Role of capsaicin-sensitive fibres, TRPV1 receptors and tachykinins in PAR2 activation-induced joint inflammation and nociception

Zsuzsanna Helyes

Department of

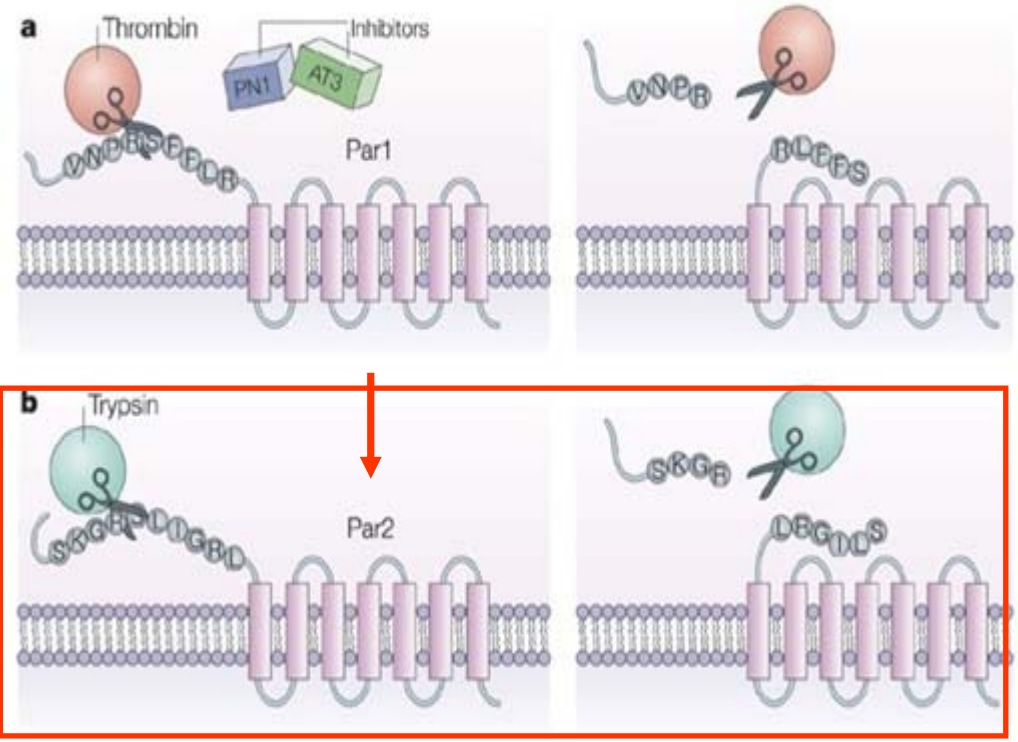
Pharmacology and Pharmacotherapy

University of Pécs, Hungary

Canadian Pain Society Meeting, 2010

BACKGROUND

Protease-activated receptor 2 (PAR2)



- G-protein coupled receptors (PAR1-4)

- **Activation:** proteolytic cleavage of the extracellular domain (trypsin, tryptase)

- **Localization:**
  - *epithelial cells*
  - *endothelial cells*
  - *inflammatory and immune cells*
  - *sensory neurones*

Transient Receptor Potential Vanilloid 1 (TRPV1)

- Non-selective cation channel
- Localization:

capsaicin-sensitive primary afferent neurones

(keratinocytes, epithelial cells, urothelium)

- Activation: capsaicin, noxious heat, protons, inflammatory mediators (eg. lipoxygenase products) etc.
- Sensitization: prostaglandins, bradykinin, etc.

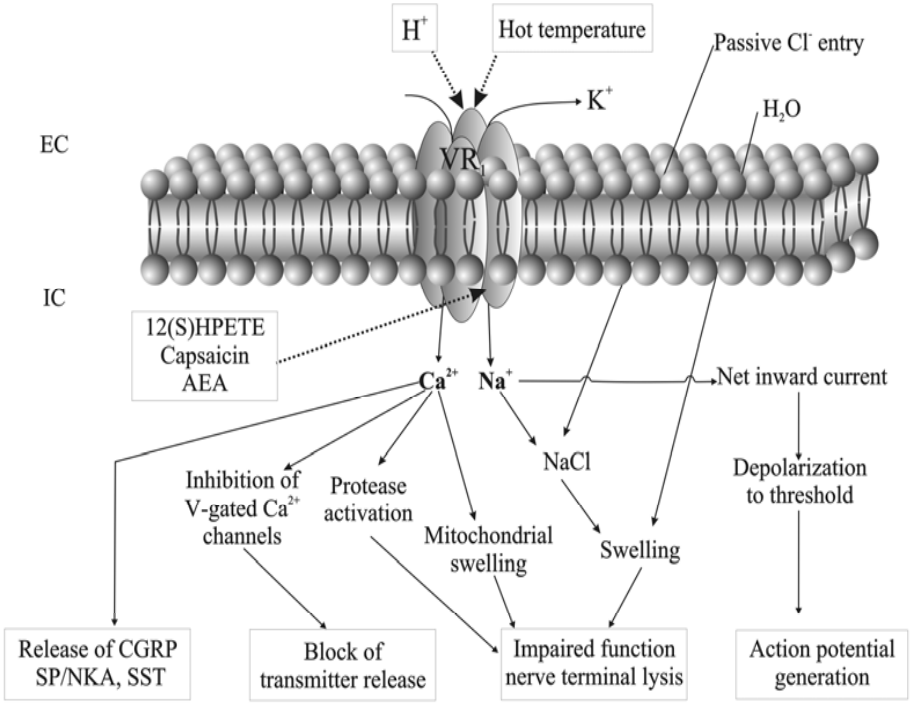
- Na^+ and Ca^{2+} influx

↓

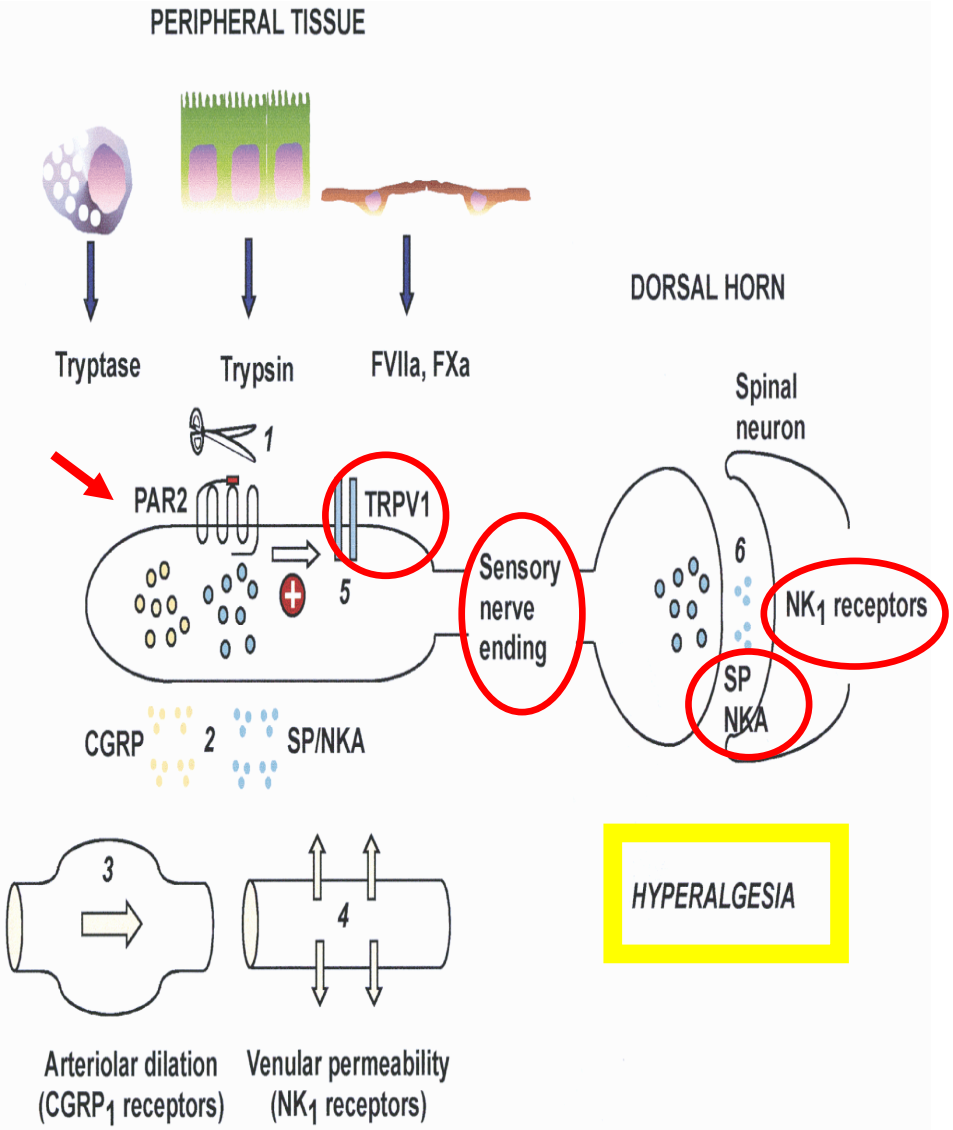
action potential

↓

neuropeptide release (tachykinins, CGRP, somatostatin, PACAP)



Involvement of PAR2 and TRPV1 receptors in neurogenic inflammation and thermal hyperalgesia



(Cottrell et al. 2003. Biochem. Soc. Trans.)

Role of capsaicin-sensitive fibres in PAR-2 activation-induced

- **inflammation and nociception: reduced response after capsaicin desensitization**
  (Shu et al. 2005; Gu and Lee 2006; Shimizu et al. 2007; Paszcuk et al. 2009)
- **scratching behaviour: diminished after TRPV1 antagonist**
  (Costa et al. 2008)
- **thermal hyperalgesia: smaller after TRPV1 antagonist**
  (Amadesi et al. 2004)
- **urinary bladder contractions: inhibited by TRPV1 antagonist**
  (Shimizu et al. 2007)

In vivo data on intraarticular PAR-2 activation:

Synovial hyperaemia, oedema
(Ferrel et al. 2003; Busso et al. 2007)

AIMS:

Investigation of the involvement of

- **TRPV1 receptors**
- **capsaicin-sensitive peptidergic afferents**
- **the tachykinin system**

in PAR2 receptor activation-induced knee joint inflammation and consequent mechanical hyperalgesia

EXPERIMENTAL MODELS- I.

Syntethic PAR2-activating peptide:
SLIGRL-NH_2

Rat
(Wistar)

1.) 100 µg/100 µl SLIGRL-NH_2

2.) 500 µg/kg i.p. ***TRPV1 receptor antagonist (SB366791)*** + SLIGRL-NH2

into the right knee joint

Mouse
(C57BL/6 wildtype = **WT**
TRPV1 deficient = **TRPV1$^{-/-}$**)

1.) 100 µg/50 µl SLIGRL-NH_2
into the knee joint

2.) For comparison:
Injection of 100 µg/50 µl SLIGRL-NH_2
intraplantarly

Control: inactive peptide- ***LRGILS-NH_2*** on the contralateral side
Measurements: every hour throughout 6 h

EXPERIMENTAL MODELS- II.

Mouse

Natural PAR2-activating agent: Mast Cell Tryptase (MCT)

1.) C57BL/6 wildtype = **WT**

2.) Resiniferatoxin (RTX) pretreatment
30-70-100 μg/kg s.c.
⇒ selective destruction of capsaicin-sensitive peptidergic sensory nerves

TRPV1 deficient = **TRPV1$^{-/-}$**
TAC1 deficient (SP, NKA) = **TAC1$^{-/-}$**
NK1 deficient = **NK1$^{-/-}$**

⇓

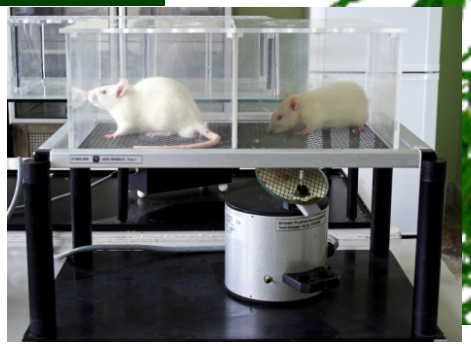
100 μg/50 μl MCT 2 weeks later
into the knee joint

Control: saline on the contralateral side
Measurements: every hour throughout 6 h

INVESTIGATIONAL TECHNIQUES-I.

Rat knee joint inflammation

1.) Touch sensitivity of the plantar surface of the paw–
dynamic plantar aesthesiometer



Secondary mechanical allodynia/hyperalgesia

2.) Mechanonociceptive threshold of the paw –
analgesimeter

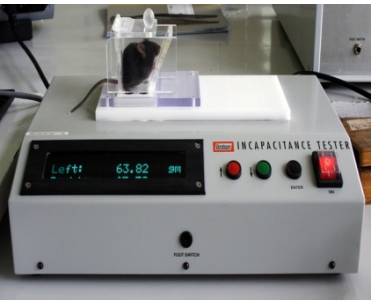
3.) Spontaneous weight distribution–
incapacitance tester

4.) Inflammatory cytokine concentration in the joint homogenate (IL-1β)– *ELISA*

INVESTIGATIONAL TECHNIQUES-II.

Mouse

A. Knee joint inflammation



1.) Spontaneous weight distribution– *incapacitance tester*

2.) Inflammatory cytokine concentration (IL-1β)– *ELISA*

3.) Knee diameter (⇒swelling) – *digital micrometer*

II. Paw inflammation

3.) Paw volume – *plethysmometer*

4.) Touch sensitivity – *aesthesiometer*

Secondary mechanical hyperalgesia

Primary mechanical hyperalgesia

RESULTS

I. RATS

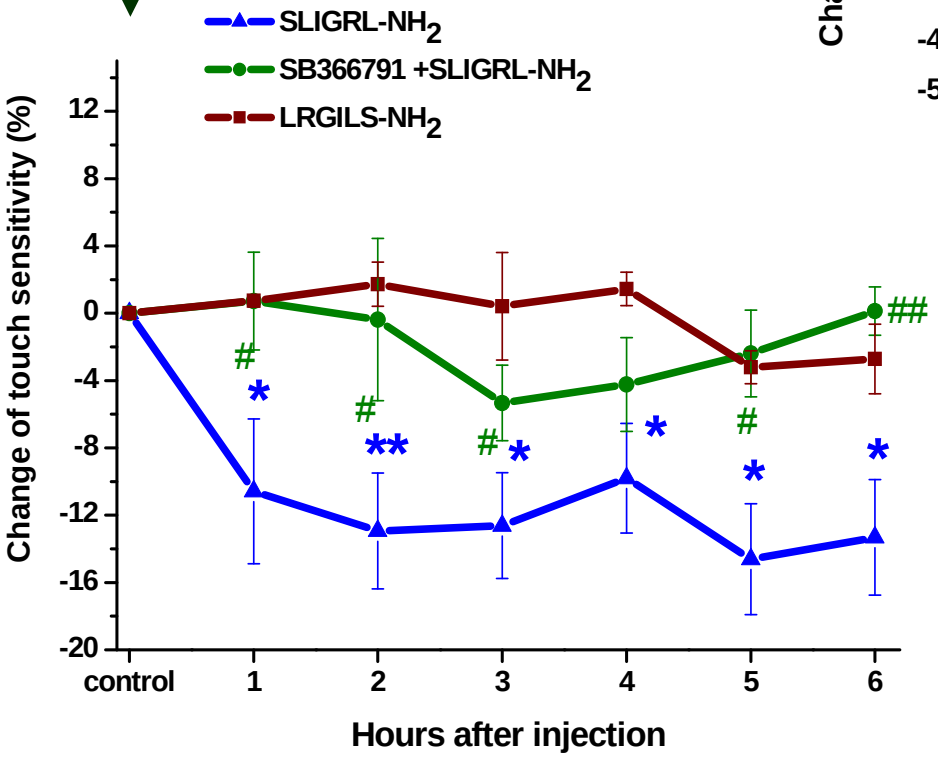
-Effects of SLIGRL-NH_2 in the knee

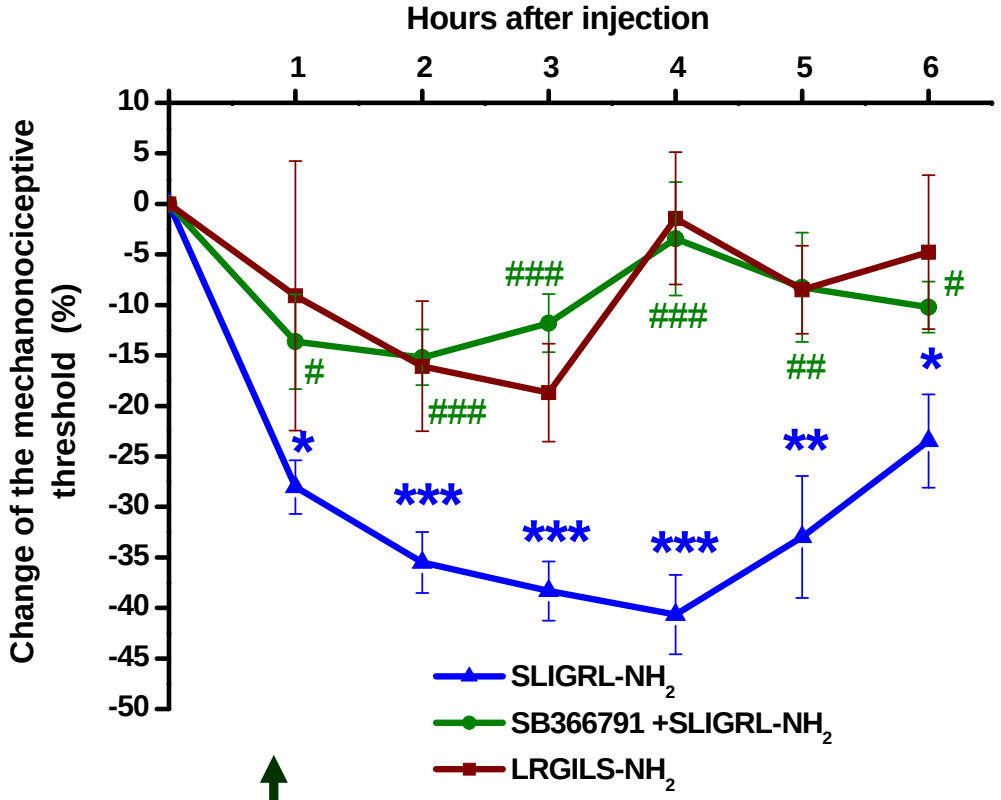
-Role for TRPV1?

(with *antagonist*)

Rat joint

Secondary mechanical allodynia



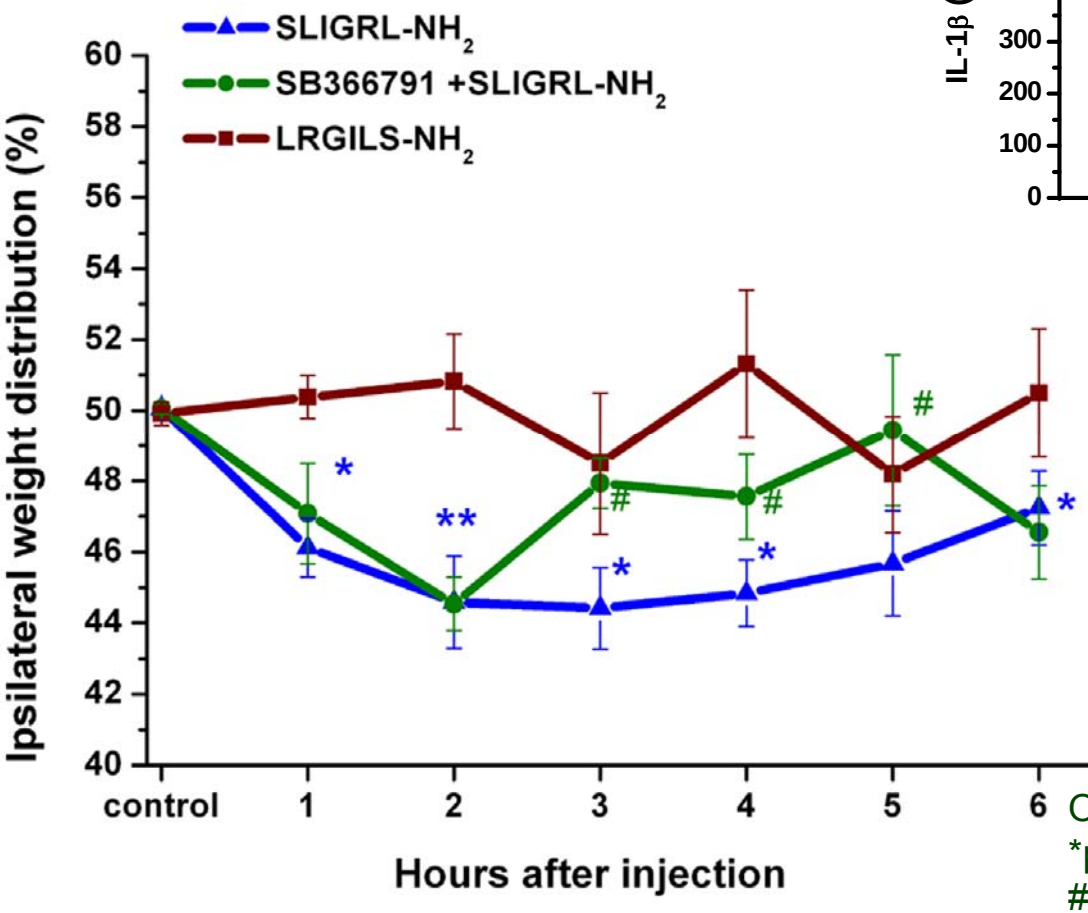


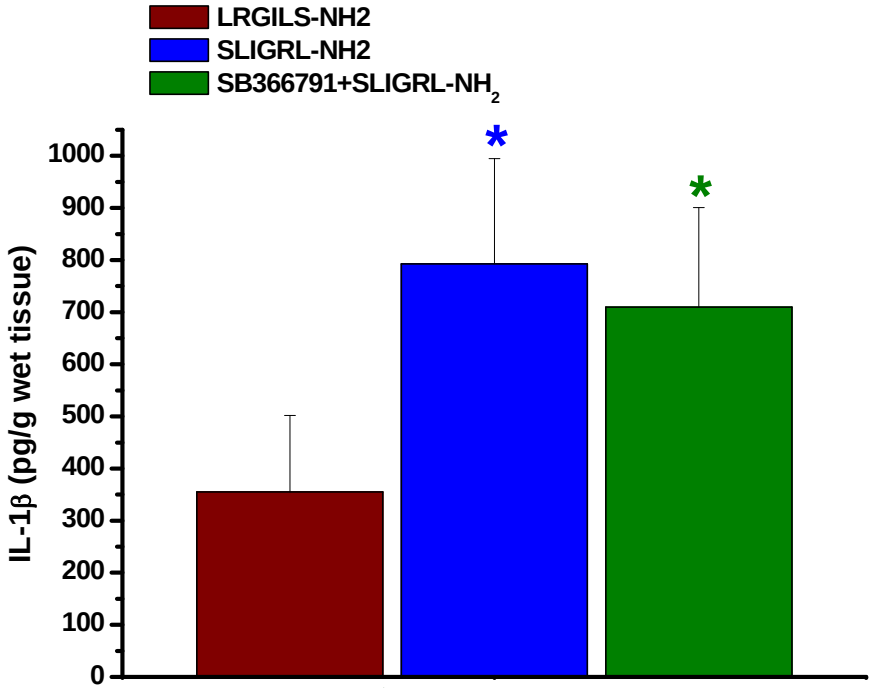
Secondary mechanical hyperalgesia

One way ANOVA + Bonferroni's post test
*p<0.05; **p<0.01, ***p<0.001 vs. inactive peptide
#p<0.05, # # p<0.01, # # # p<0.05 vs. active peptide
n=6-10/group

Rat joint

Spontaneous weight bearing





IL-1β

One way ANOVA + Bonferroni's post test
*p<0.05; **p<0.01 vs. inactive peptide,
#p<0.05 vs. active peptide n=6-10/group

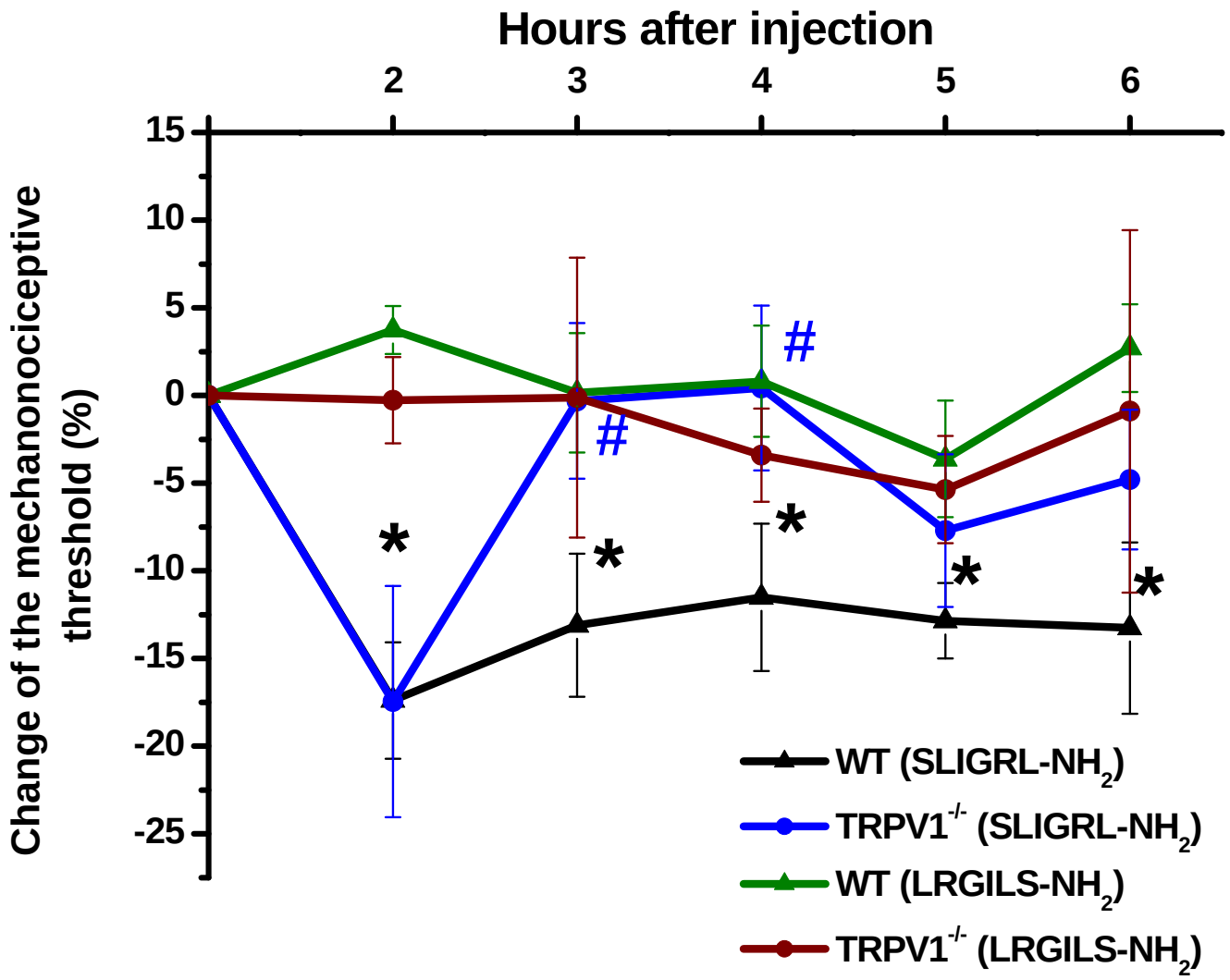
II. MICE

-Effects of SLIGRL-NH_2 in the knee compared to the paw

- Role for TRPV1? (with *gene-deleted mice*)

Mouse joint

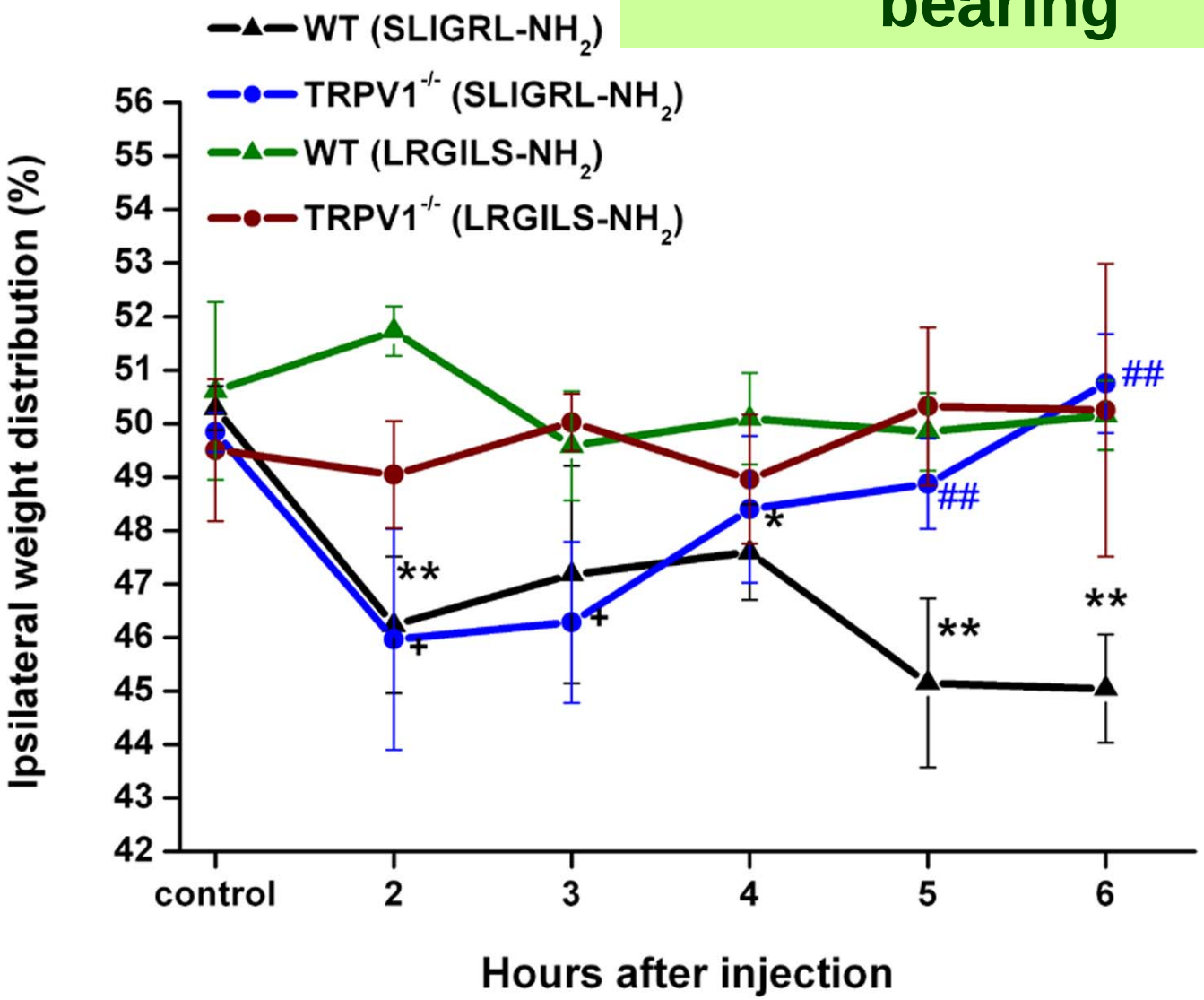
Secondary mechanical hyperalgesia



One way ANOVA + Bonferroni's post test
*p<0.05, **p<0.01 vs. WT inactive peptide; +p<0.05 vs, $TRPV1^{-/-}$ inactive peptide
#p<0.05, # # p<0.05 vs. WT; n=6-10/group

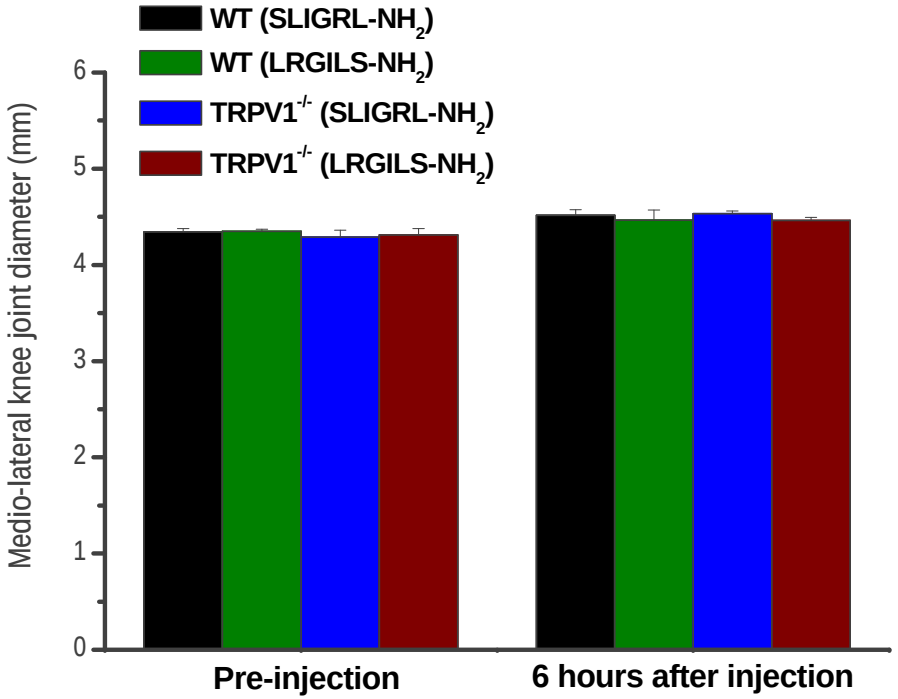
Mouse joint

Spontaneous weight bearing



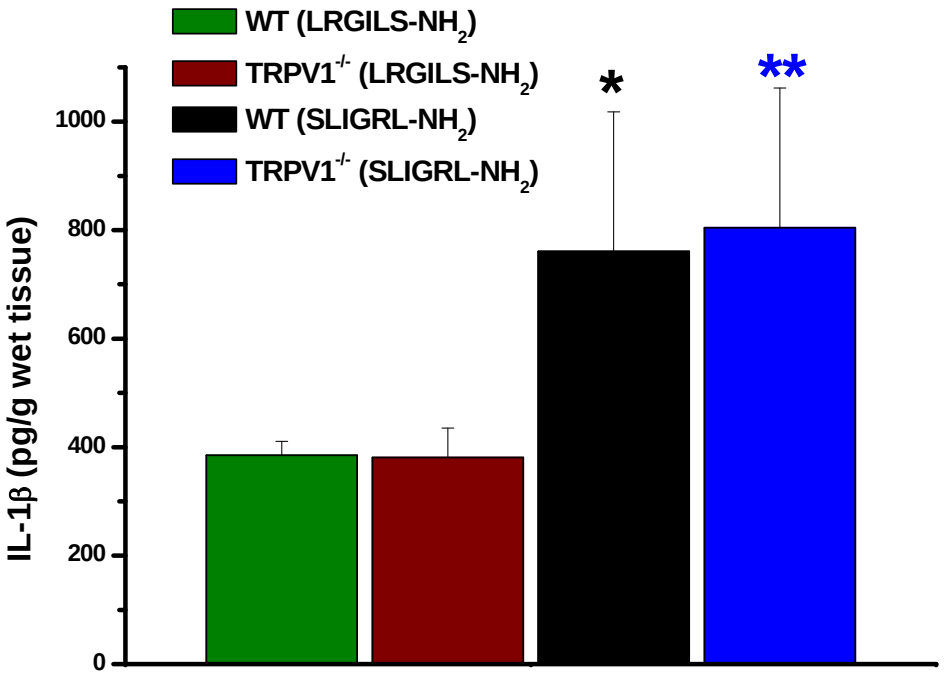
One way ANOVA + Bonferroni's post test
*p<0.05, **p<0.01 vs. WT inactive peptide; +p<0.05 vs, TRPV1$^{-/-}$ inactive peptide
#p<0.05, # # p<0.05 vs. WT; n=6-10/group

Mouse joint



Knee diameter

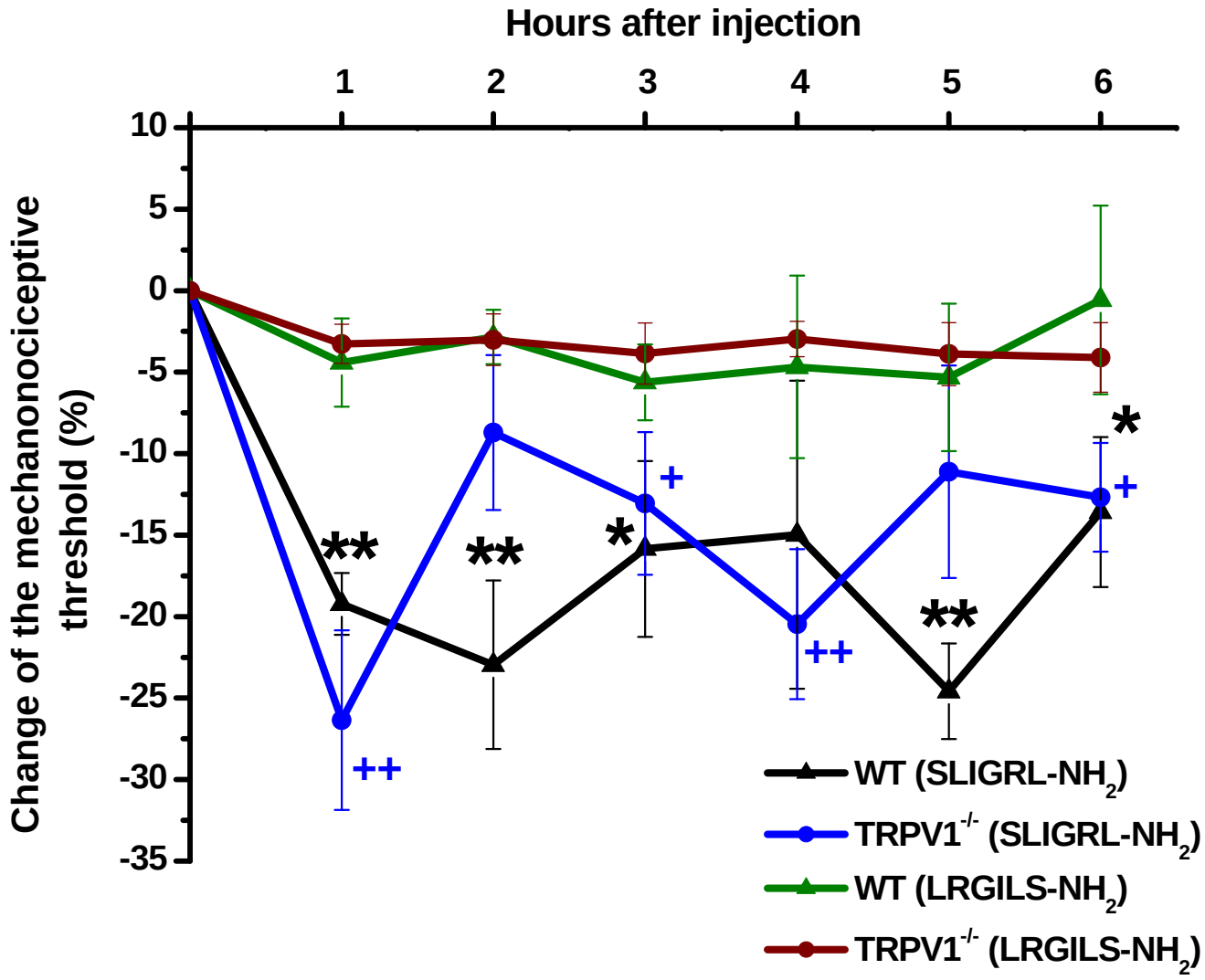
IL-1β



One way ANOVA + Bonferroni's post test
*p<0.05; **p<0.01 vs. inactive peptide;
n=6-8/group

Mouse paw

Primary mechanical hyperalgesia

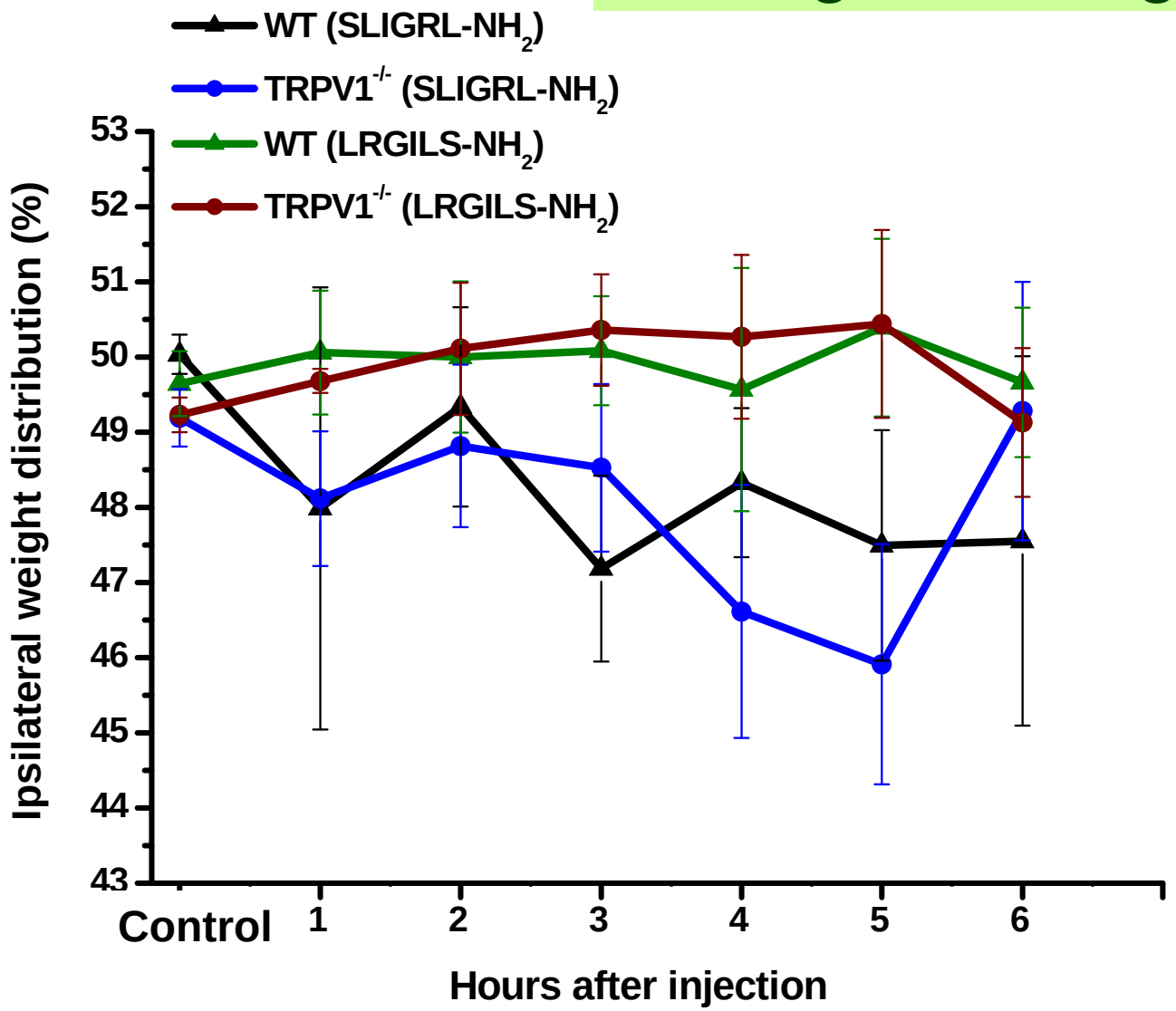


One way ANOVA + Bonferroni's post test

*p<0.05, **p<0.01 vs. WT inactive peptide; +p<0.05 vs, $TRPV1^{-/-}$ inactive peptide; n=6-10/group

Mouse paw

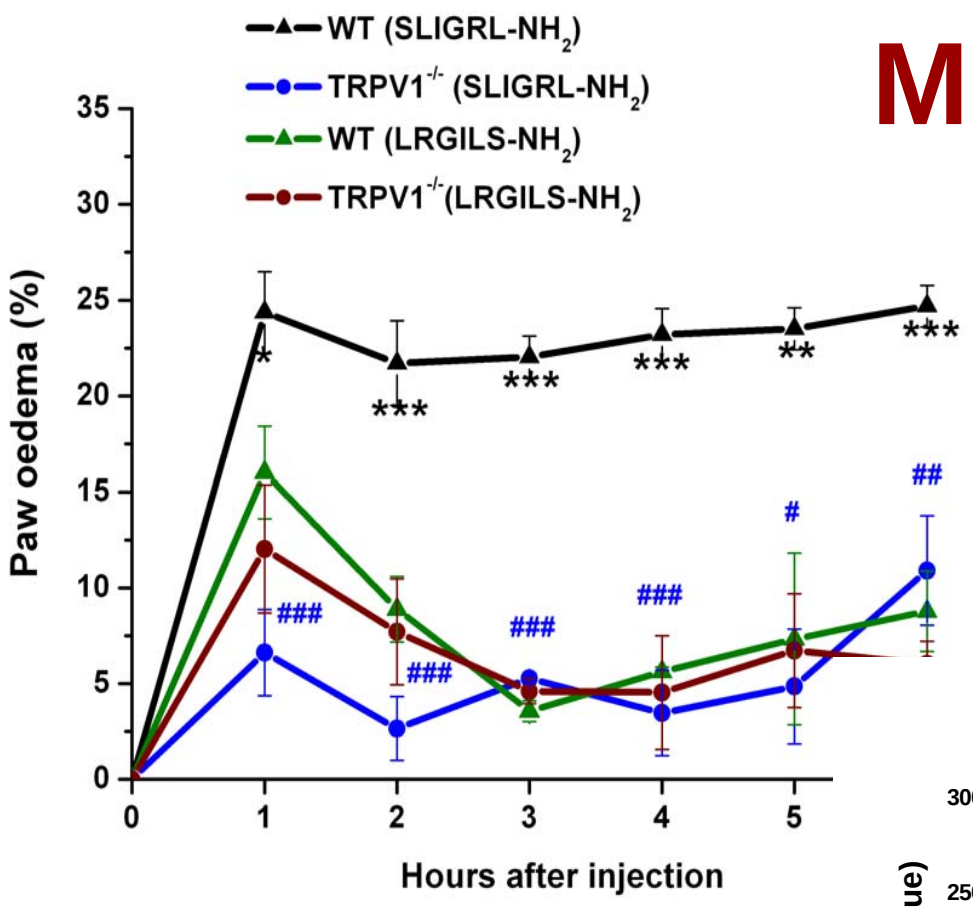
Spontaneous weight bearing



One way ANOVA + Bonferroni's post test

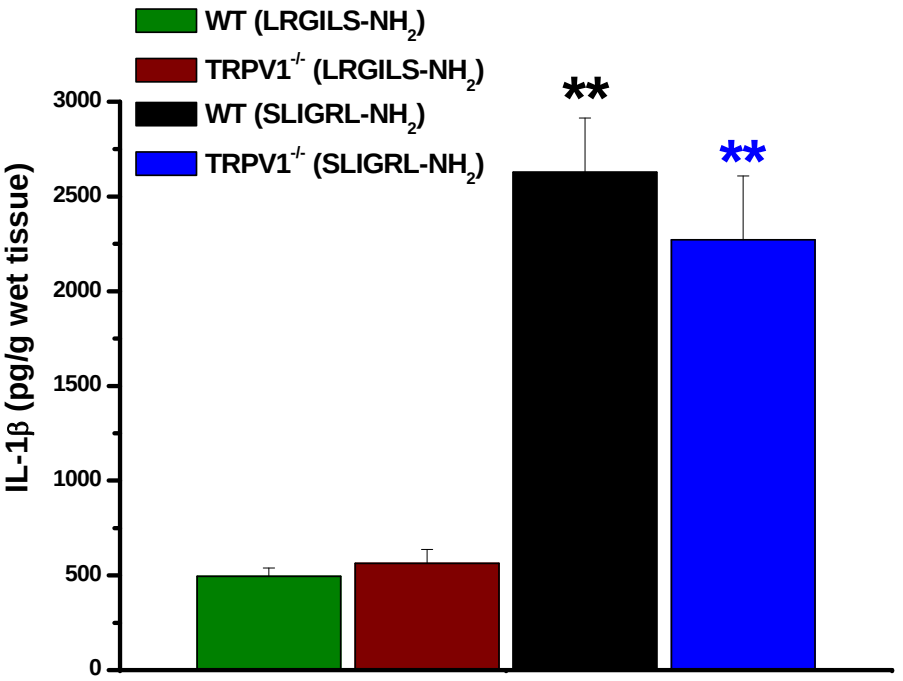
*p<0.05, **p<0.01 vs. WT inactive peptide; +p<0.05 vs, TRPV1$^{-/-}$ inactive peptide; n=6-10/group

Mouse paw



Oedema

IL-1β



One way ANOVA + Bonferroni's post test

*p<0.05, **p<0.01, ***p<0.001 vs. WT inactive peptide;
#p<0.05, # # p<0.05 vs. WT; n=6-10/group

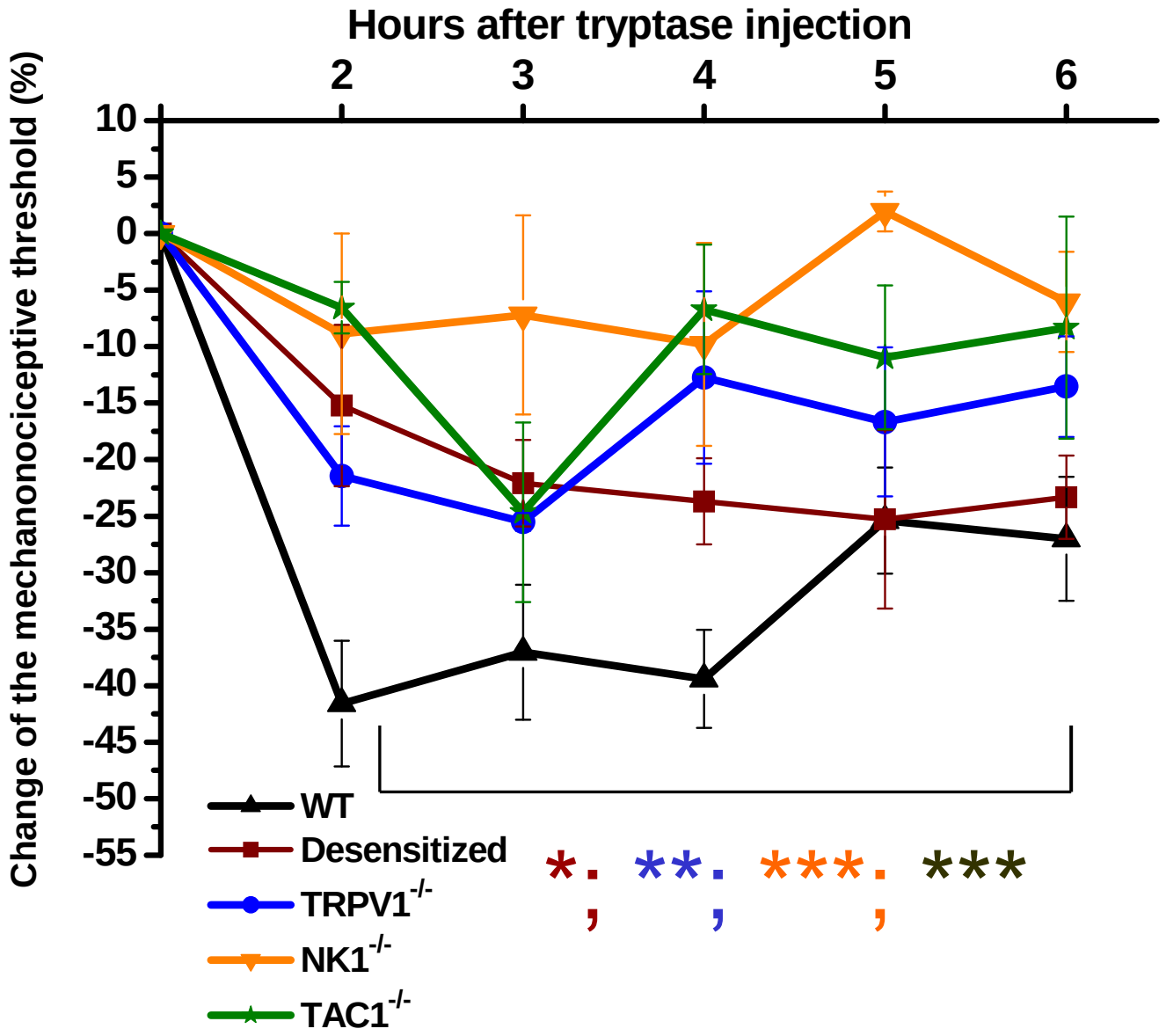
III. MICE

- Effect of MCT in the knee
- Role for
  - ❖ capsaicin-sensitive nerves
  - ❖ TRPV1 channels and
  - ❖ tachykinins?

    (with *desensitization* and *gene-deleted mice*)

Mouse joint

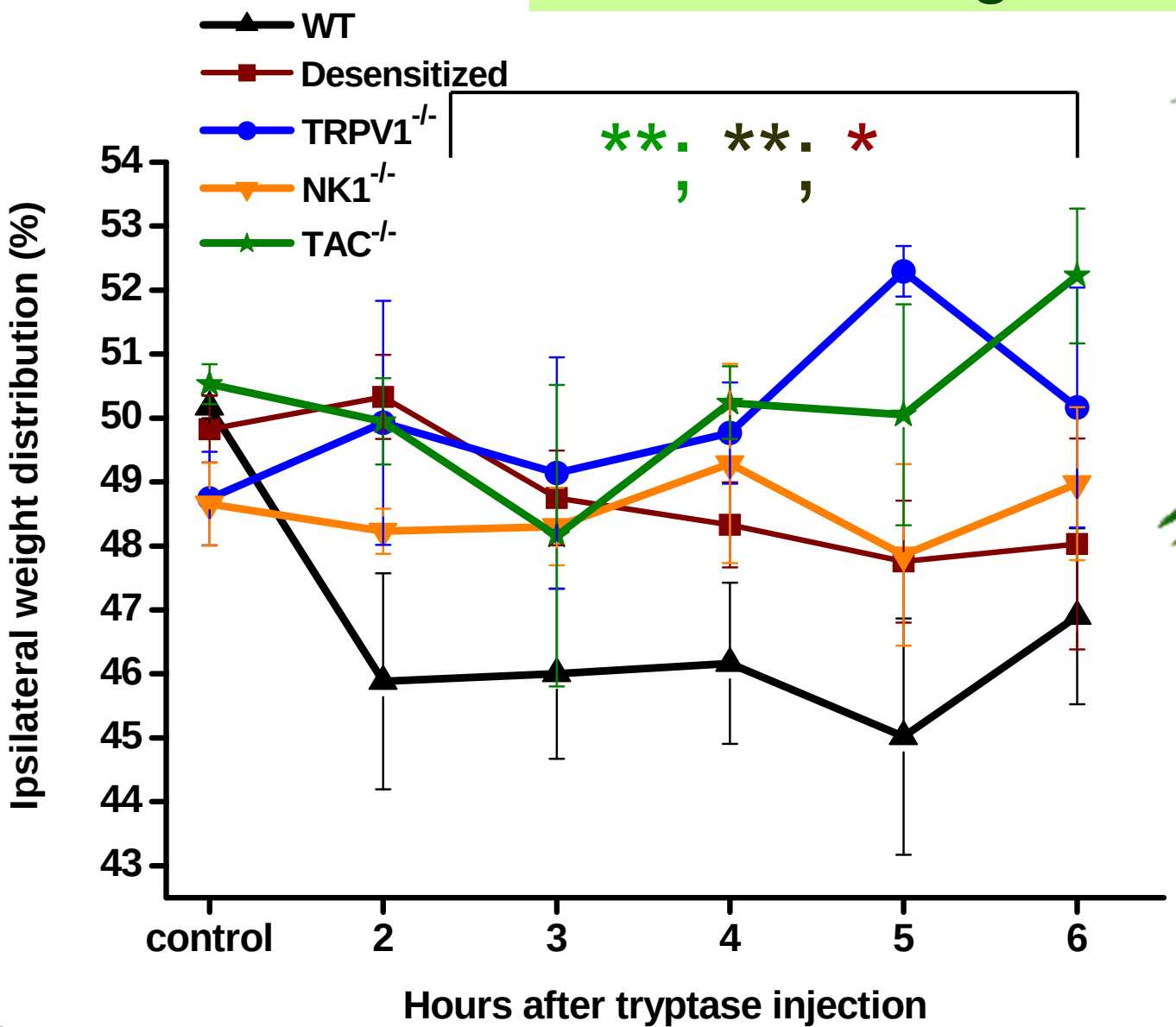
Secondary mechanical hyperalgesia



One way ANOVA + Dunnett's post test; *p<0.05, **p<0.01; ***p<0.001 vs. WT; n=6/group

Mouse joint

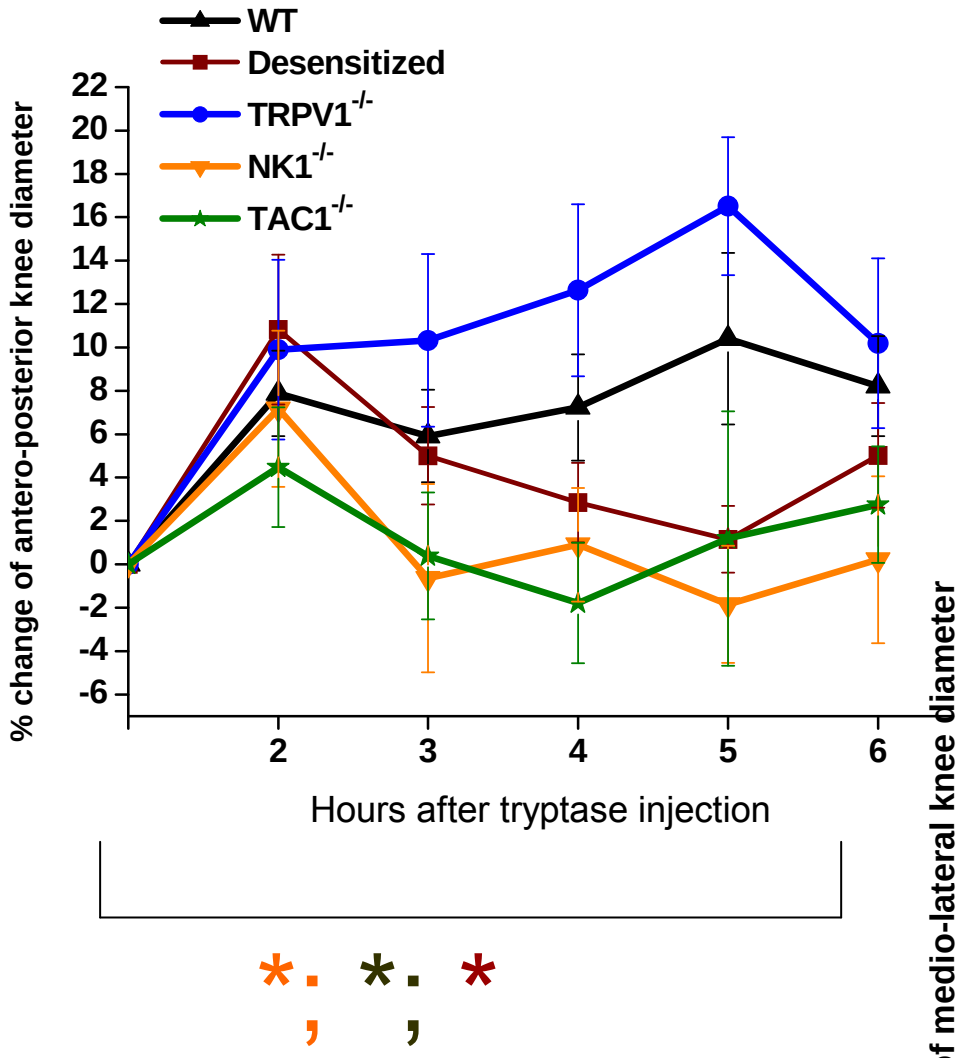
Spontaneous weight bearing



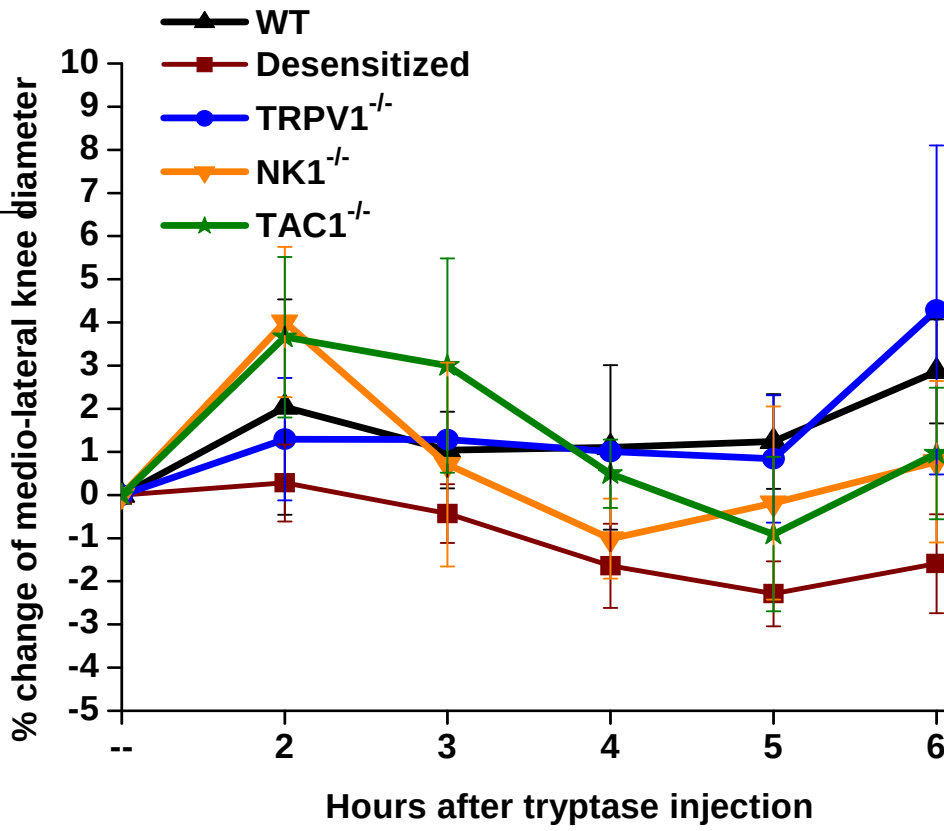
One way ANOVA + Dunnet's post test; *p<0.05, **p<0.01 vs. WT vs. WT; n=6/group

Mouse joint

Knee diameter

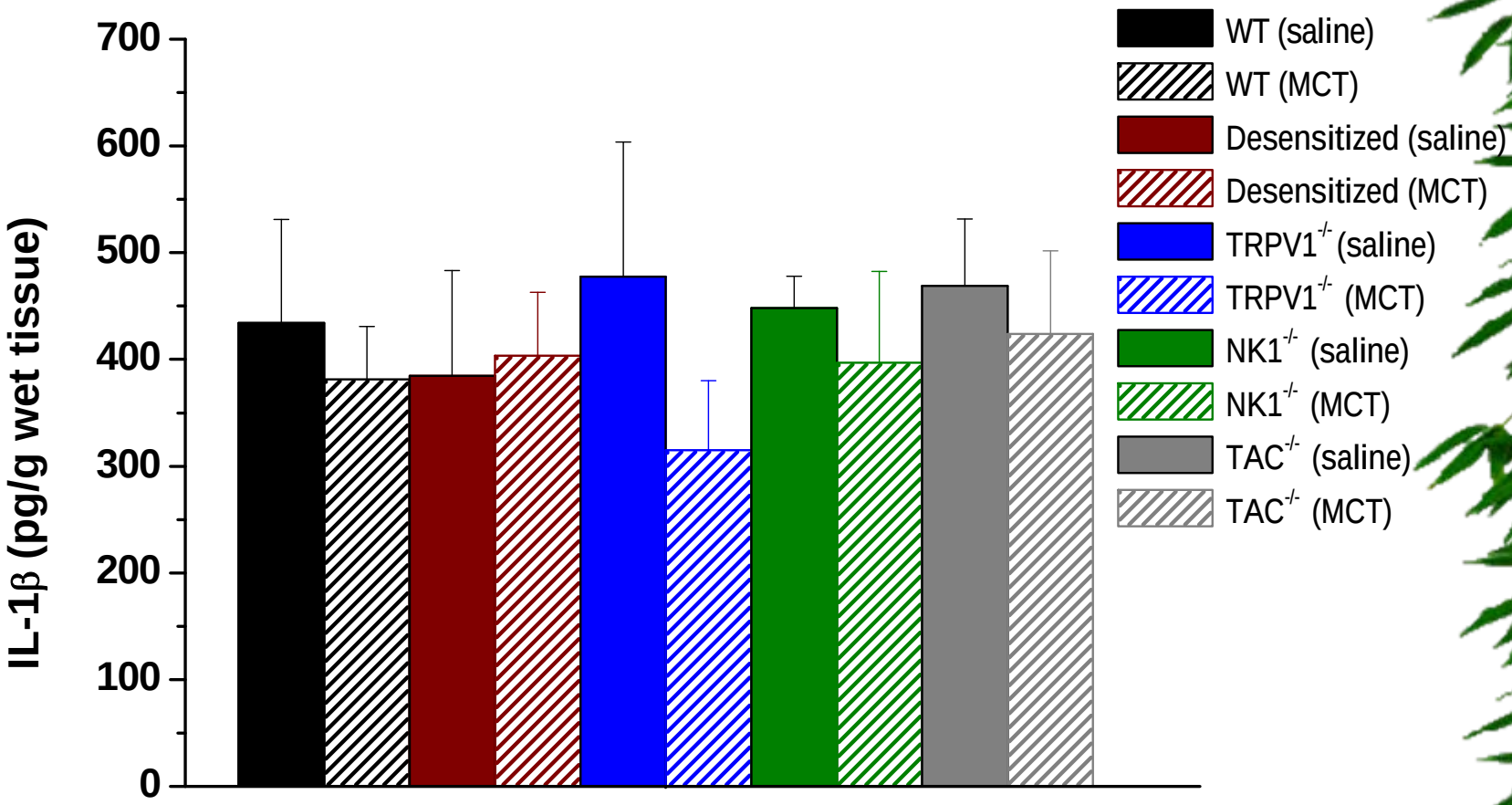


p<0.05 vs. WT; n=6-8/group
one way ANOVA + Dunnett's post test



Mouse joint

IL-1β

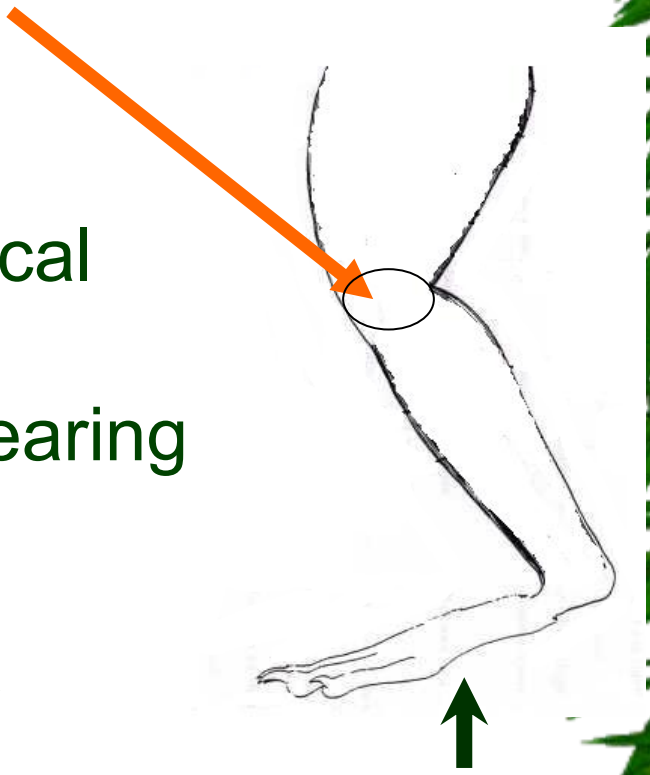


One way ANOVA + Bonferroni's post test
*p<0.05; **p<0.01 vs. inactive peptide; n=6-8/group

SUMMARY I.

Rat knee joint

PAR2 activation (**SLIGRL-NH_2**)



- significant secondary mechanical hyperalgesia, allodynia and
- decreased ipsilateral weight bearing

- which are abolished/ markedly decreased (after 2 h)
by the TRPV1 receptor antagonist pretreatment

SUMMARY II.

Mice: PAR2 activation (**SLIGRL-NH2**)

1.) **Knee joint:**

-**Secondary** mechanical hyperalgesia

-Decreased ipsilateral weight bearing

⇓

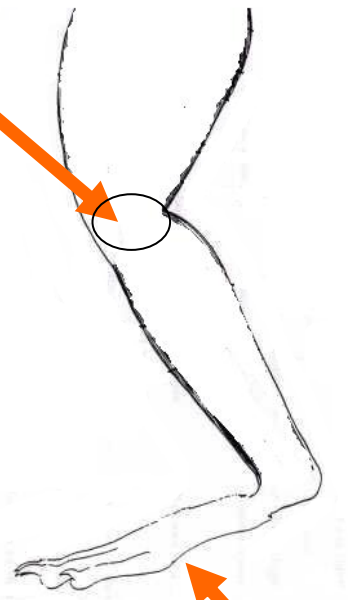
Significantly smaller in *TRPV1-/-* mice

-IL-1β increase

⇓

Similar in TRPV1$^{-/-}$ mice

-No joint swelling



2.) **Paw**:

-**Primary** mechanical hyperalgesia

-Impaired ipsilateral weight bearing

⇓

Similar in TRPV1$^{-/-}$ mice

-***Paw oedema***

⇓

50% smaller in TRPV1-/- mice

SUMMARY III.

Mouse knee joint: comparison of

Synthetic PAR2 activating agent **SLIGRL-NH_2**	Natural PAR2 activating **MCT**
❖ No joint swelling	❖ 8-10% joint swelling
❖ 50% IL-1β increase	❖ No IL-1β increase
❖ 15-20 % secondary mechanical hyperalgesia	❖ 30-40 % secondary mechanical hyperalgesia
❖ Impaired weight distribution	❖ Impaired weight distribution

SUMMARY IV.

Mouse knee joint : PAR2 activation (**MCT**)

- **Secondary** mechanical hyperalgesia
- **Impaired** ipsilateral **weight bearing**

⇓

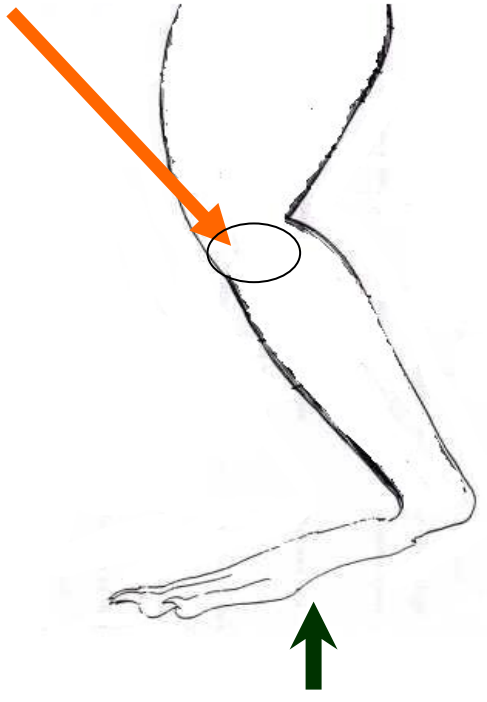
Significantly decreased in RTX-desensitized, TRPV1$^{-/-}$, TAC1$^{-/-}$ and NK1$^{-/-}$ mice

- Moderate **joint swelling**

⇓

Significantly smaller in TAC1$^{-/-}$ and NK1$^{-/-}$ mice

Similar in TRPV1$^{-/-}$ mice



- No IL-1β increase

CONCLUSIONS I.

PAR2 activation in the knee joint ➡ **inflammation**

a.) **secondary mechanical hyperalgesia/allodynia** (involve central mechanisms and sensitization)

b.) **spontaneously decreased ipsilateral weight distribution**

mediated by capsaicin-sensitive fibres, TRPV1 receptors, TAC1 gene-encoded tachykinins (SP, NKA) and NK1 receptors

⇓

-*TRPV1 on peptidergic afferents is likely to be activated/sensitized by mediators released through PAR2 stimulation*

-*Central mechanisms are suggested through SP and NK1 receptors*

CONCLUSIONS II.

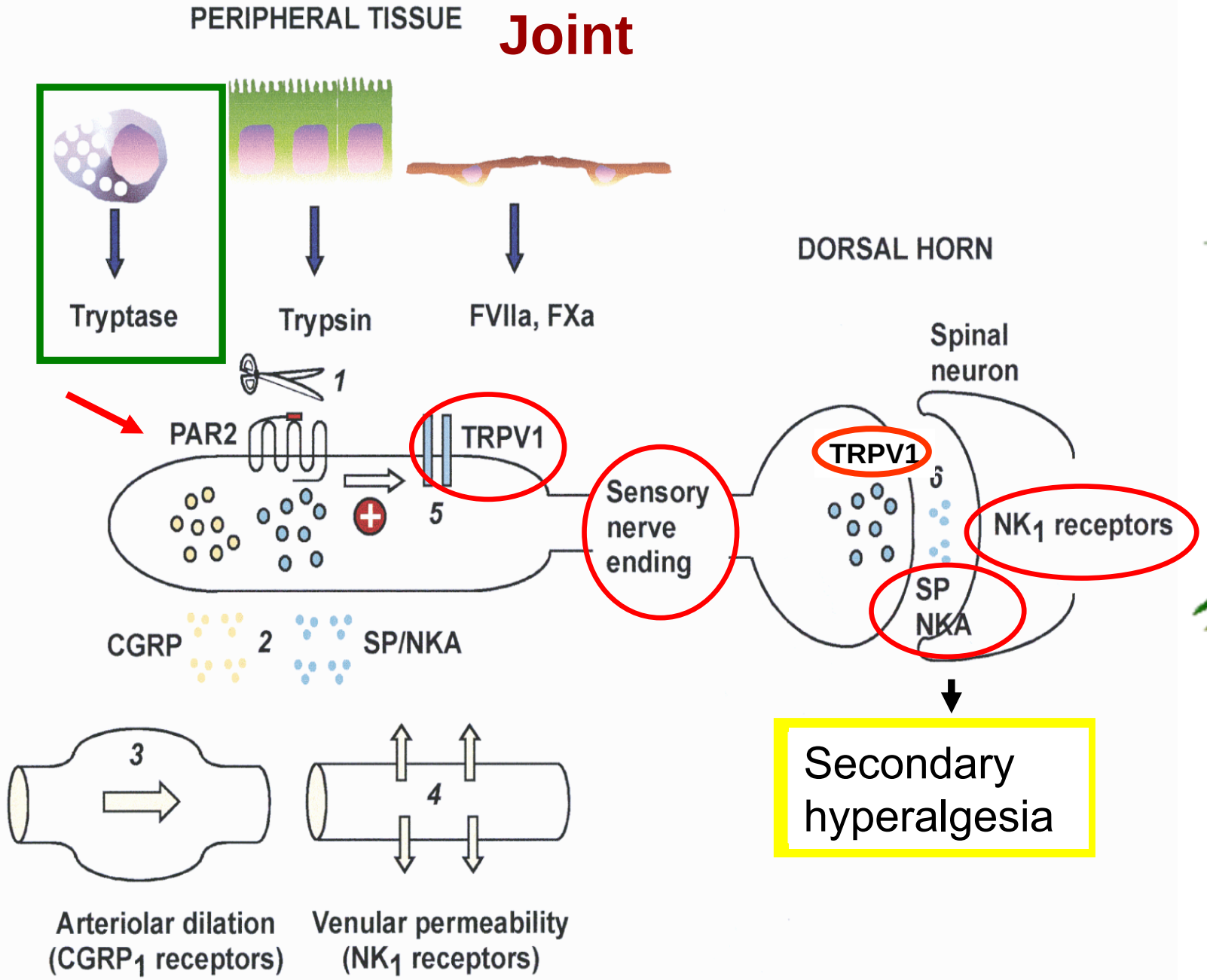
Intraplantar PAR2 activation ➡ **paw inflammation**

a.) **paw swelling**: TRPV1 receptor-mediated

b.) **primary mechanical hyperalgesia** (predominantly mediated by peripheral mechanisms)

c.) **spontaneously decreased unilateral weight distribution**

in which TRPV1 receptor activation does not play a role

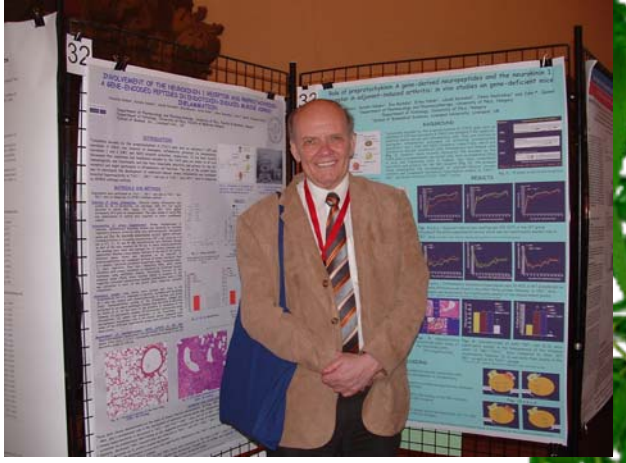


NEUROGENIC INFLAMMATION

(Cottrell et al. 2003. Biochem. Soc. Trans.)

ACKNOWLEDGEMENTS

Thank you for your attention!



Our research group:

Prof. János Szolcsányi
Katalin Sándor
Adrienn Markovics
Éva Borbély
Erika Pintér
Ágnes Kemény
Krisztián Elekes

Jason J. McDougall
(Dept. Physiol. Biophysics, University of Calgary, Canada)
John P. Quinn (University of Liverpool, UK)

EURÓPA KULTURÁLIS FŐVÁROSA
2 0 1 0

Pécs 2010
European Capital of Culture