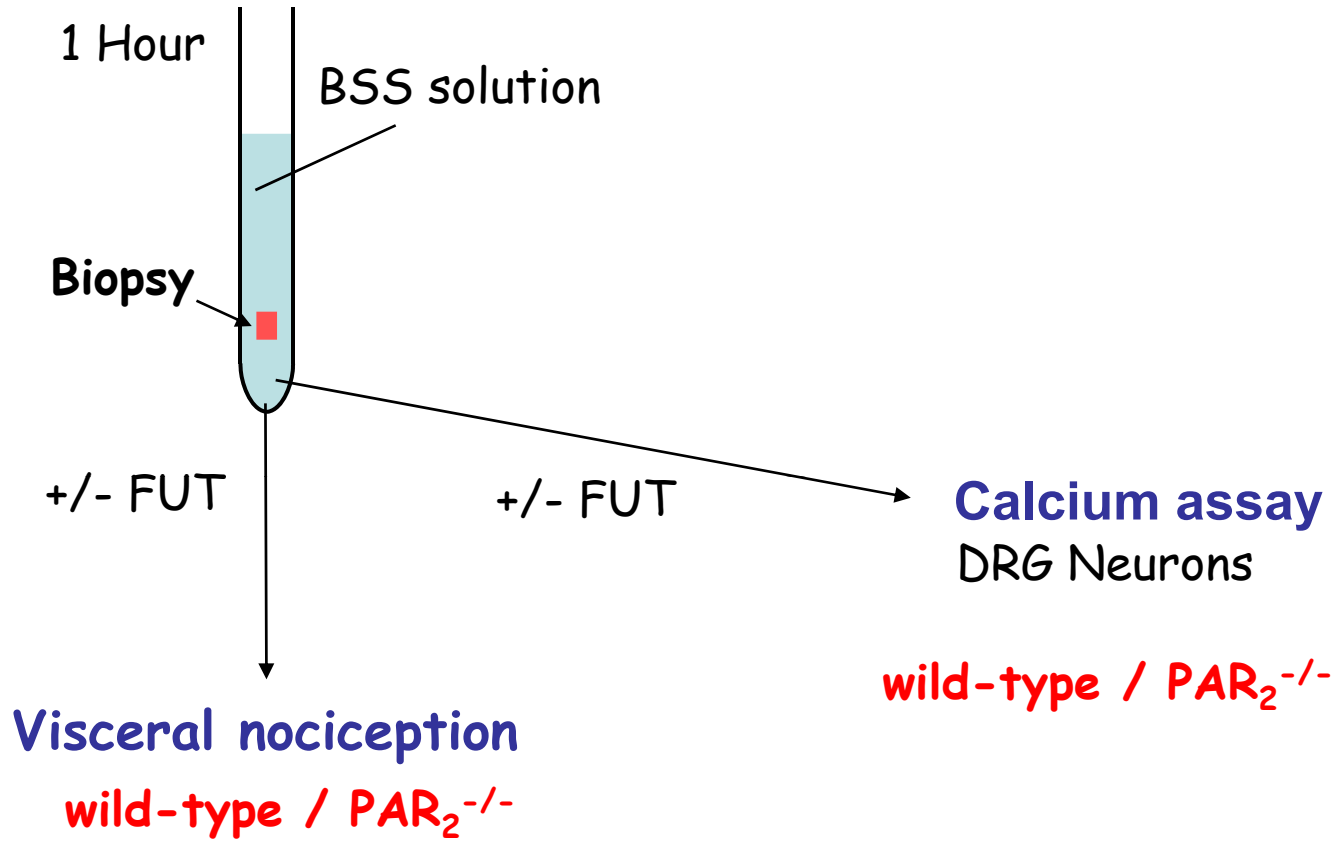
Irritable Bowel Syndrome: association with increased proteolytic activity

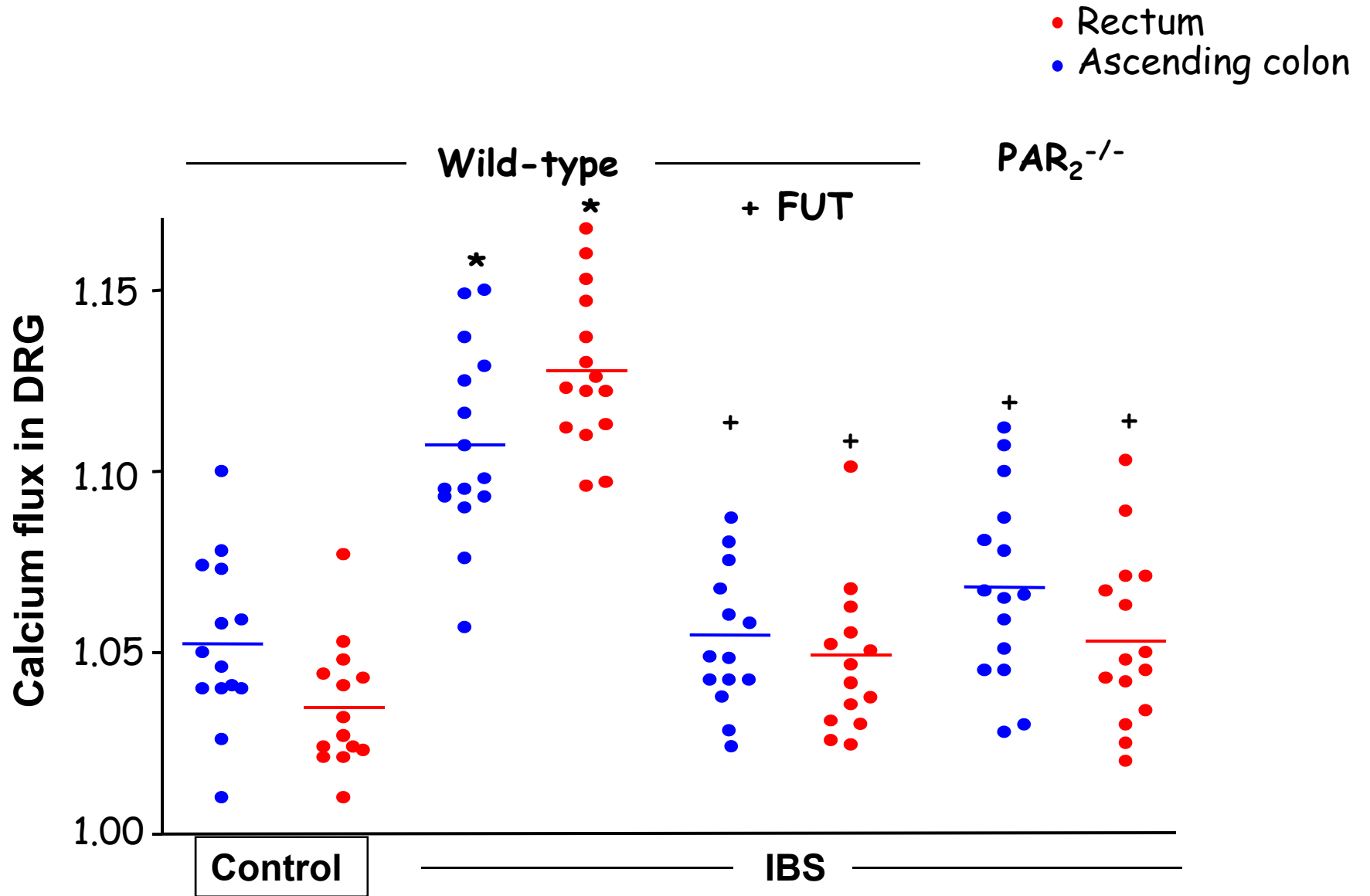
Cenac et al. *J. Clin. Invest.* 2007



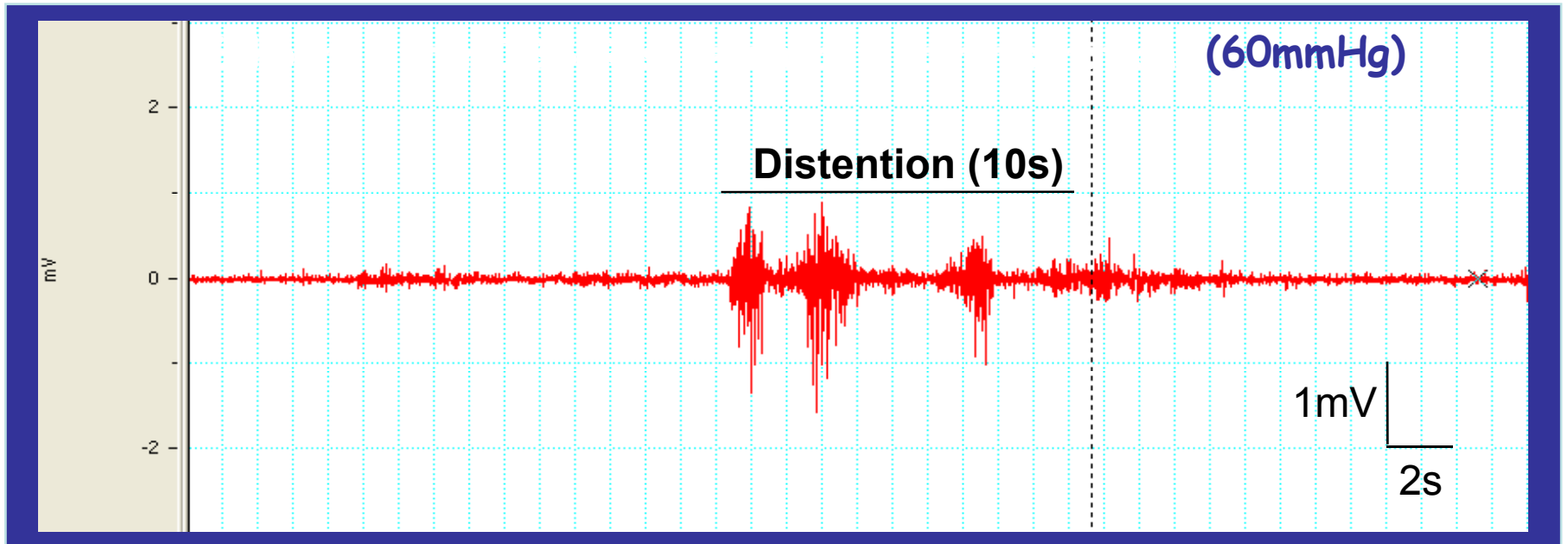
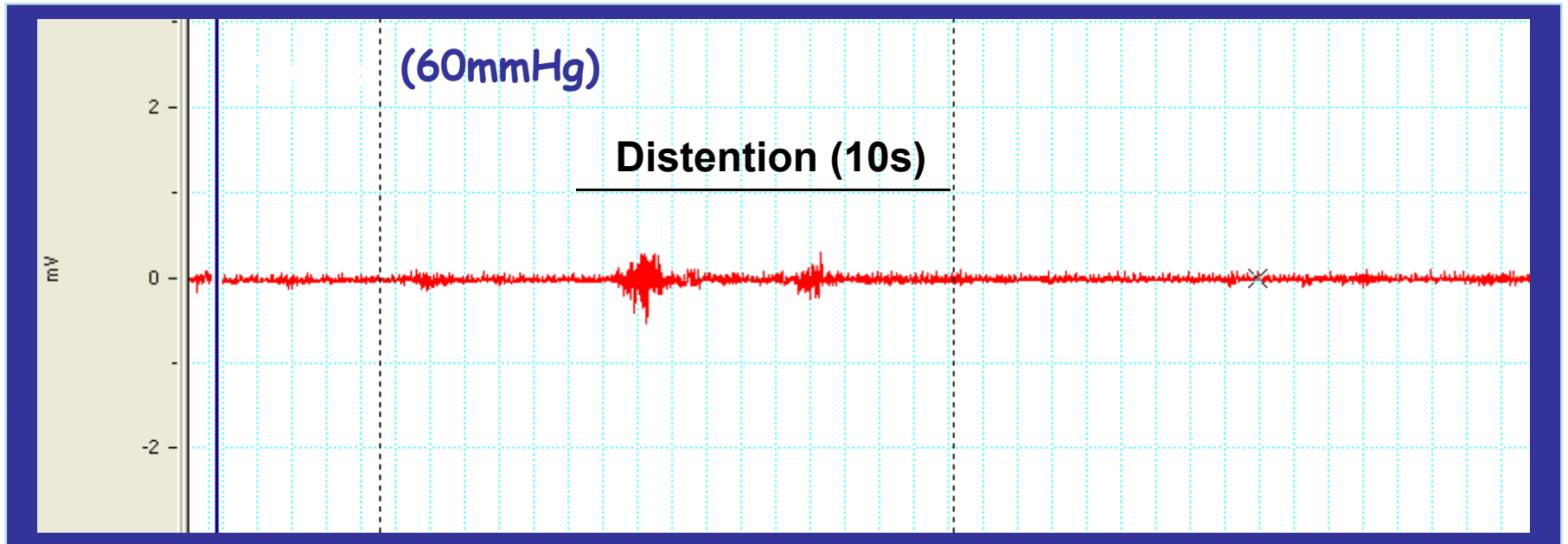
* significantly different from corresponding control values

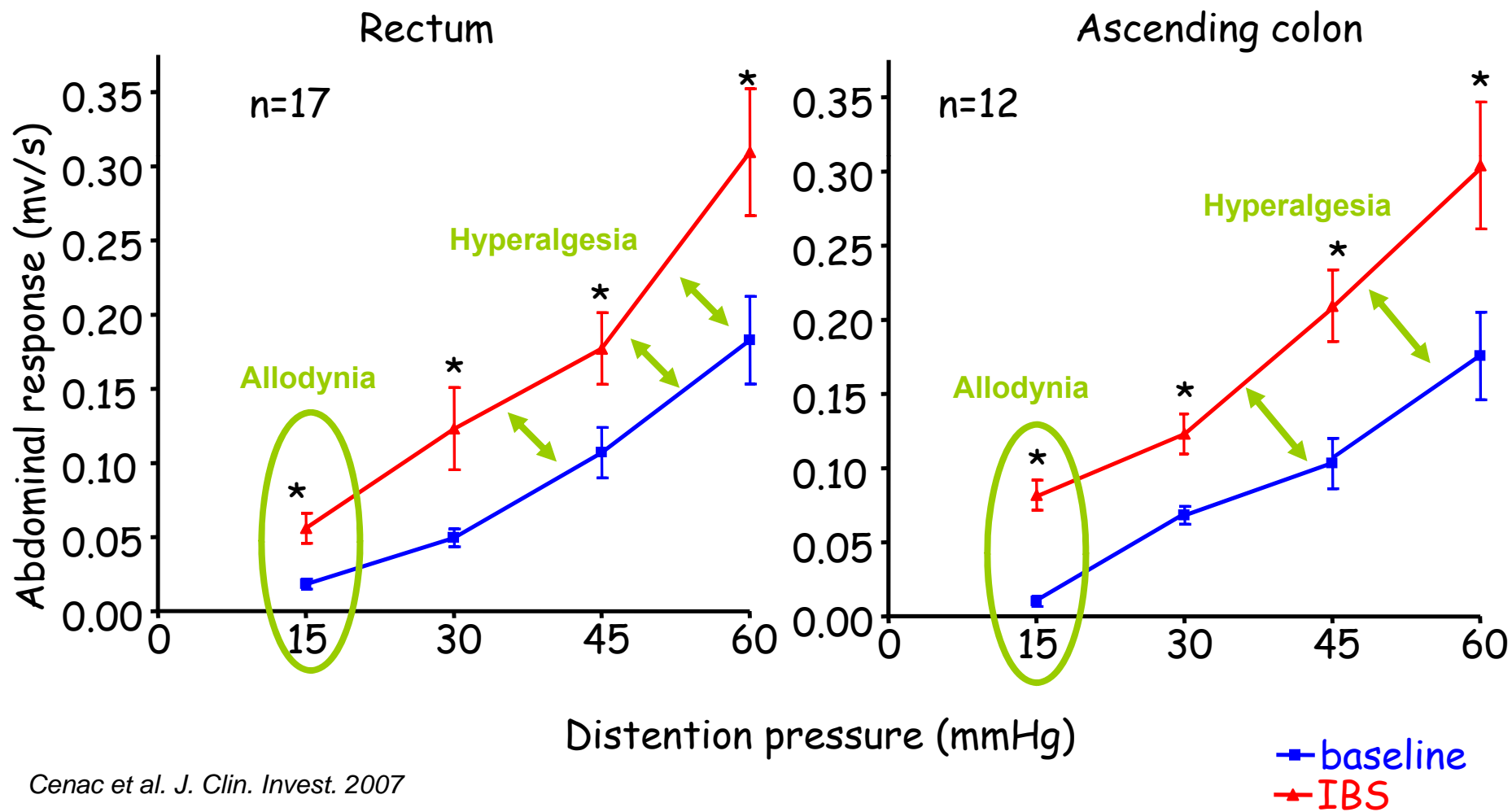
Protocol





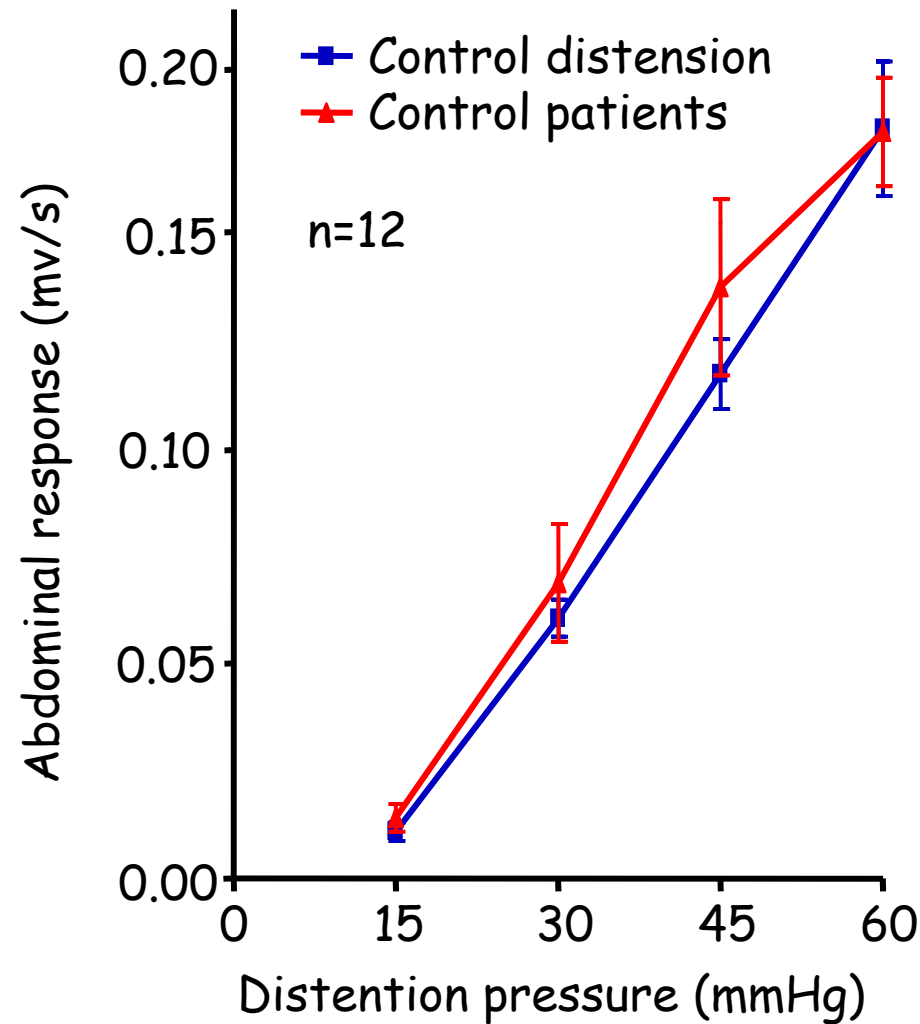
Abdominal Muscle Record during Distention

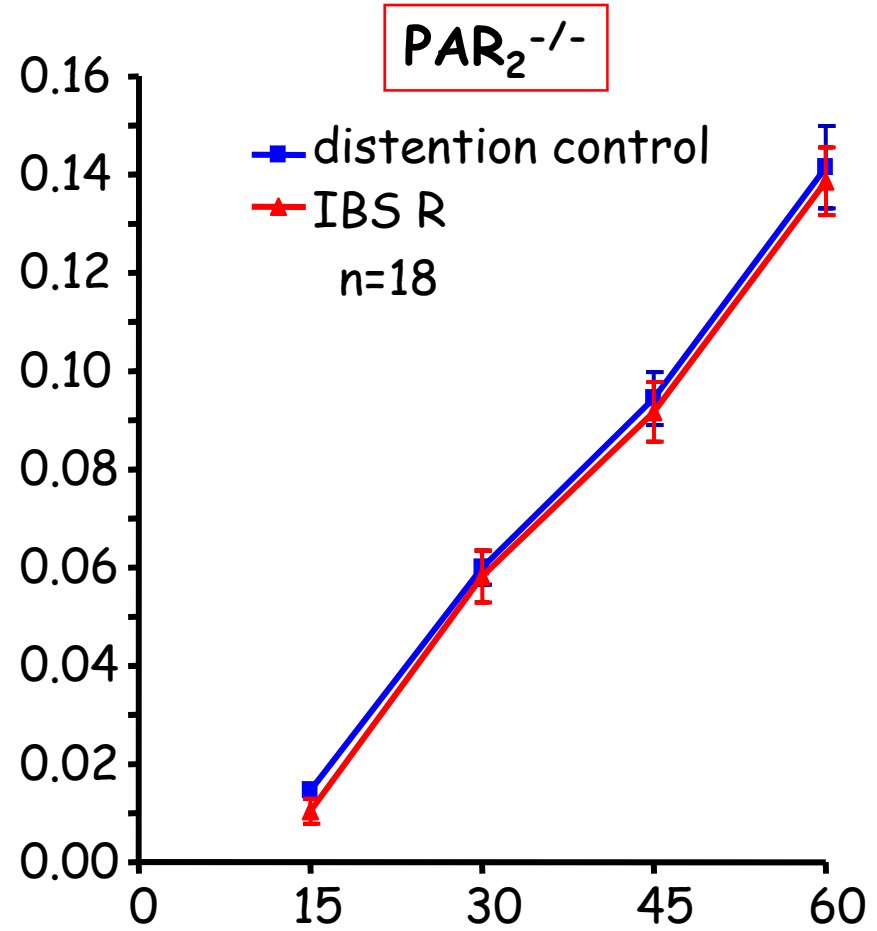
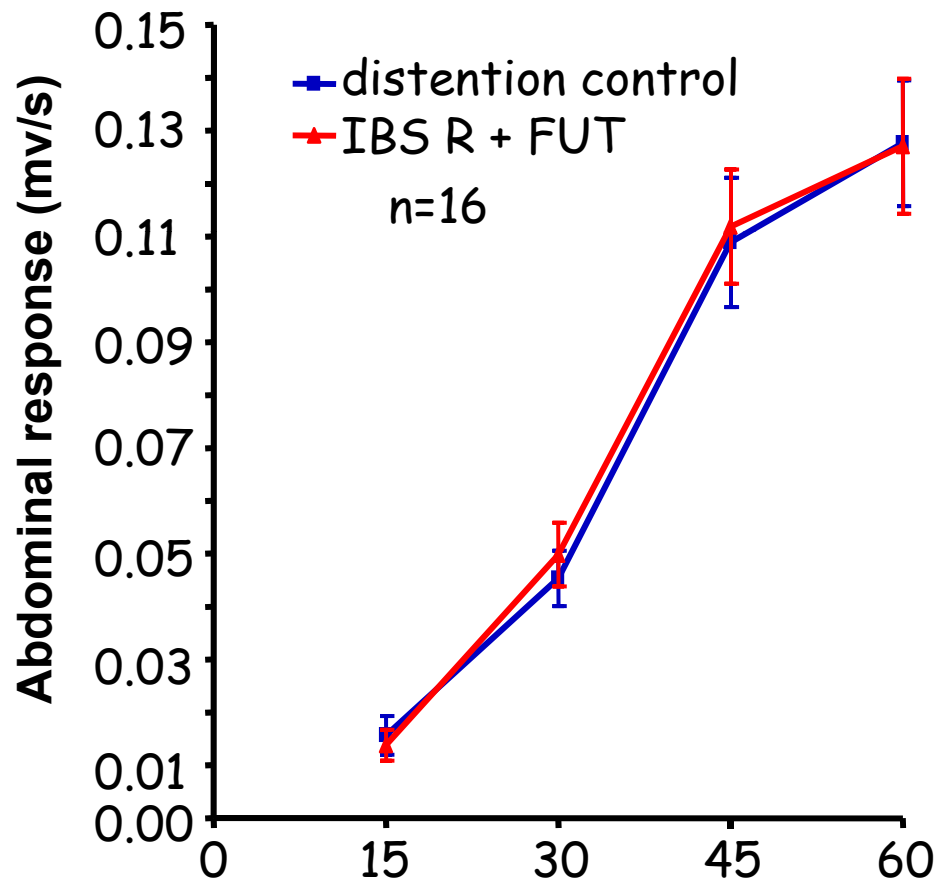




Administration of IBS biopsy supernatants mediate visceral hyperalgesia

Cenac et al. J. Clin. Invest. 2007



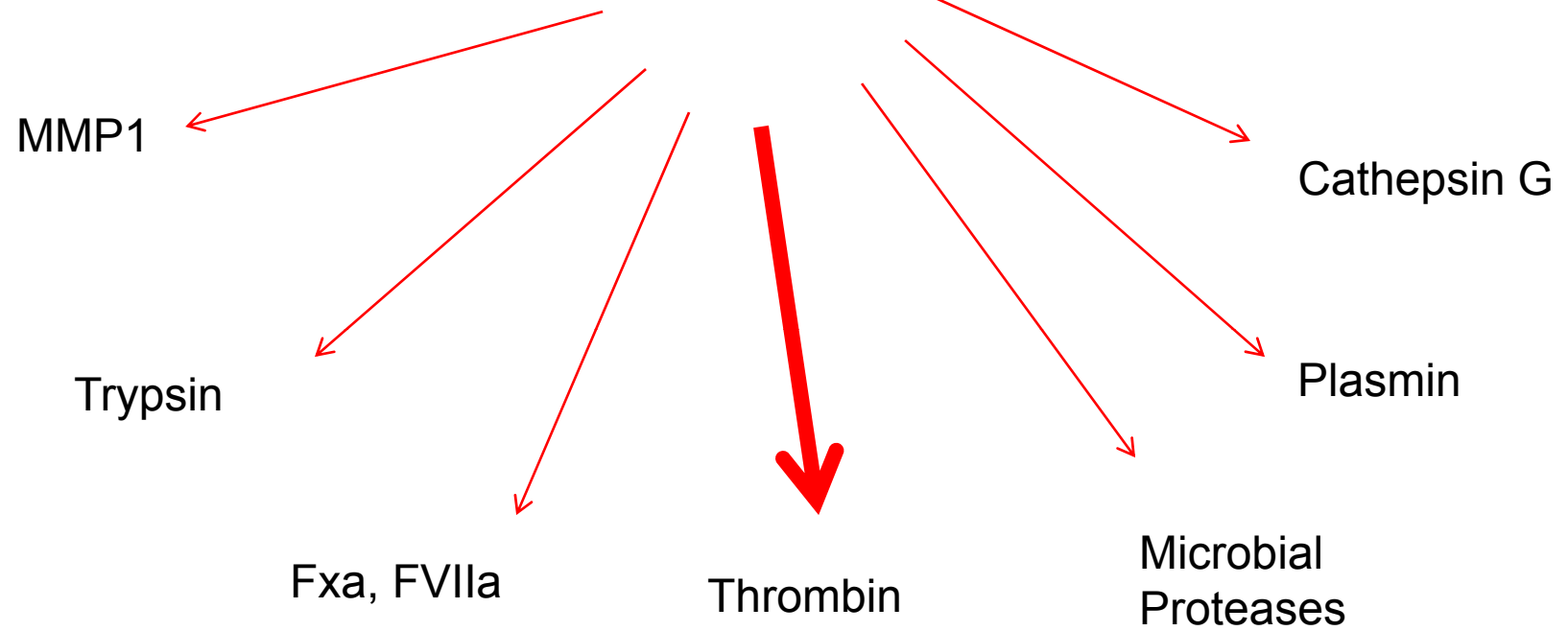


Conclusions

Calcium mobilization in sensory neurons, allodynia and hyperalgesia in response to IBS supernatants are mediated by PAR-2 activation and proteolytic activity.

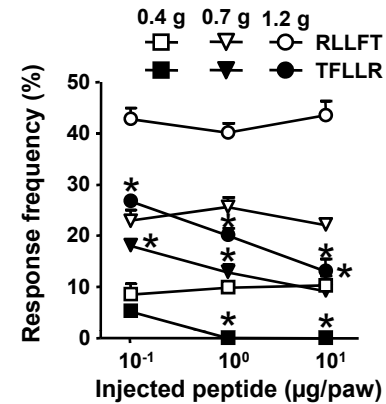
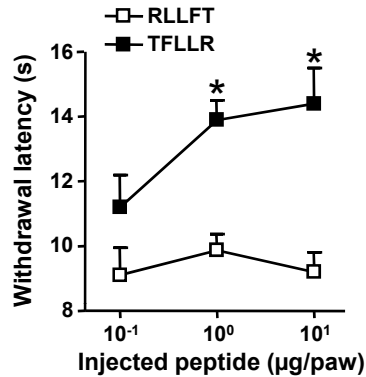
PAR₂ activation by colonic proteases plays a prominent role in IBS-associated pain symptoms.

PAR-1

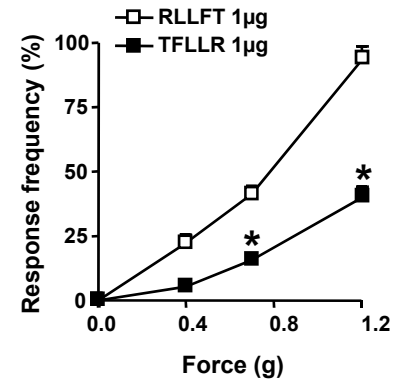
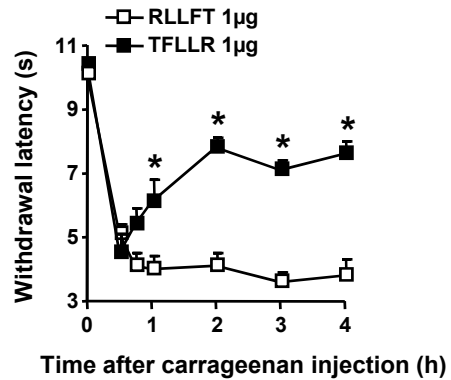


Intraplantar injection of PAR1-AP reduces nociceptive responses

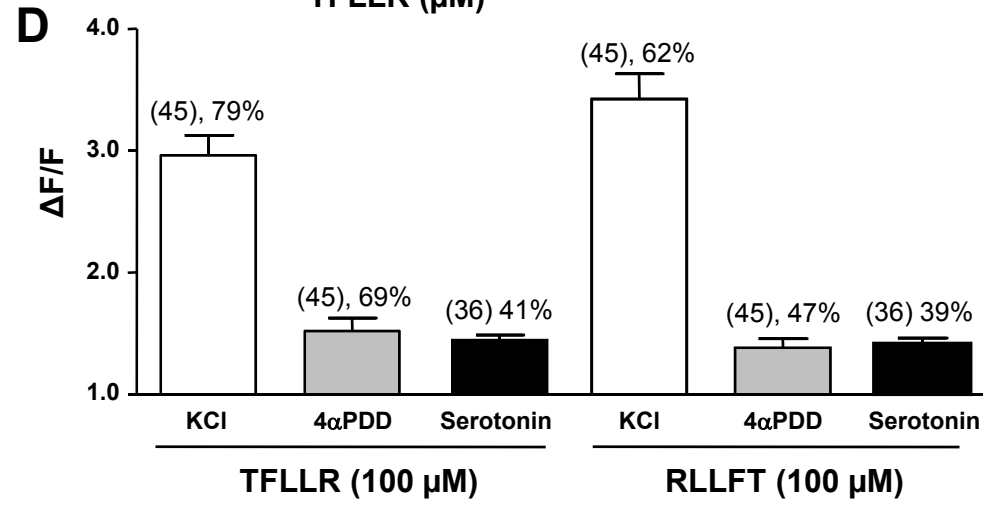
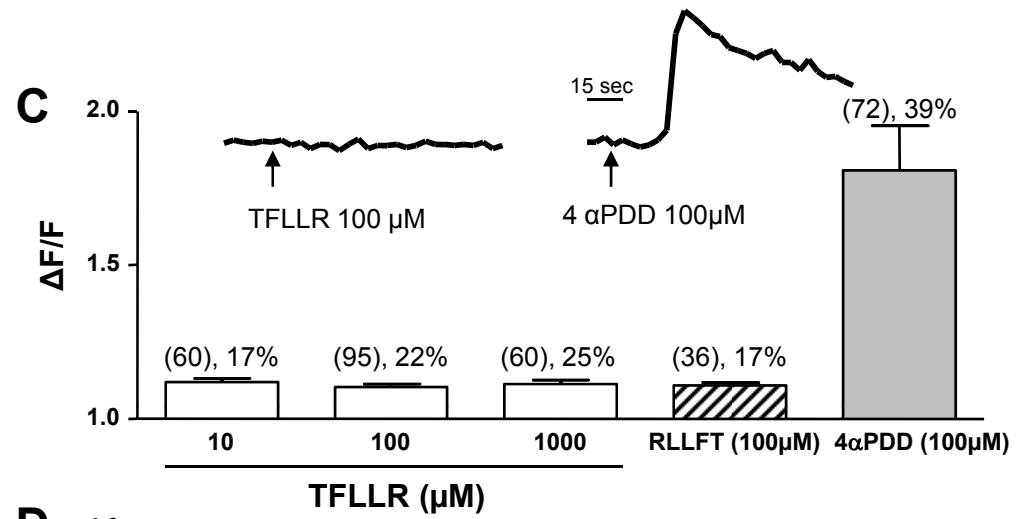
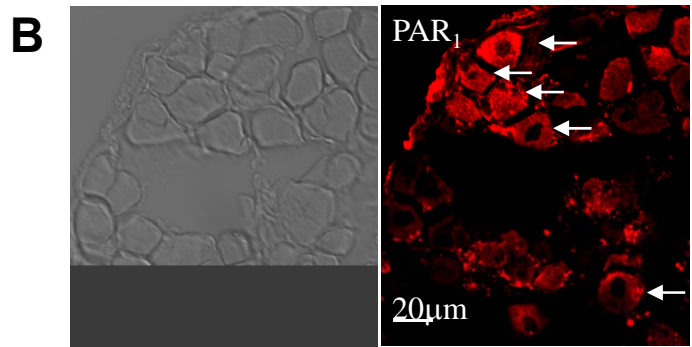
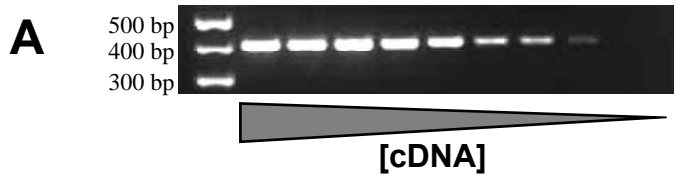
Nociception
Basal conditions



Nociception
Inflammatory
conditions

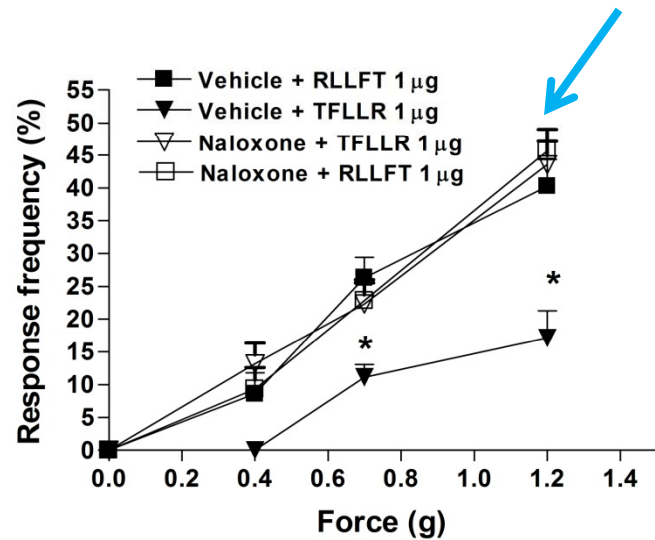
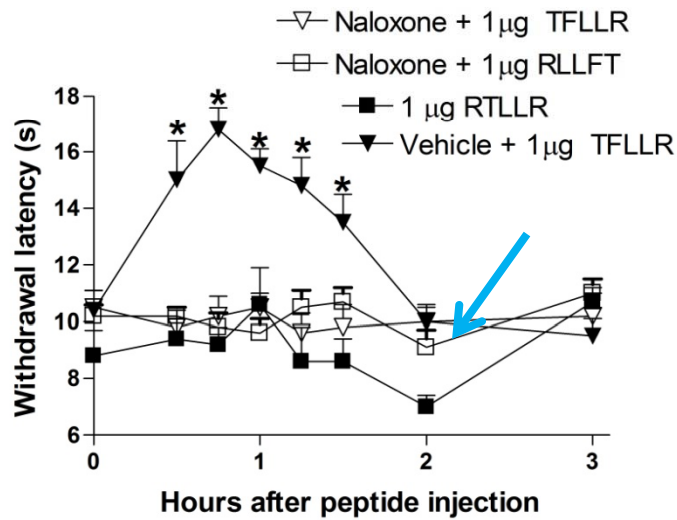


PAR1-AP signalling in DRG neurons

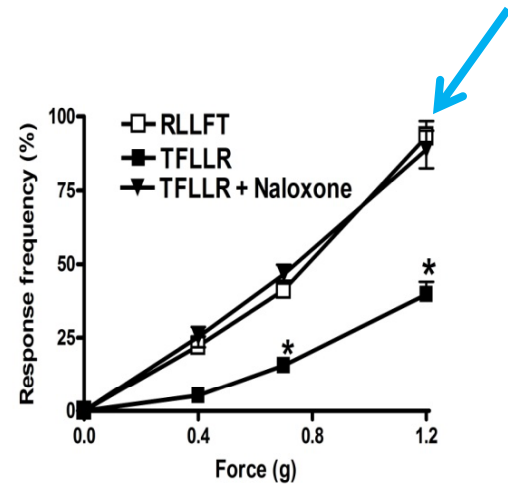
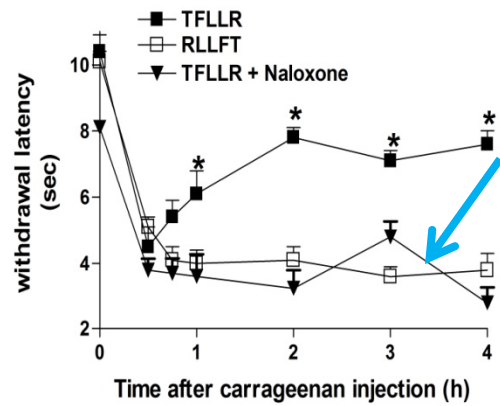


PAR1-AP-induced decreased nociceptive response is dependent on opioid receptor activation

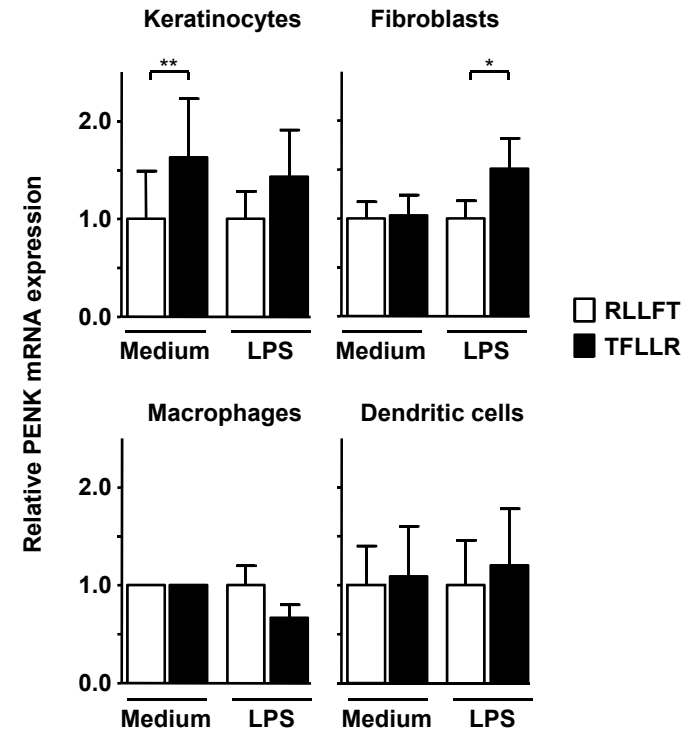
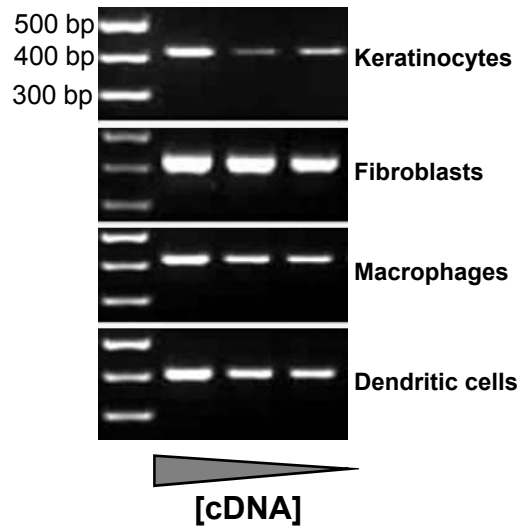
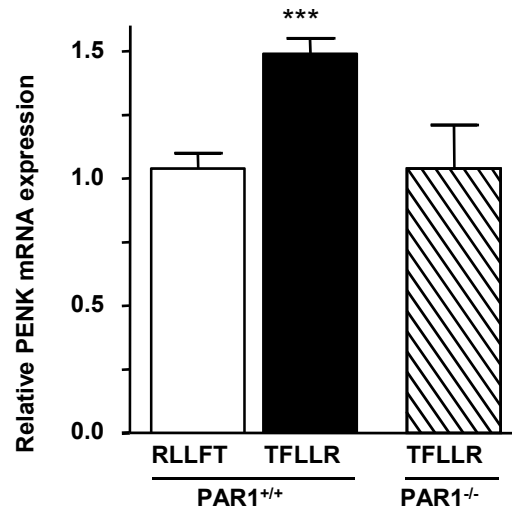
Nociception
Basal conditions



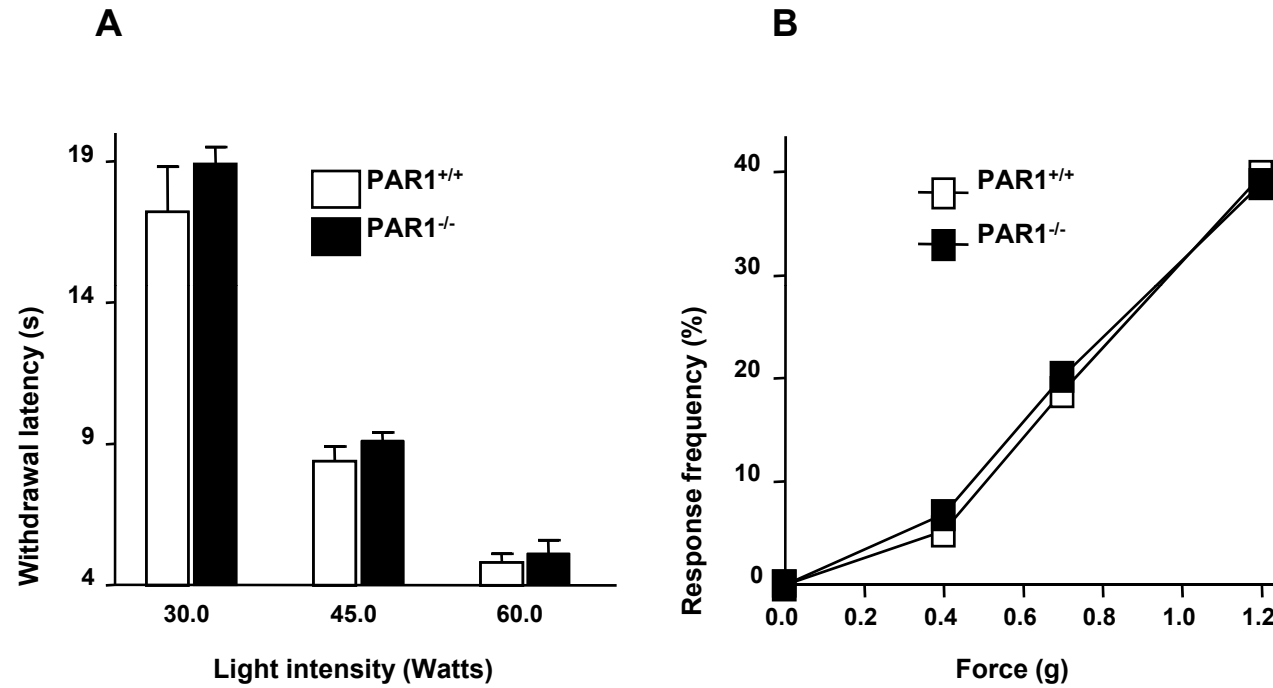
Nociception
Inflammatory
conditions



PENK is overexpressed in response to PAR1-AP

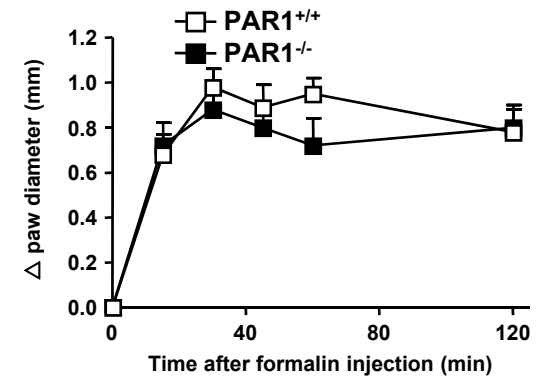
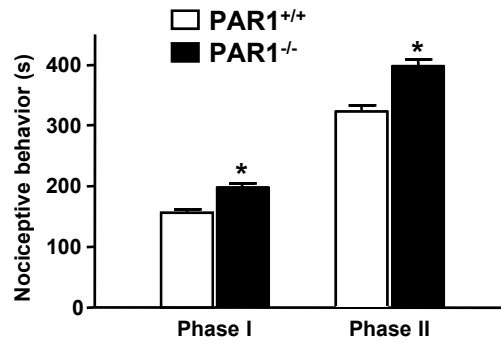
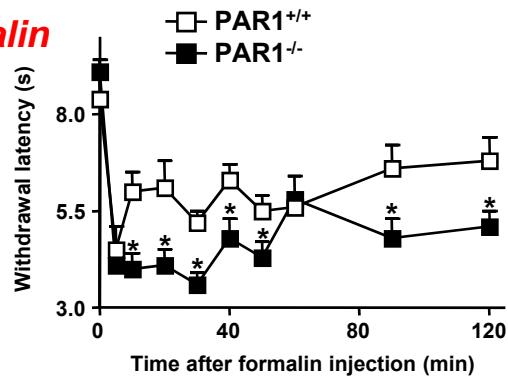


PAR-1 deficiency does not modify nociceptive responses

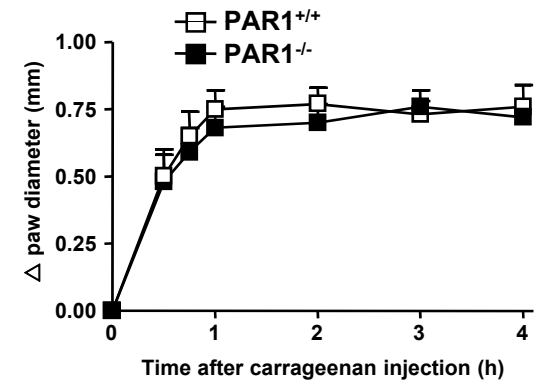
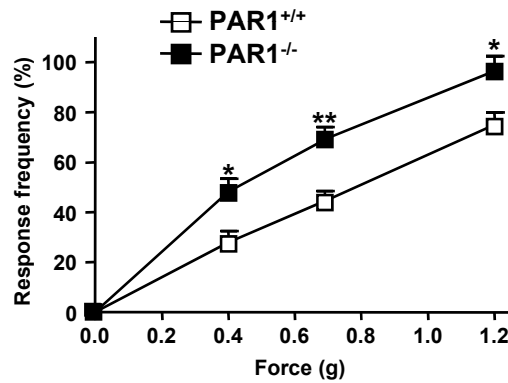
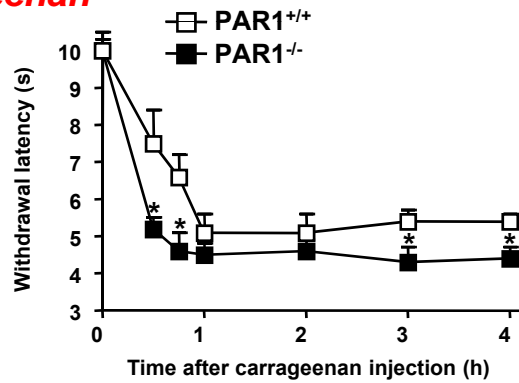


PAR-1 deficiency enhanced inflammatory hyperalgesia

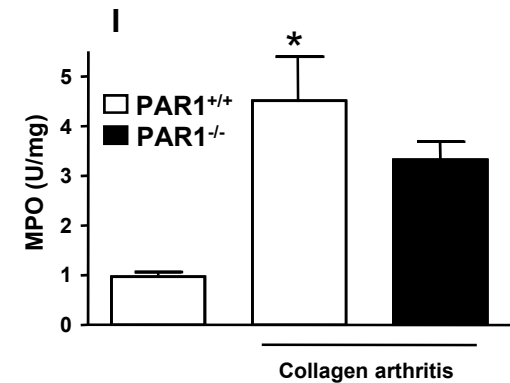
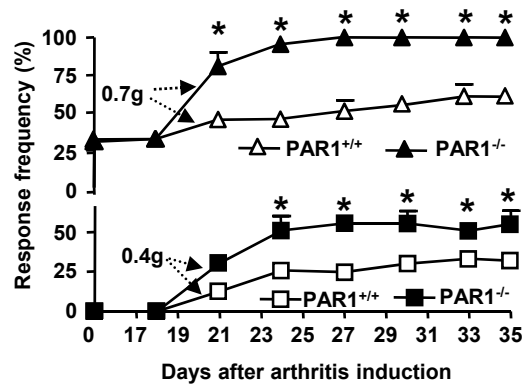
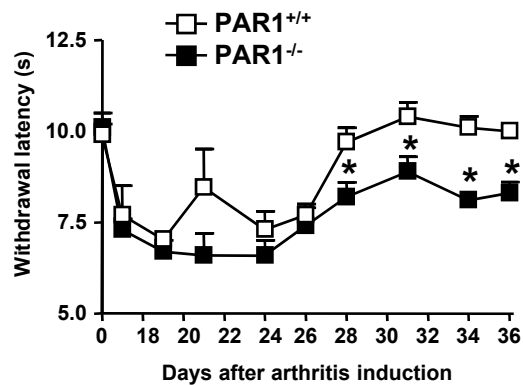
Formalin



Carrageenan



Arthritis



Analgesic

Opioids

PAR-1

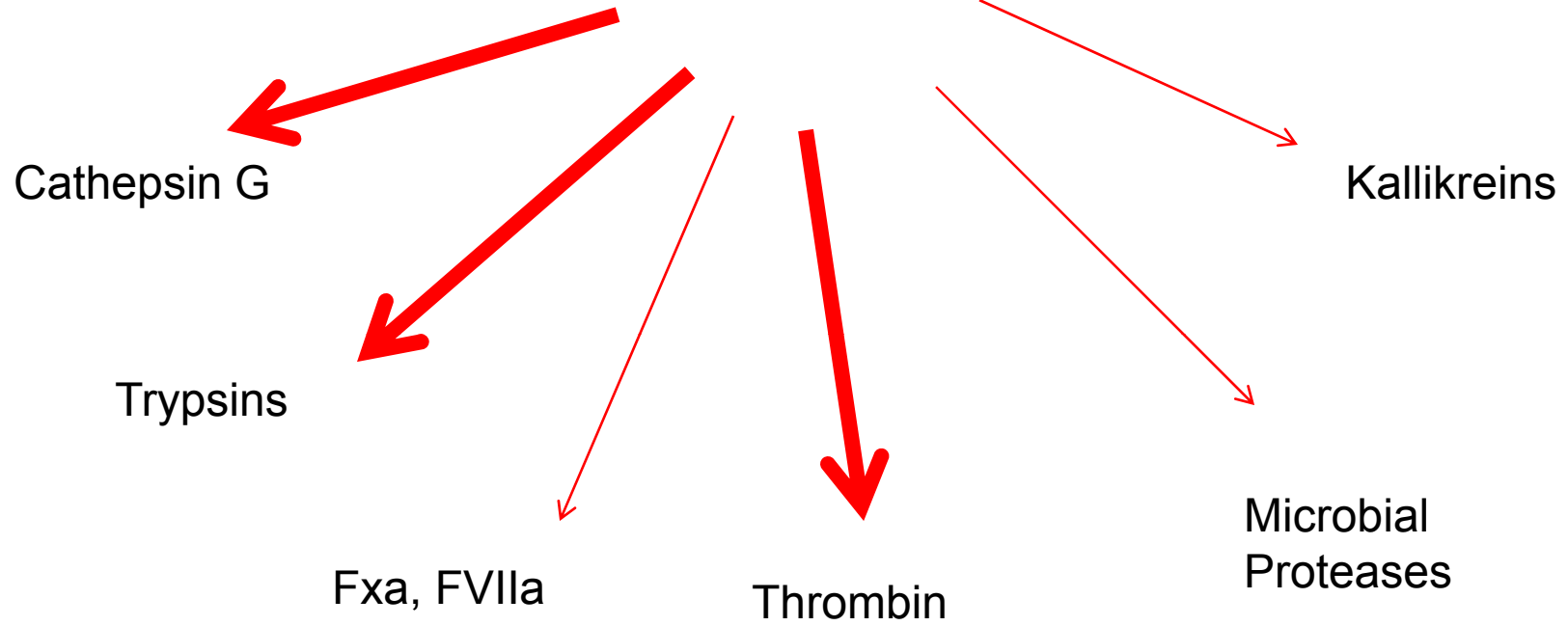
DRG neurons signalling

PENK release

- keratinocytes
- fibroblasts

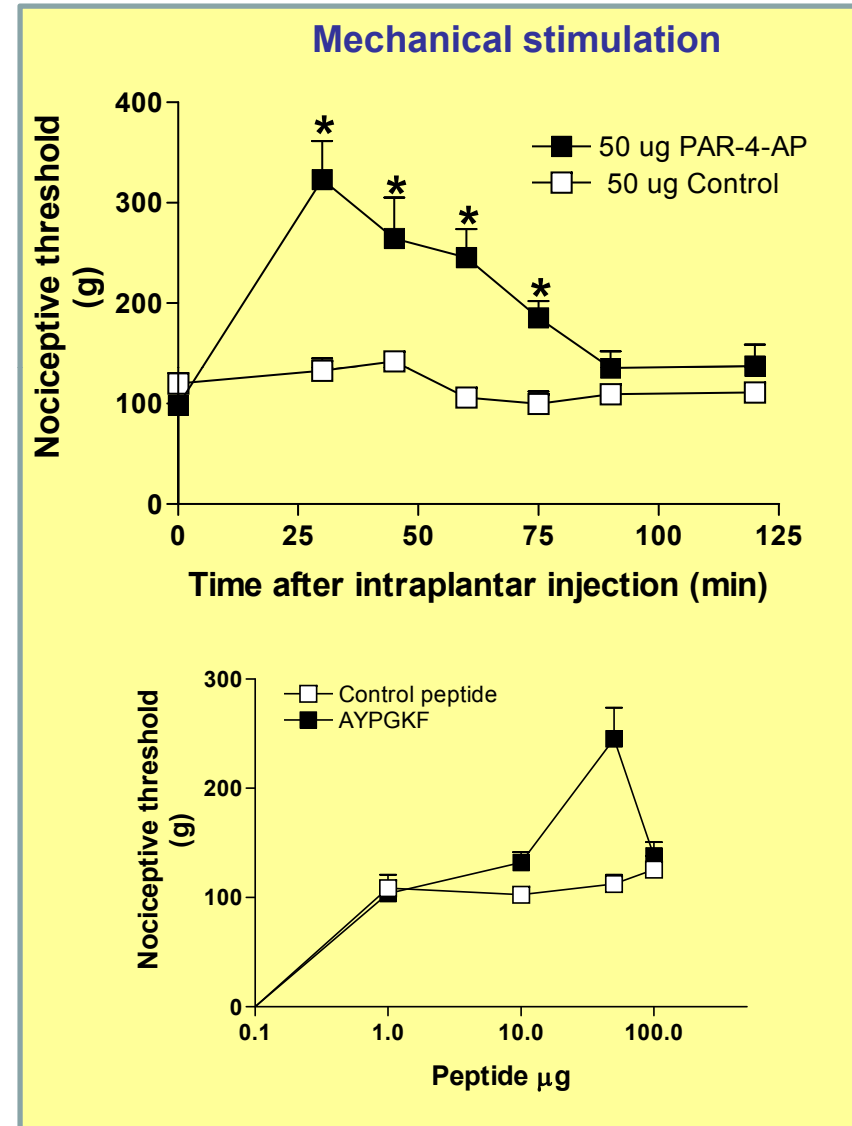
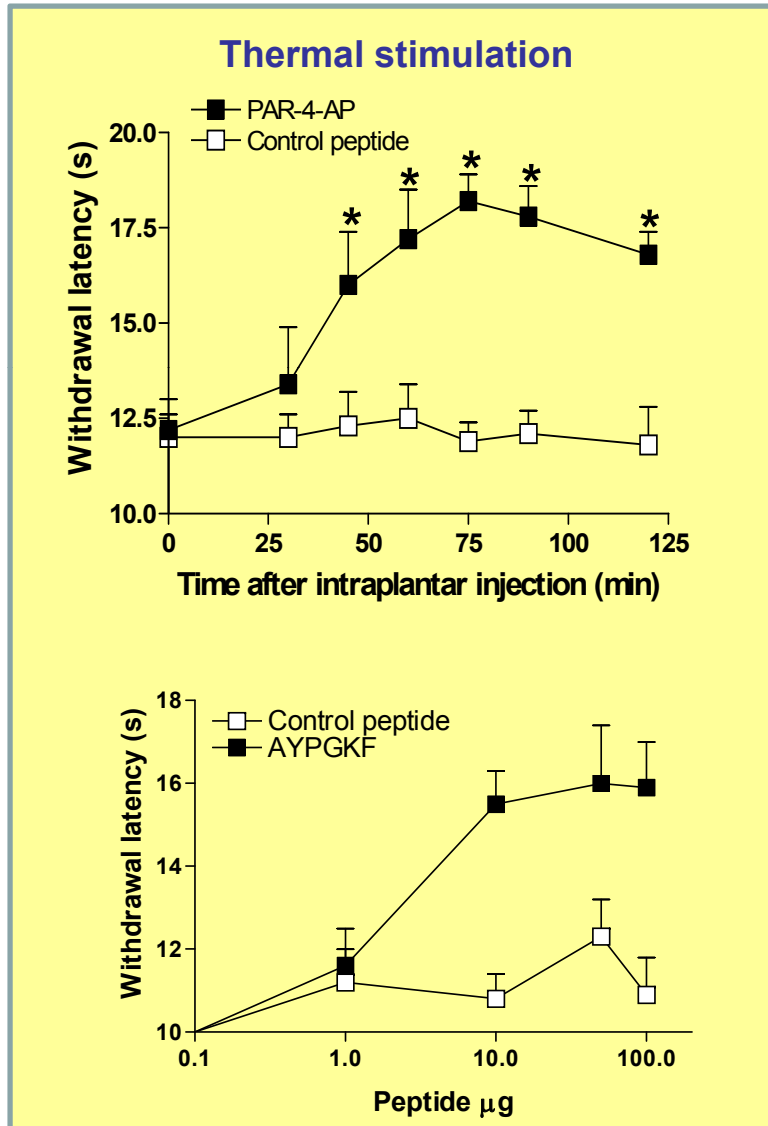
Endogenous control of inflammatory hypersensitivity / pain

PAR-4



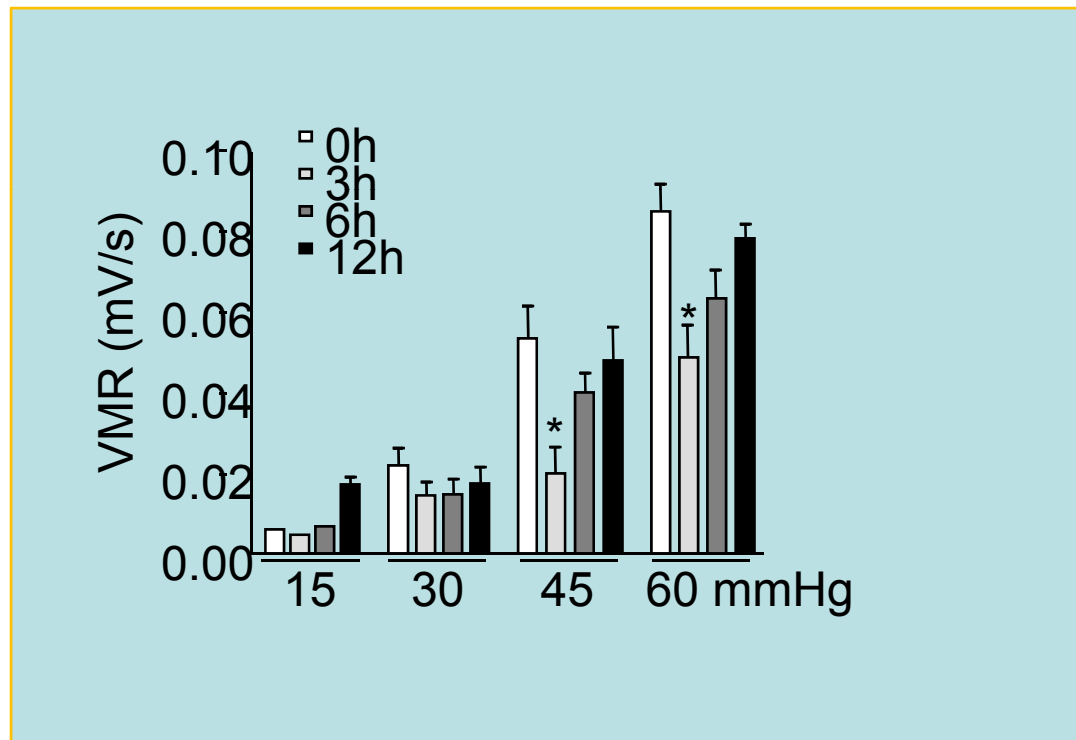
Antinociceptive effects of PAR₄ agonist

Somatic nociception



Antinociceptive effects of PAR₄ agonist

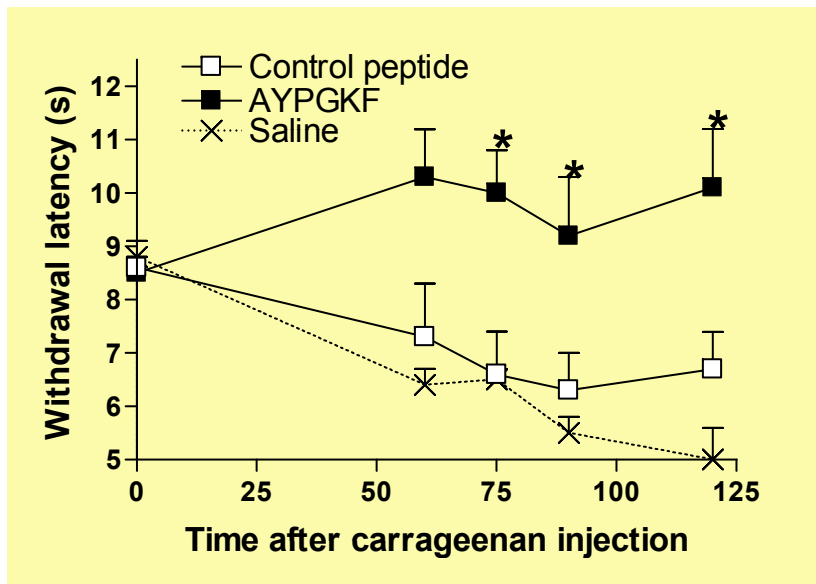
Visceral nociception



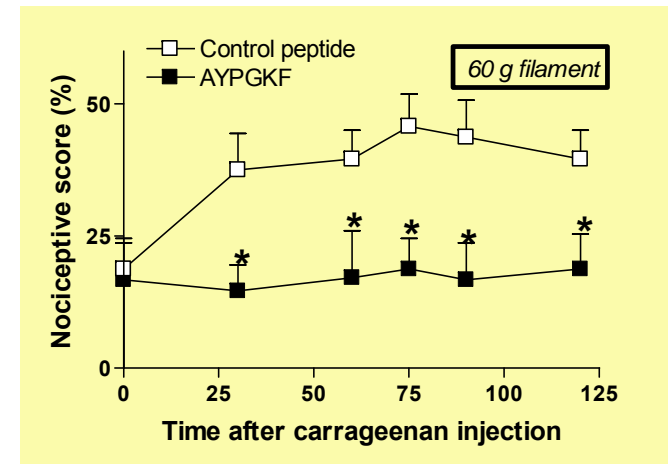
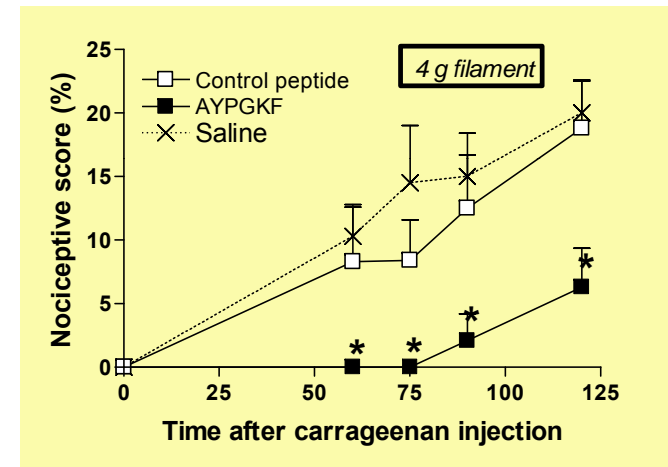
Analgesic effects of PAR₄ agonist

Somatic Hyperalgesia and Allodynia

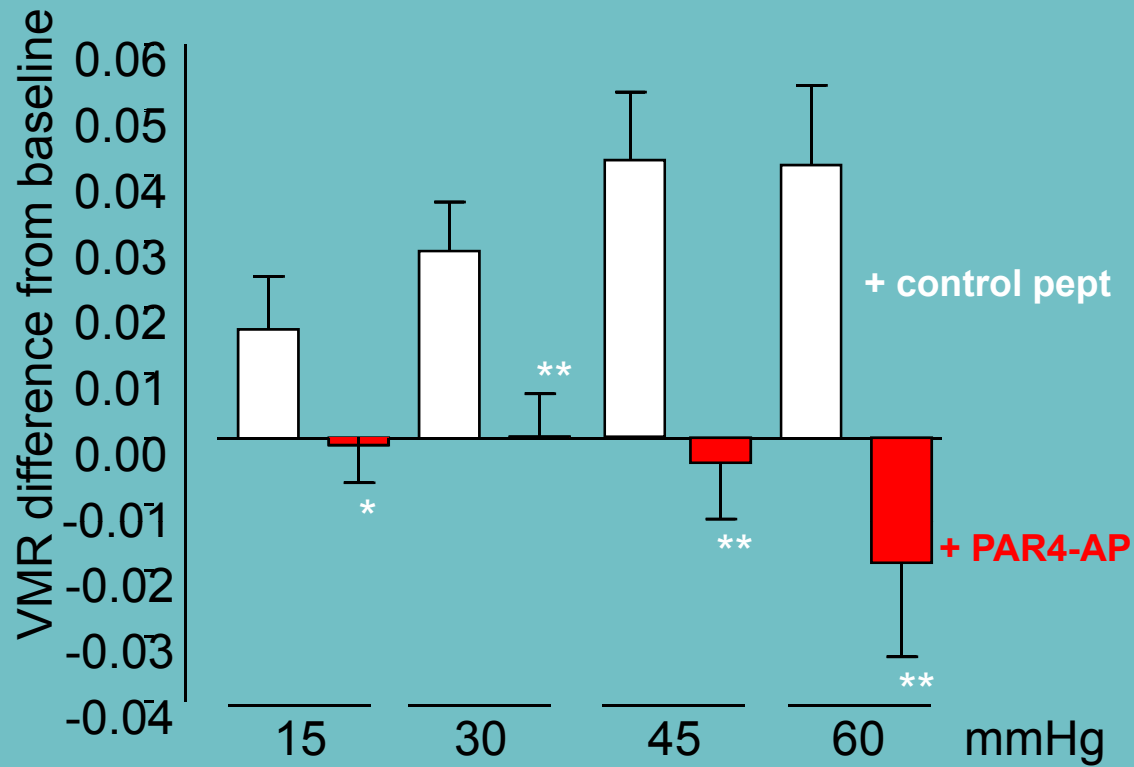
Thermal



Mechanical

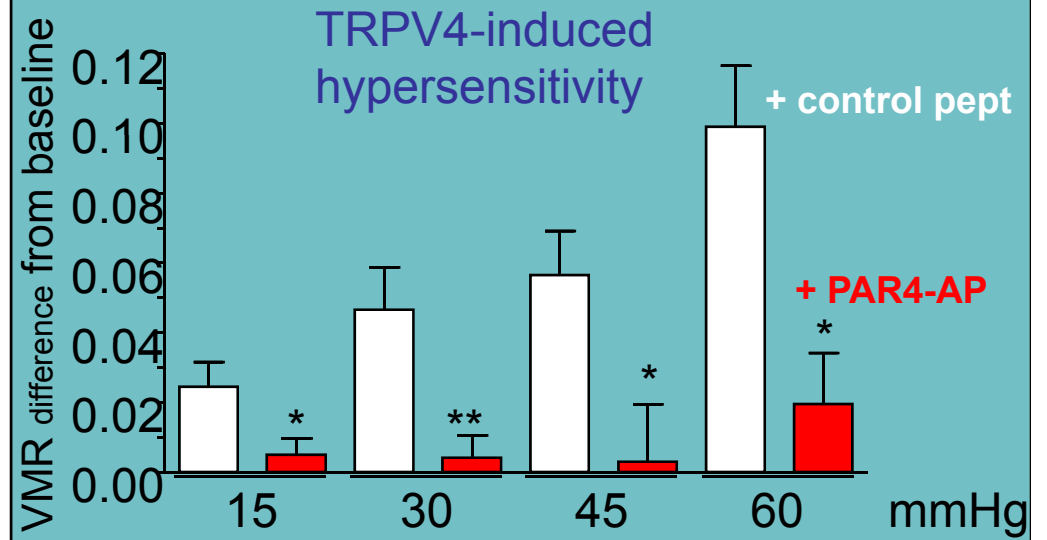


PAR2-induced visceral hypersensitivity

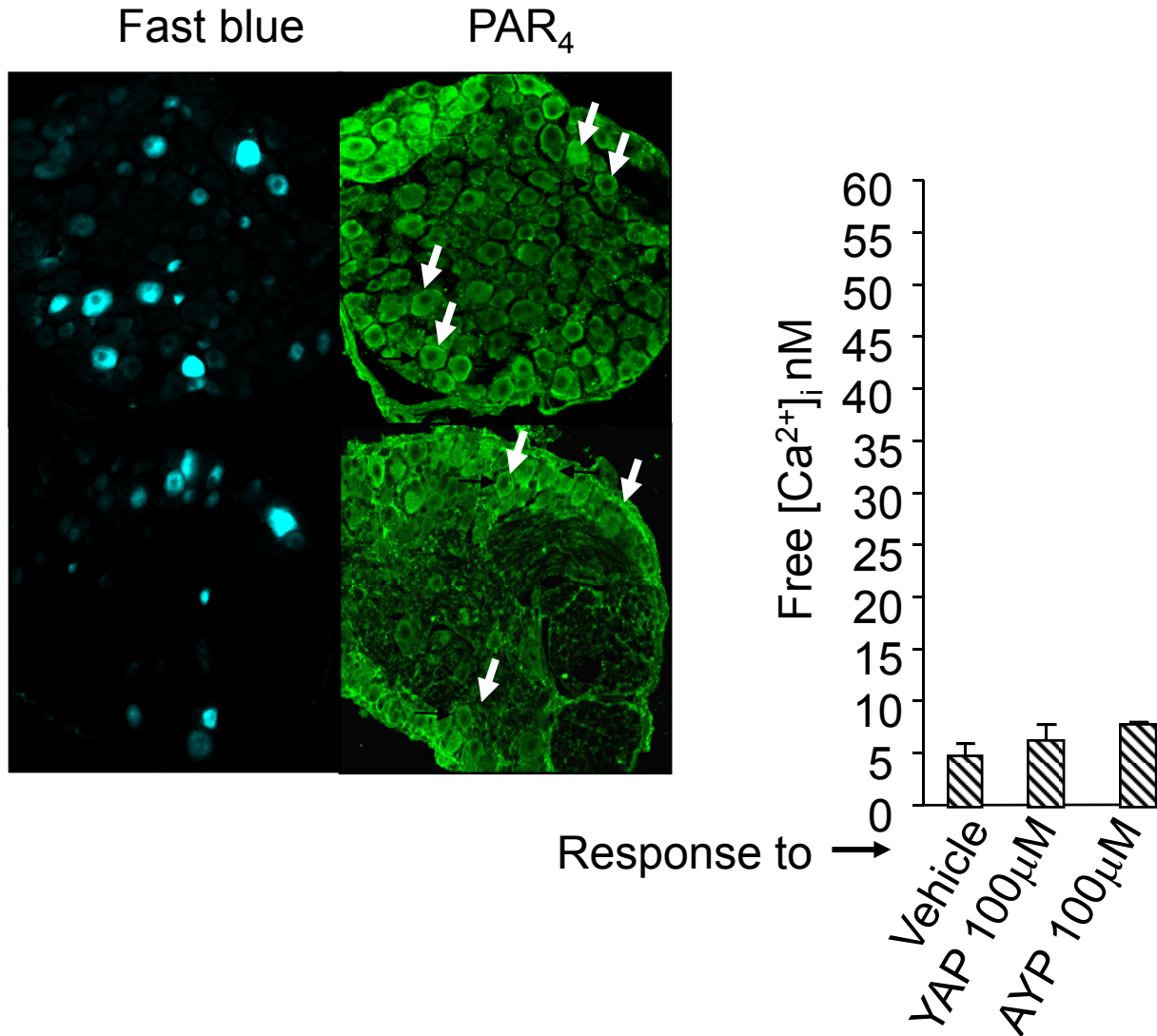


Analgesic effects of PAR₄ agonist

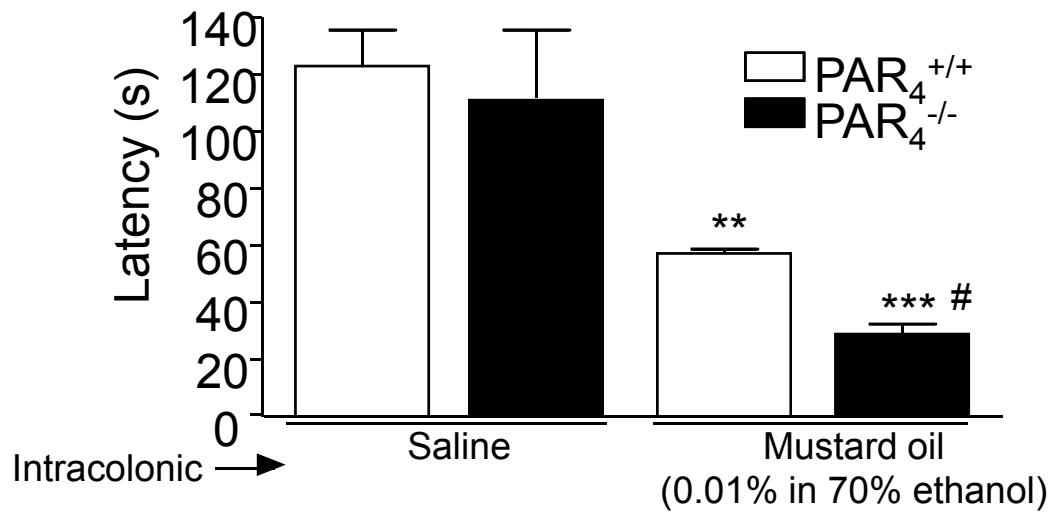
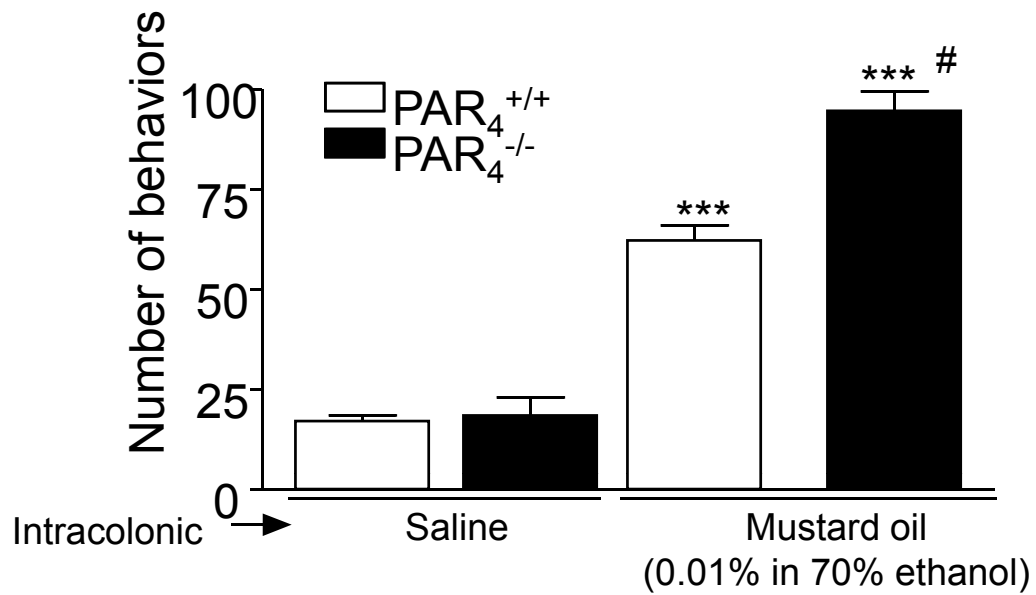
Visceral Hypersensitivity



PAR-4 signalling in colonic primary afferents



PAR-4 deficiency enhanced visceral hypersensitivity



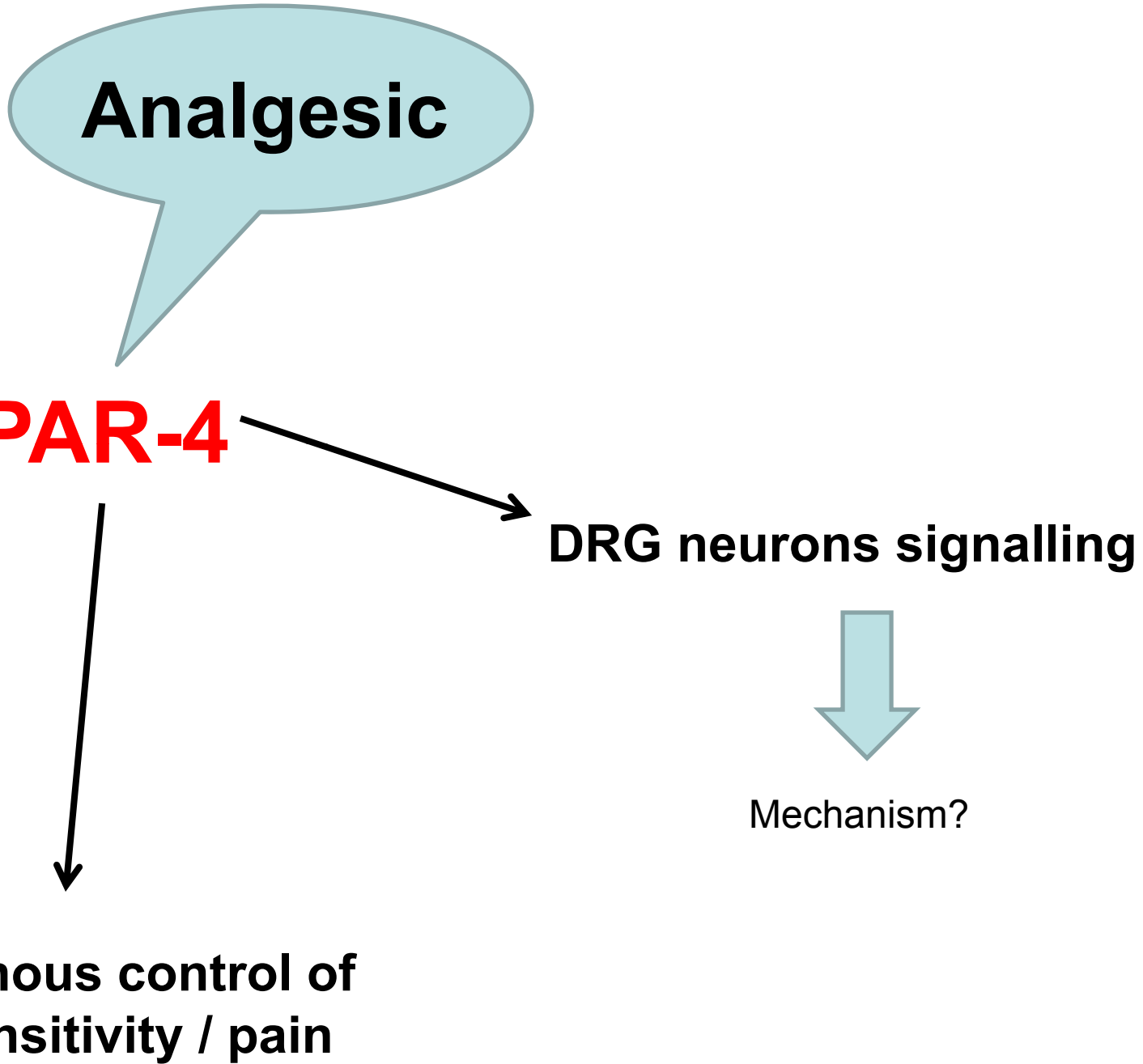
Analgesic

PAR-4

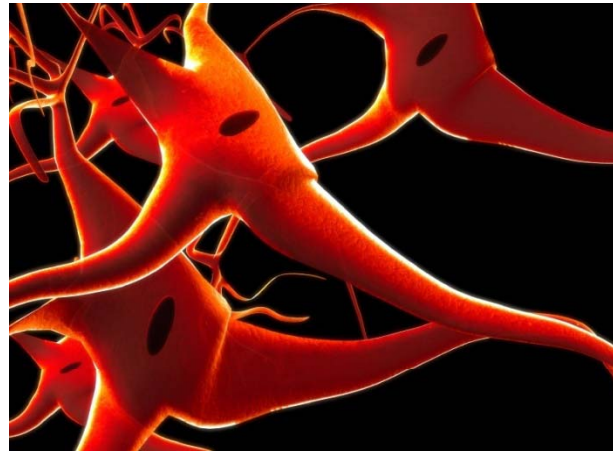
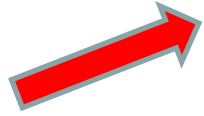
DRG neurons signalling

Mechanism?

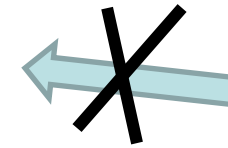
**Endogenous control of
hypersensitivity / pain**



PAR-2



Primary Afferents



PAR-1



PAR-4

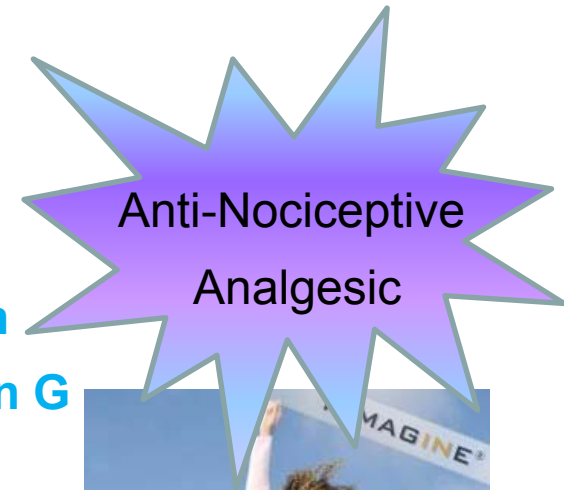


Pro-Nociceptive
Hyperalgesic
Allodynic
Pain

PROTEASES

Tryptase

**Thrombin
Cathepsin G**



Anti-Nociceptive
Analgesic



- Trypsins
- Elastase
- Proteinase-3
- Kallikreins
- Microbial proteases





INFLAMMATION RESEARCH NETWORK

Canada



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Kevin Chapman
Eric Hyun



France



Emilie d'Aldebert
Celine Auge
Alexandre De Nadai Souza
Jean-Paul Motta
Perrine Rousset
Dr. Daniela Balz-Hara
Dr. Nicolas Cenac
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