

Ion Channel Retreat, Vancouver  
July 9, 2015

## Role of ANO1 (TMEM16A) channels in inflammatory pain



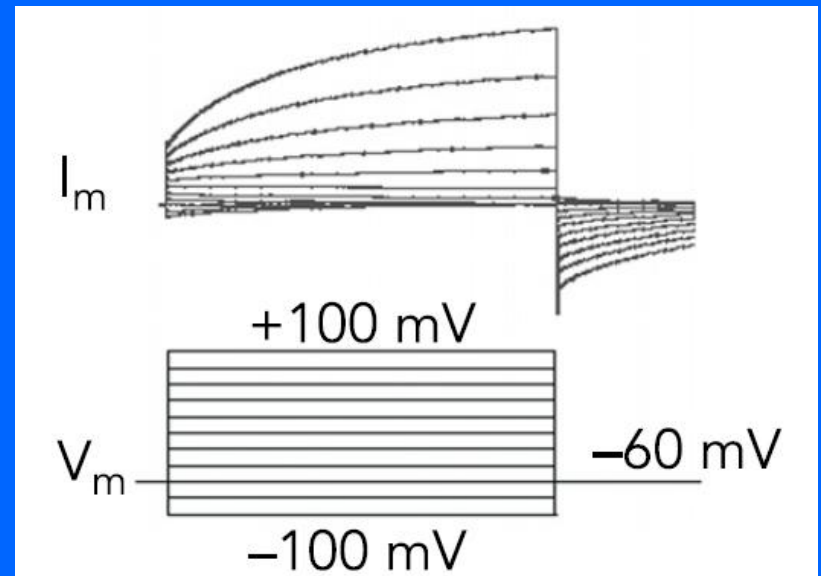
Nikita Gamper

# Physiological roles of $\text{Ca}^{2+}$ -activated $\text{Cl}^-$ channels (CaCCs) in mammalian tissues

- ✓ **Epithelia.....fluid transport, mucus secretion**
- ✓ **Smooth muscle.....contractility, tone**
- ✓ **Sensory neurons.....signal amplification, excitability  
(and other sensory cells)**

# Key features of CaCC:

- Low apparent affinity to  $\text{Ca}^{2+}$ : low  $\mu\text{M}$  range
- $P_{\text{I}} > P_{\text{Cl}}$
- Voltage dependence with outward rectification
- Slow kinetics
- Sensitivity to NPPB, DIDS, NFA, FFA



# Molecular identity CaCC remained elusive for many years

➤ CLCA?

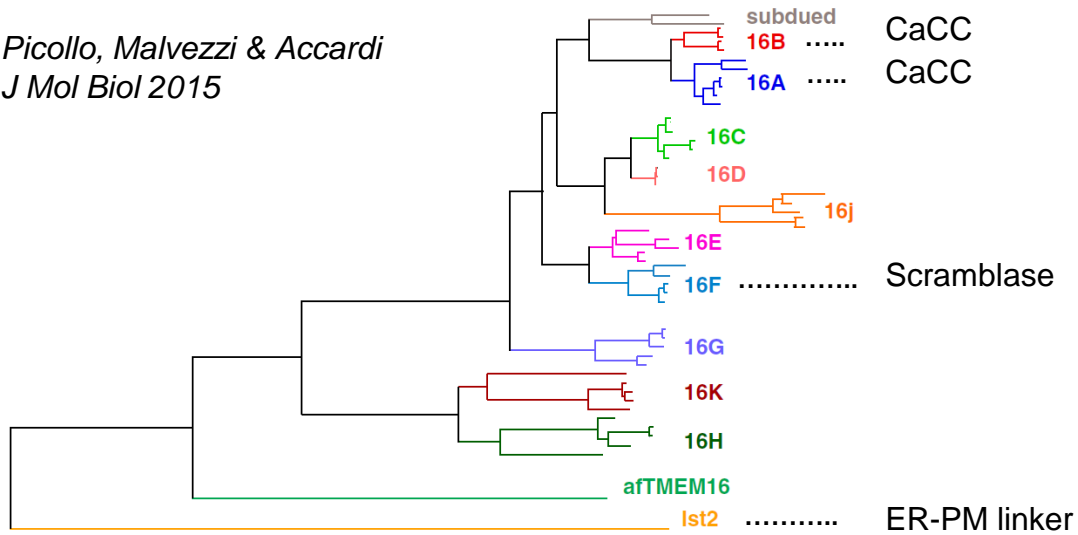
➤ CLC3?

➤ Bestrophins?

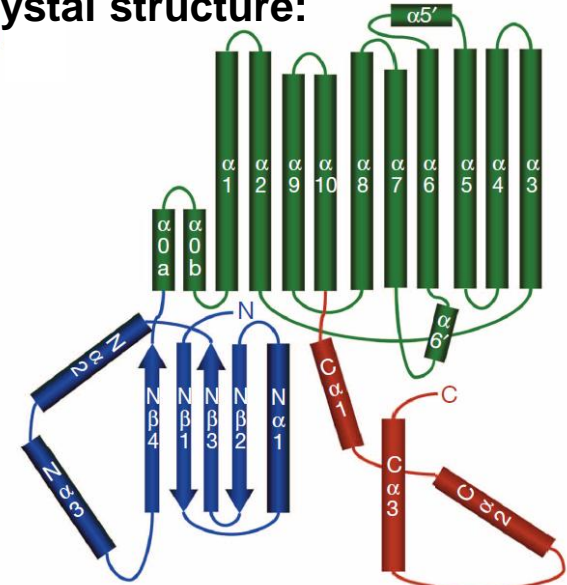
➤ **ANO1/TMEM16A**

# TMEM16 proteins in the spotlight

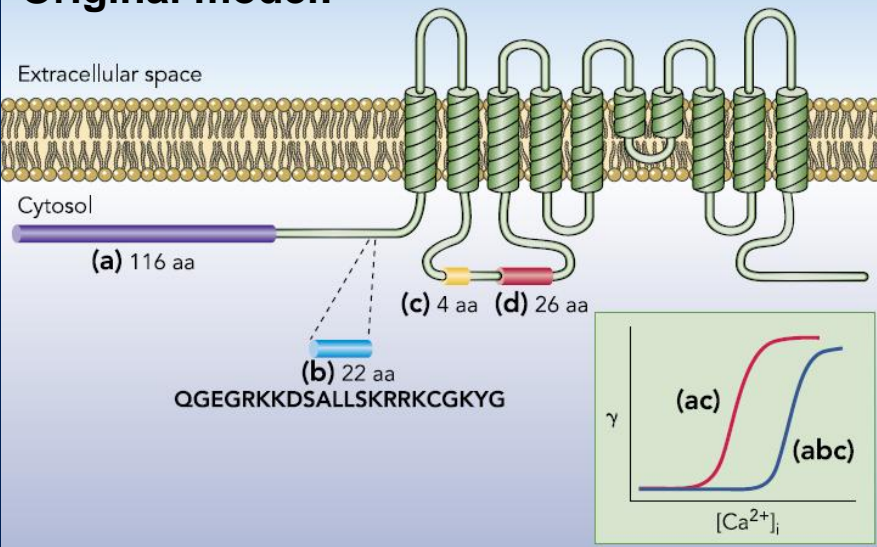
Piccolo, Malvezzi & Accardi  
*J Mol Biol* 2015



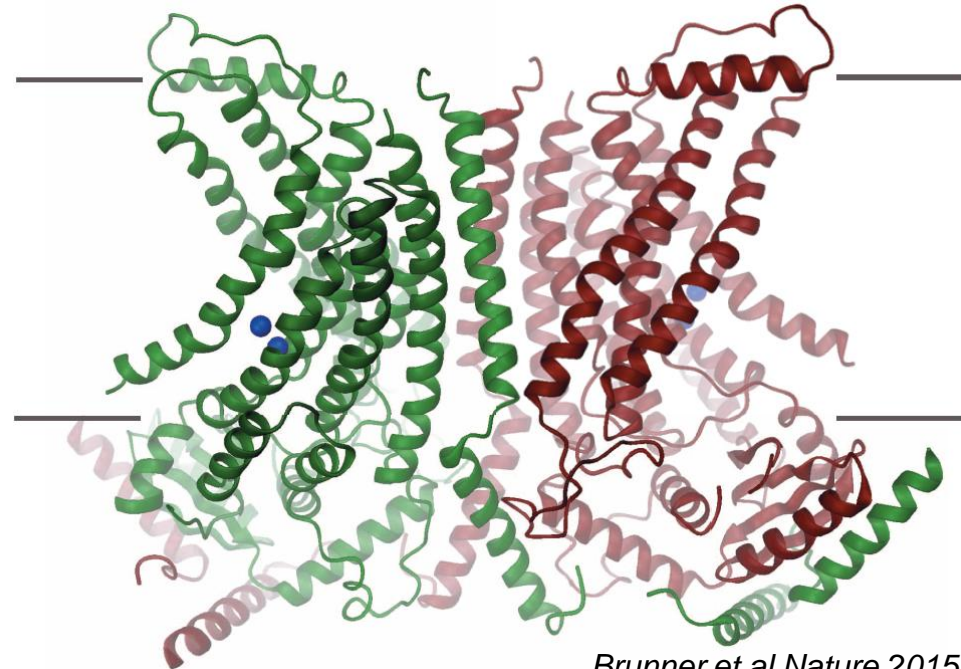
## Crystal structure:



## Original model:



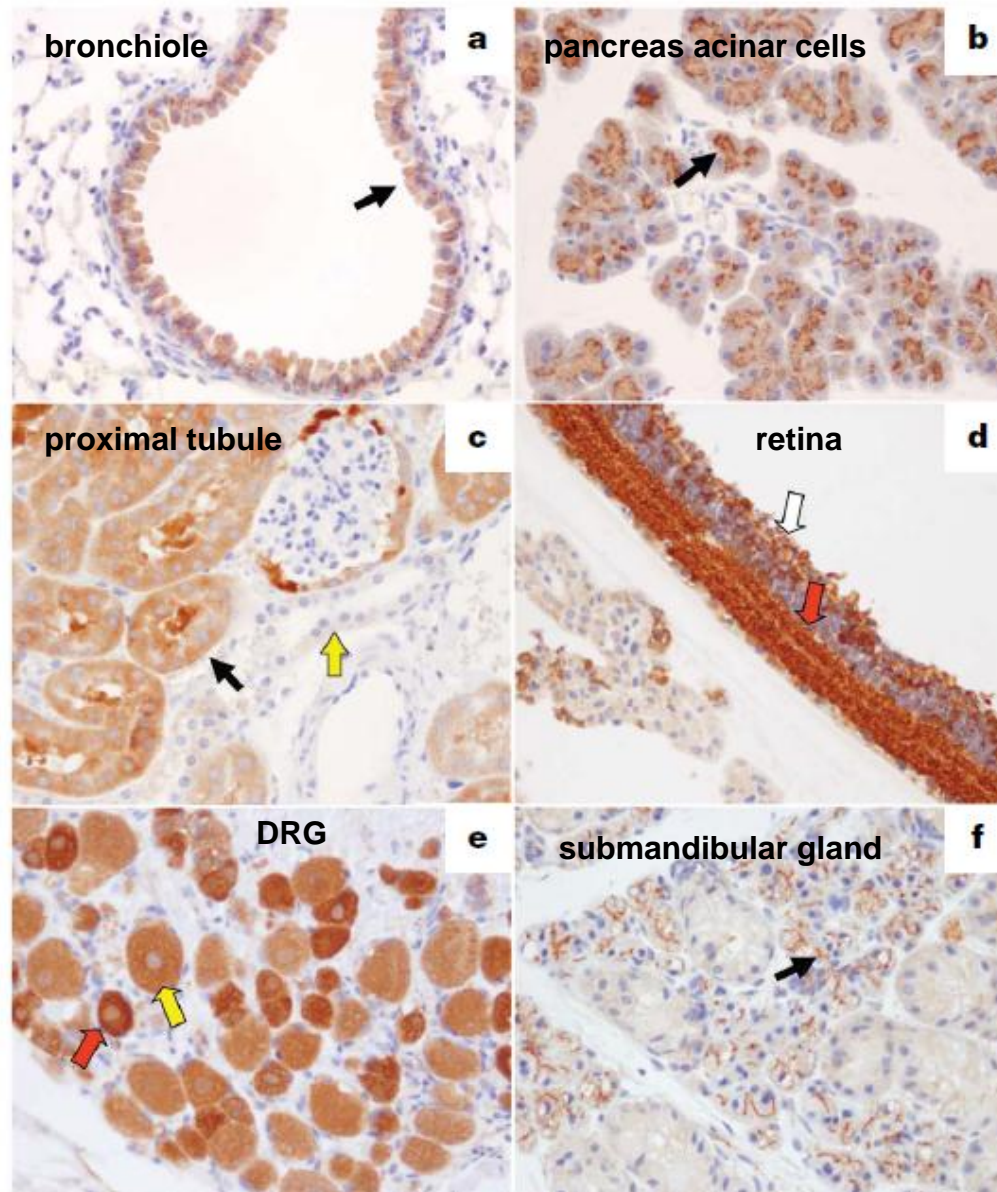
Ferrera et al. 2010 Physiology



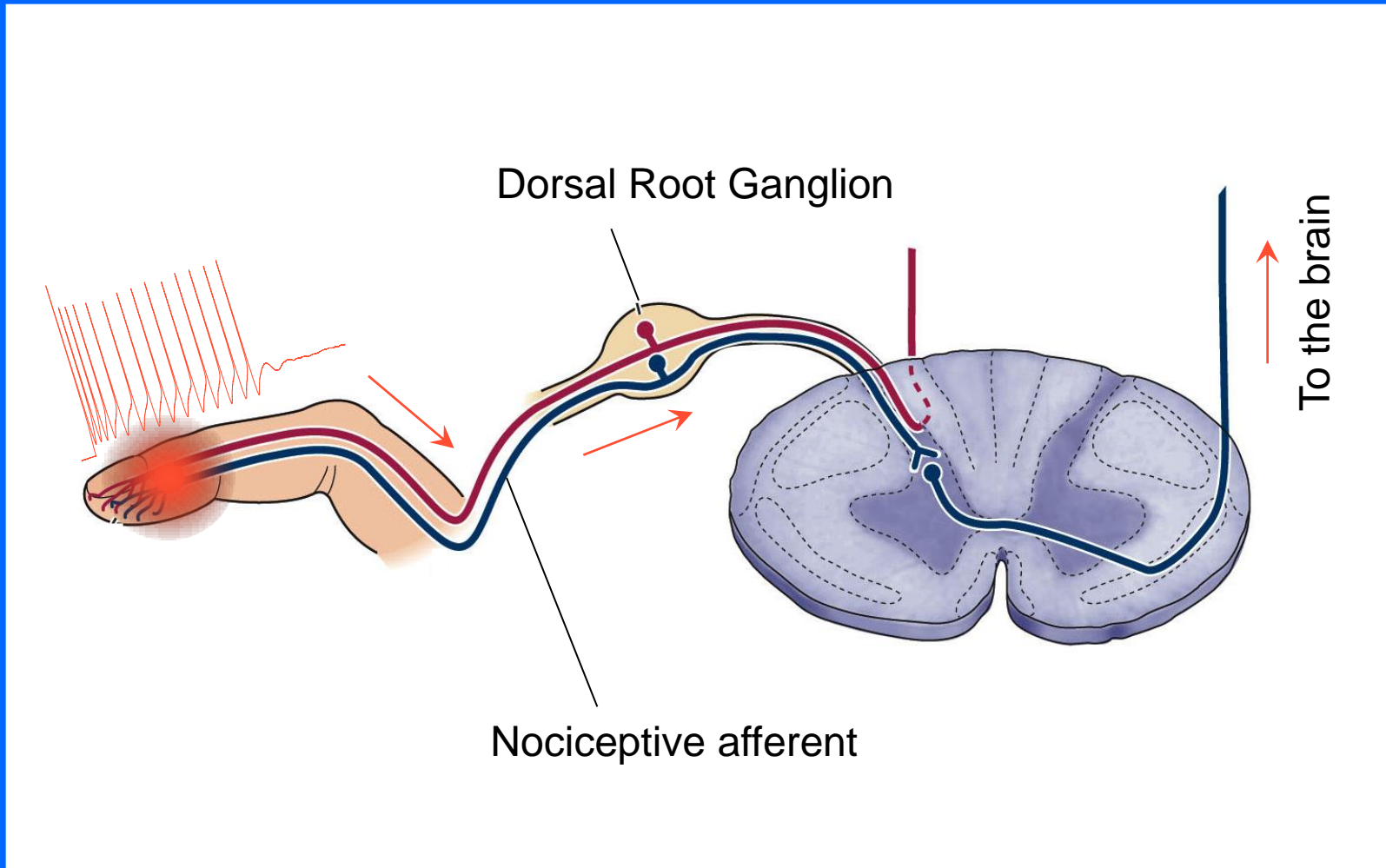
Brunner et al Nature 2015



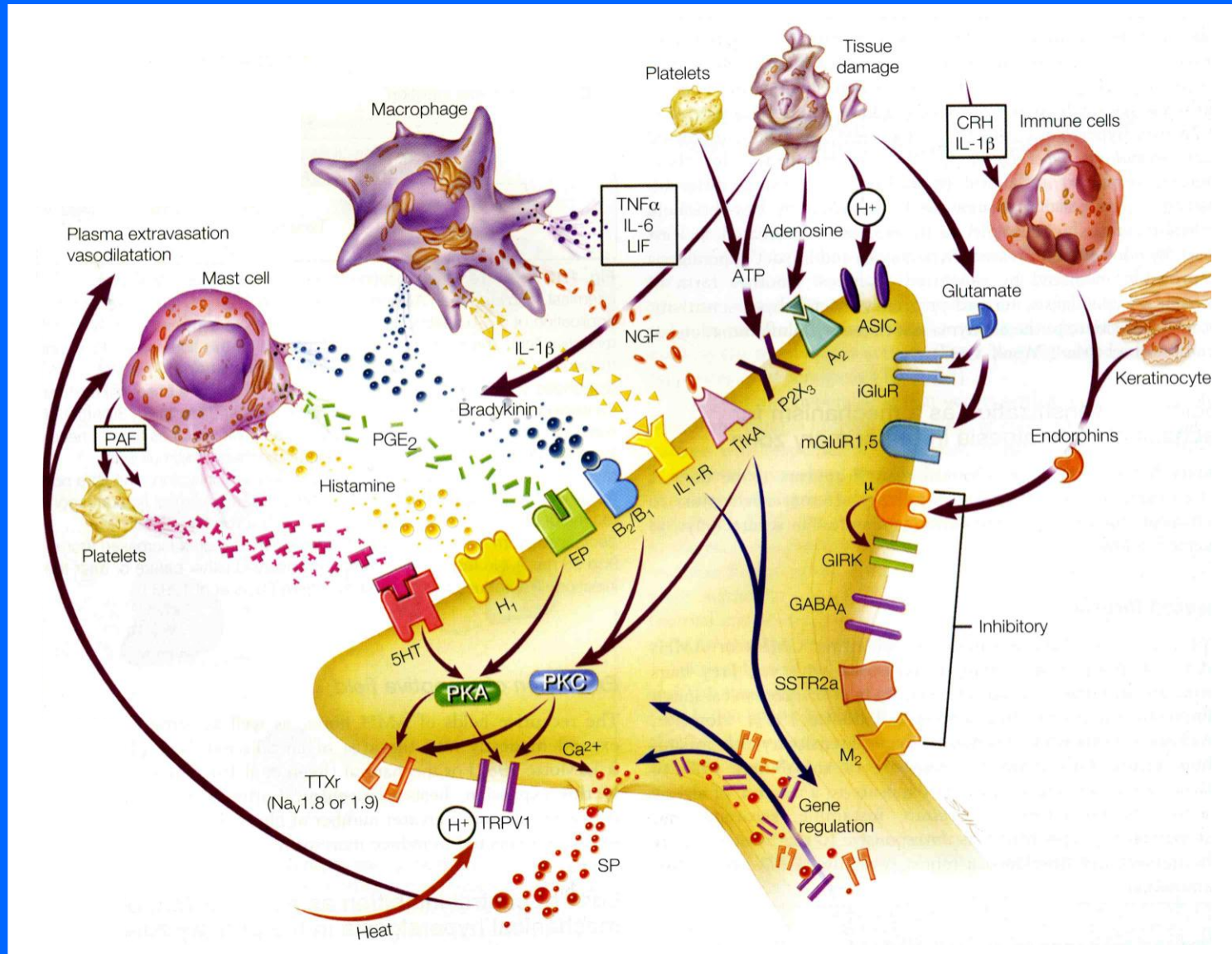
# Expression of TMEM16A various tissues



Excitability of sensory neurons is a way to communicate sensory information to the brain

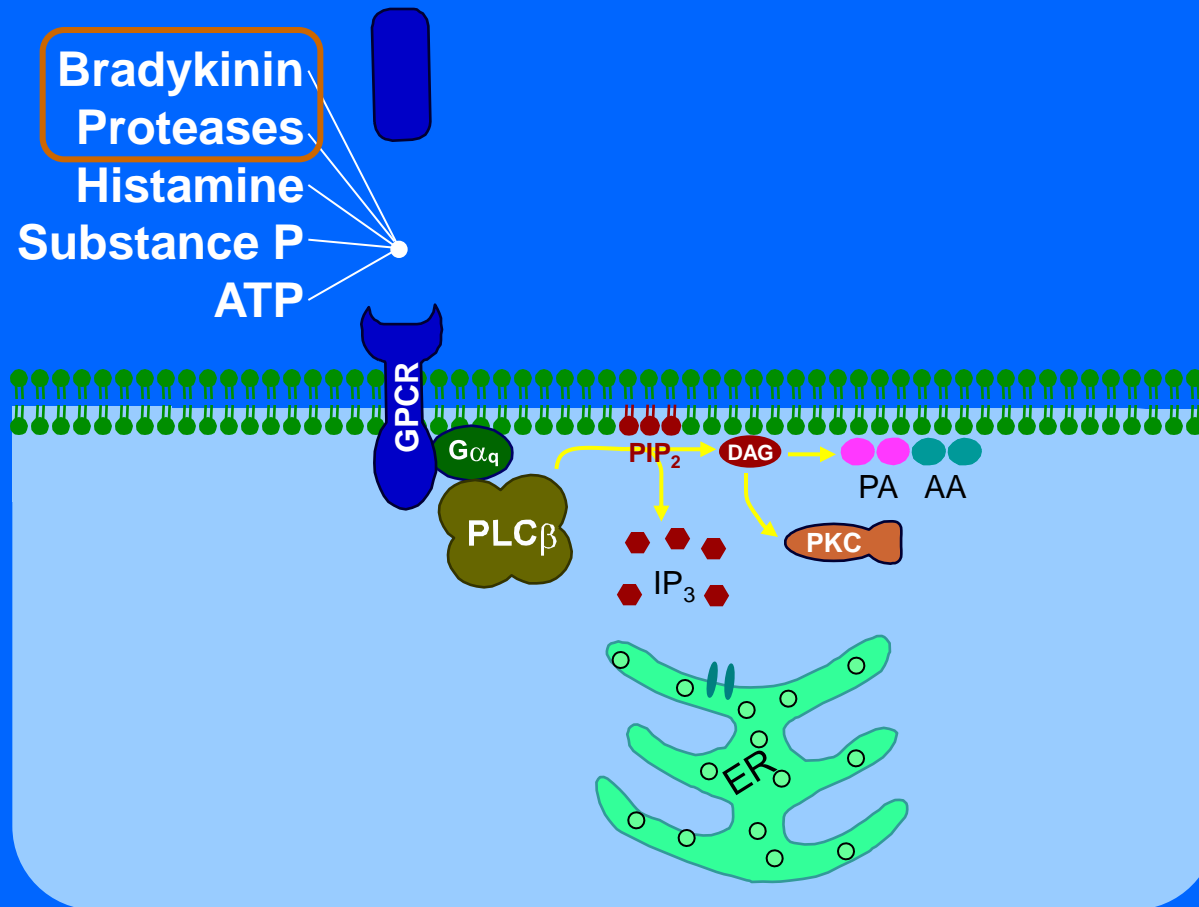


# Many inflammatory mediators excite sensory neurons through G protein coupled receptors

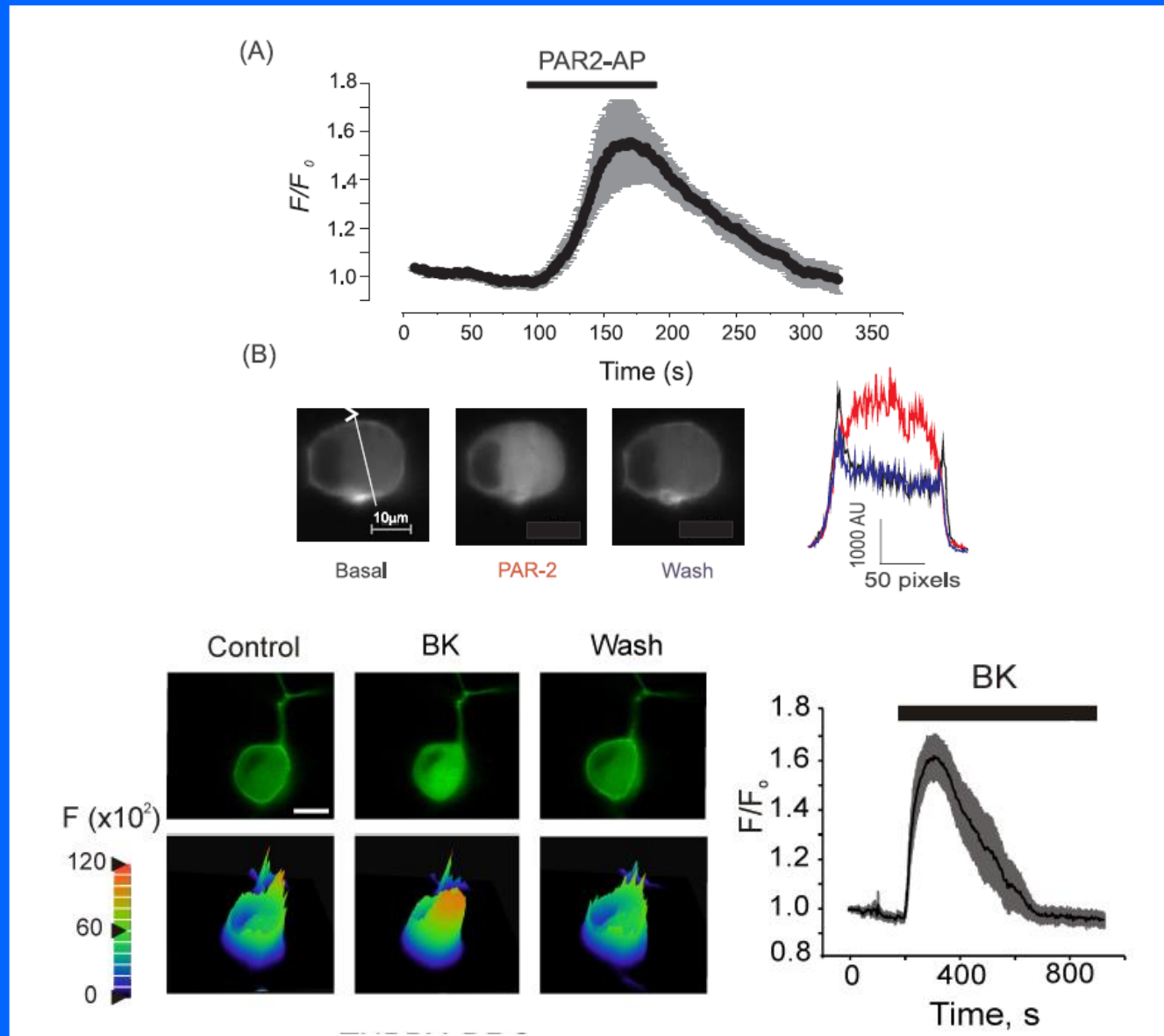




Several inflammatory mediators act through  $G_{q/11}$ -coupled receptors expressed in sensory neurons



# Both BK and PAR2-AP induces strong activation of PLC in DRG

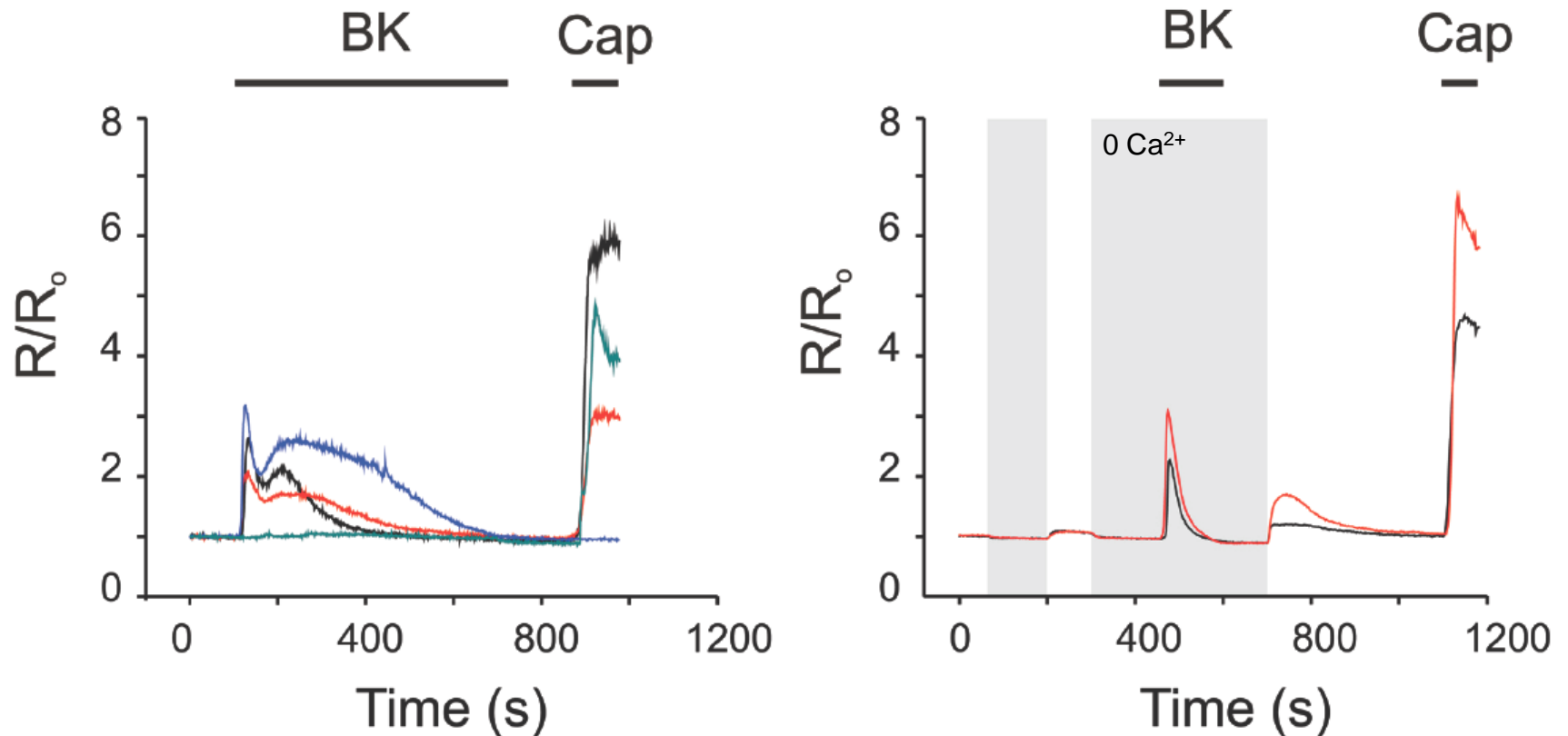


Linley et al. J Neurosci 2008

Liu et al. J Clin Invest 2010

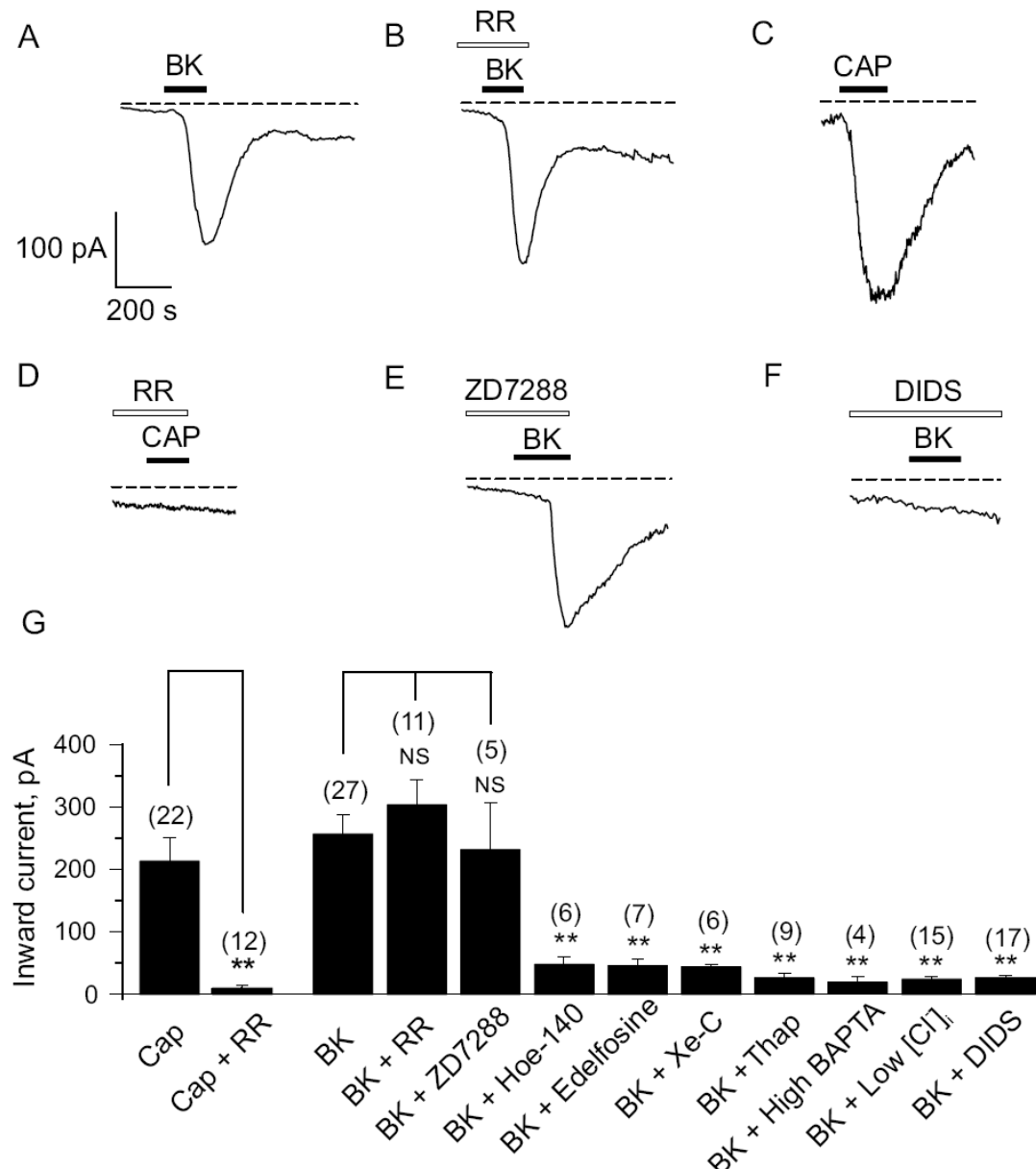
Linley et al. PNAS 2012

# Inflammatory mediators induce $\text{Ca}^{2+}$ release from the ER stores

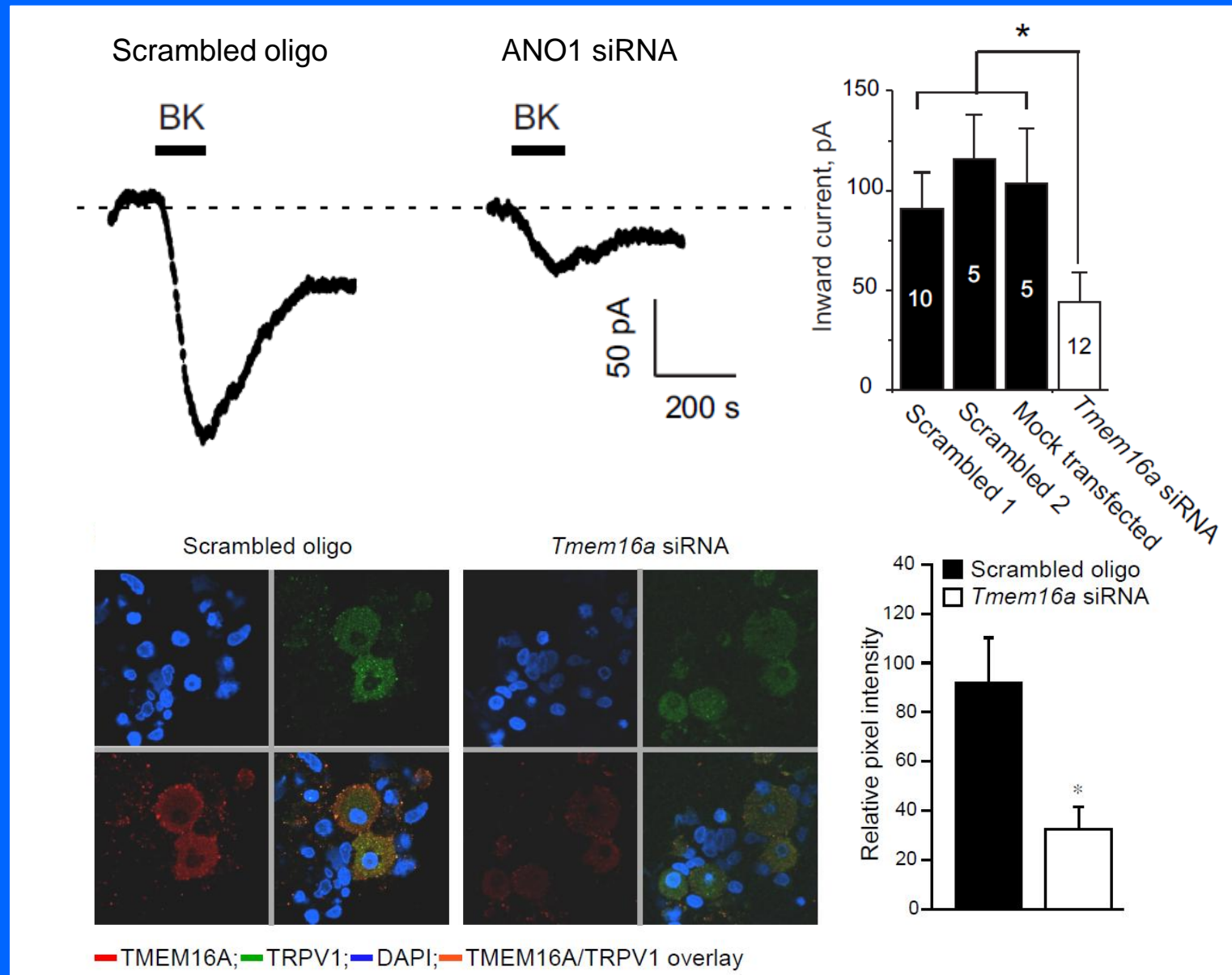


Linley et al. J Neurosci 2008  
Liu et al. J Clin Invest 2010  
Linley et al. PNAS 2012

# Inflammatory mediators activate $\text{Ca}^{2+}$ -activated $\text{Cl}^-$ channels (CaCC) in small DRG neurons

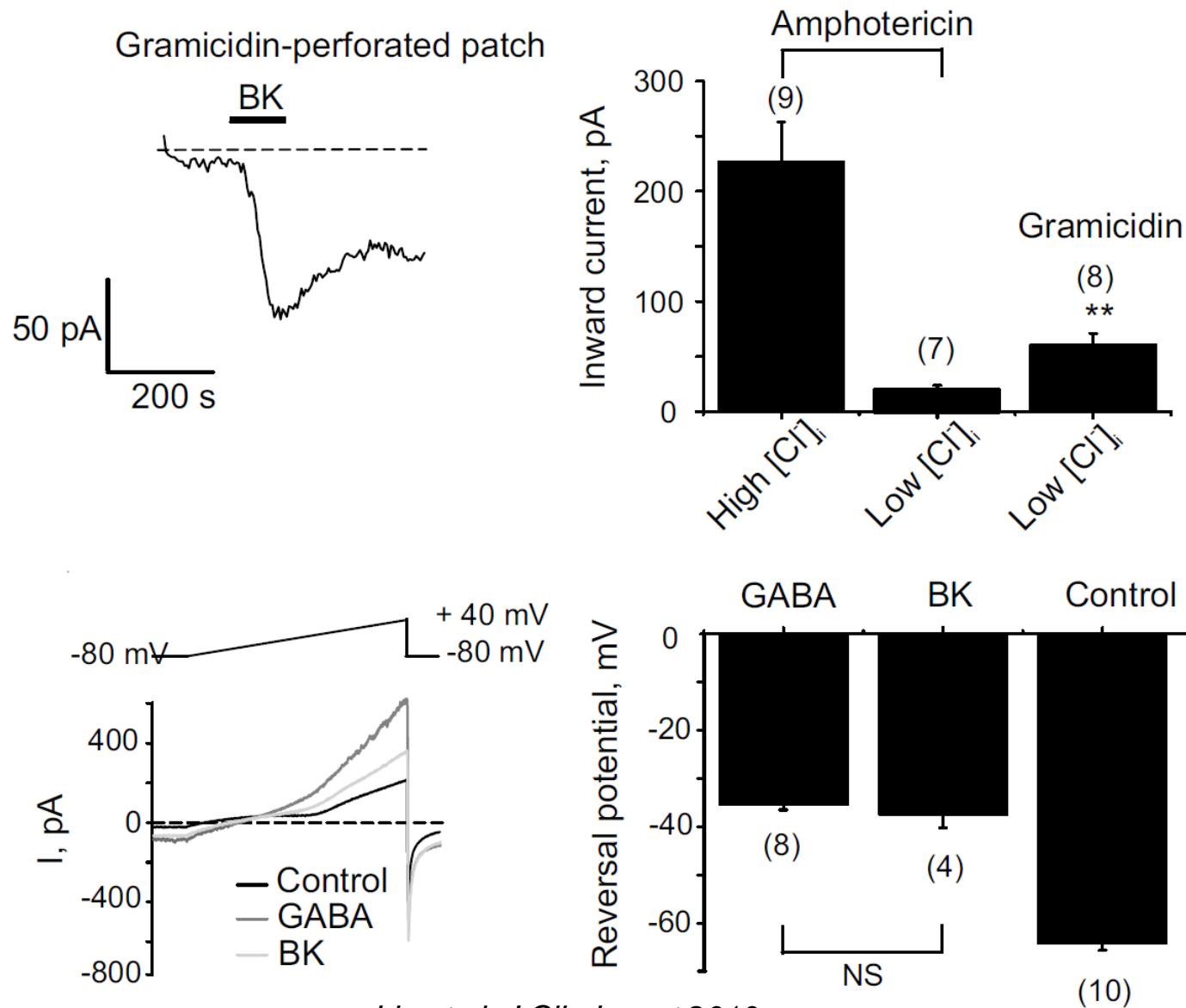


# siRNA ANO1 knock-down reduces BK-induced inward current

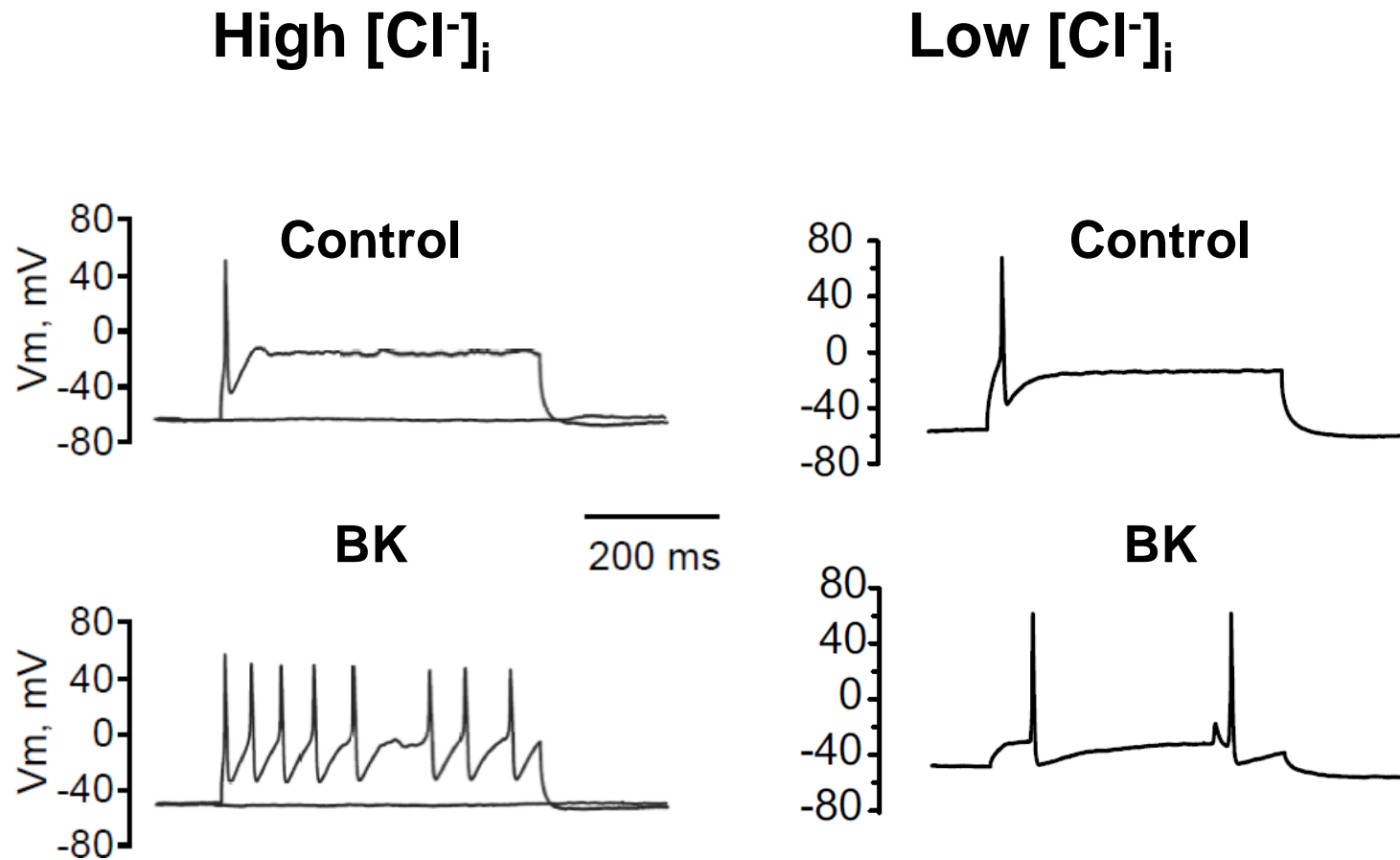




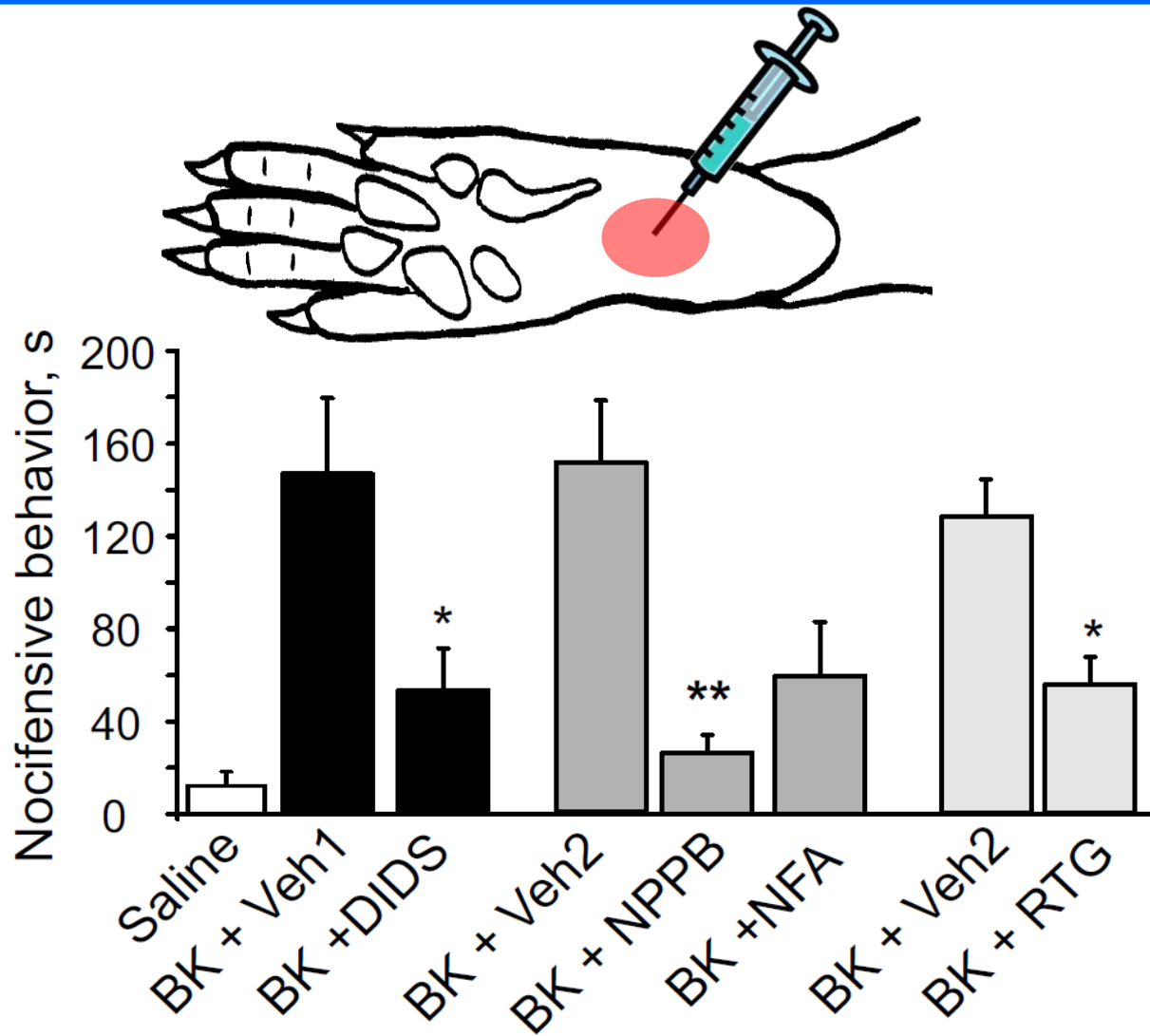
The intracellular  $\text{Cl}^-$  concentration in DRG neurons provides driving force for depolarizing  $\text{Cl}^-$  currents through  $\text{Cl}^-$  channels.



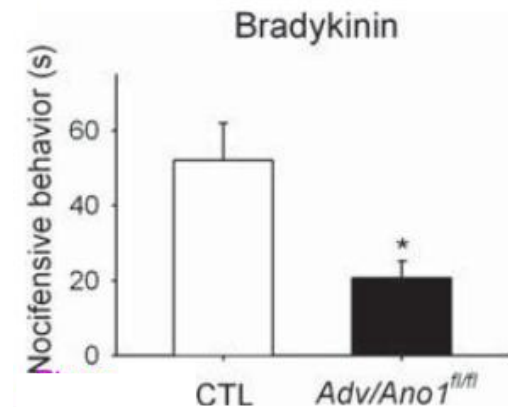
# CaCC contributes to the BK-induced excitation of DRG



# Pharmacological inhibition of CaCC in peripheral terminals of nociceptive fibres reduces BK-induced pain



Liu et al. J Clin Invest 2010

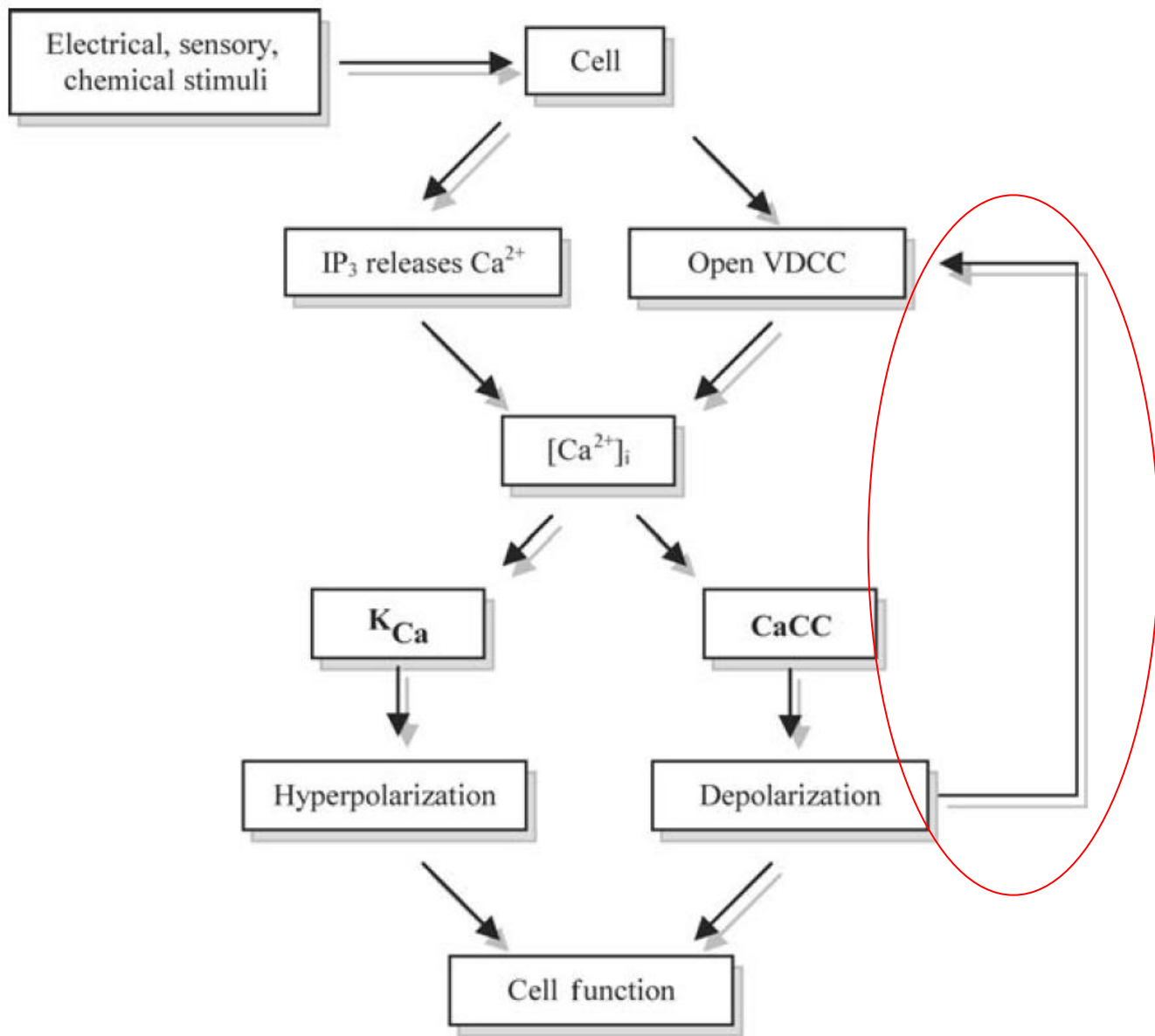


Lee et al Mol Pain 2014

## Conclusions thus far:

- ANO1 underlies CaCCs in small DRG neurons
- This channel is activated by  $\text{Ca}^{2+}$  release from the ER and this activation has an excitatory effect which may result in pain

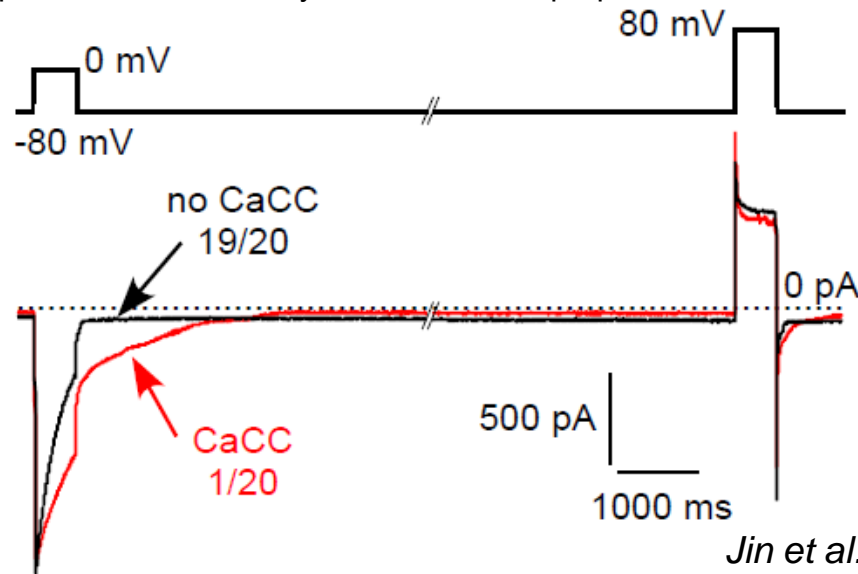
# Does it matter where $\text{Ca}^{2+}$ is coming from?





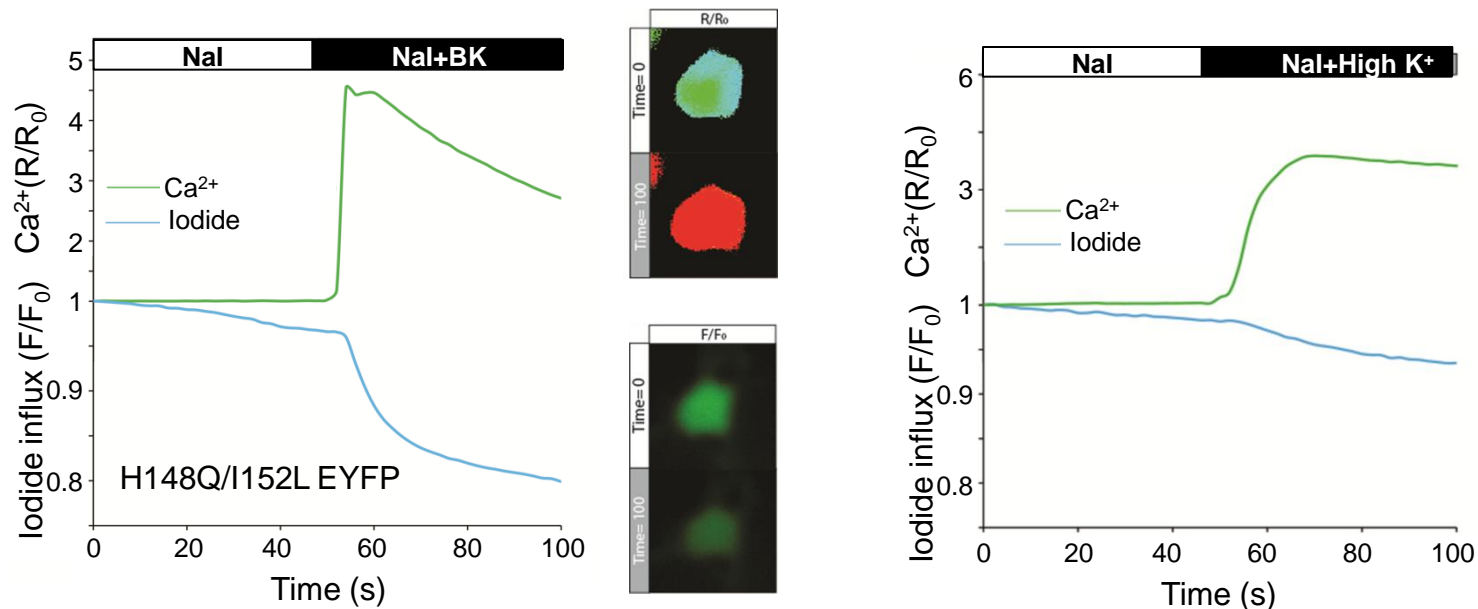
# CaCC couple **poorly** to VGCC in small DRG neurons

VGCC-coupled 'tail' CaCC is only seen in a minor proportion of small DRG neurons



*Jin et al. Science Signaling 2013*

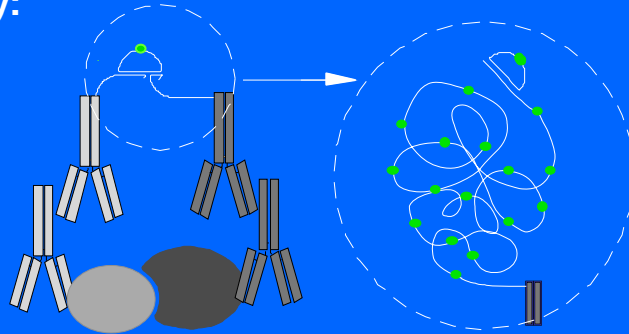
Depolarization with High- $K^+$  extracellular solution also activates CaCC poorly as compared to BK



*Shab and Gamper, unpublished*

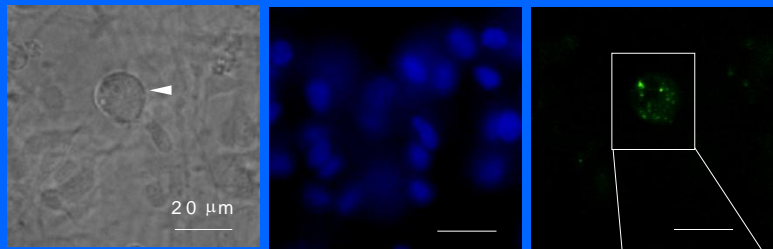
# ANO1 is in close proximity to the IP<sub>3</sub> receptors

## Proximity ligation assay:

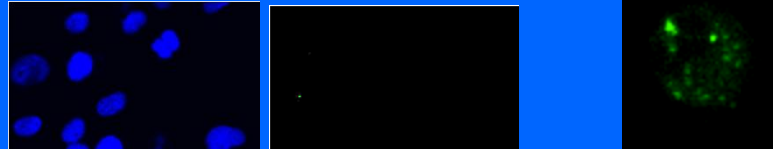


## HUVECs transfected With ANO1 and GFP:

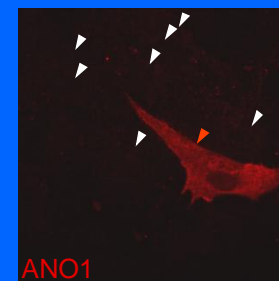
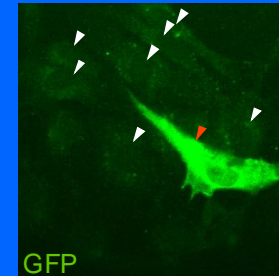
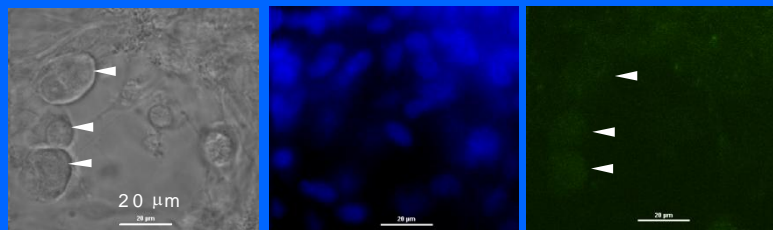
DRG: ANO1/IP<sub>3</sub>R1



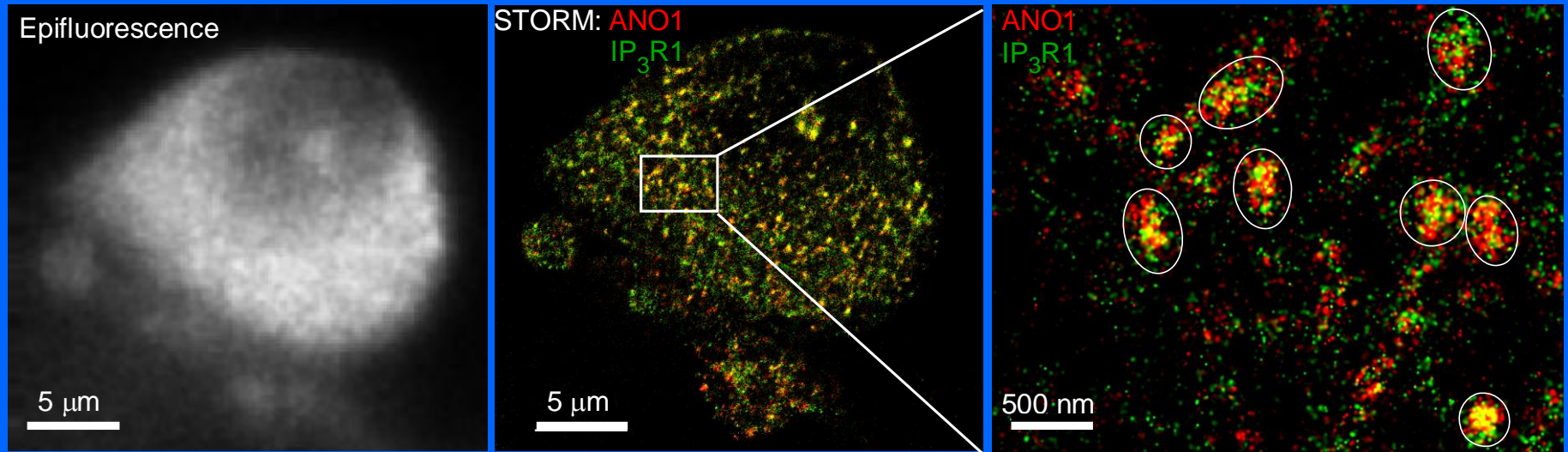
HUVEC: ANO1/IP<sub>3</sub>R1



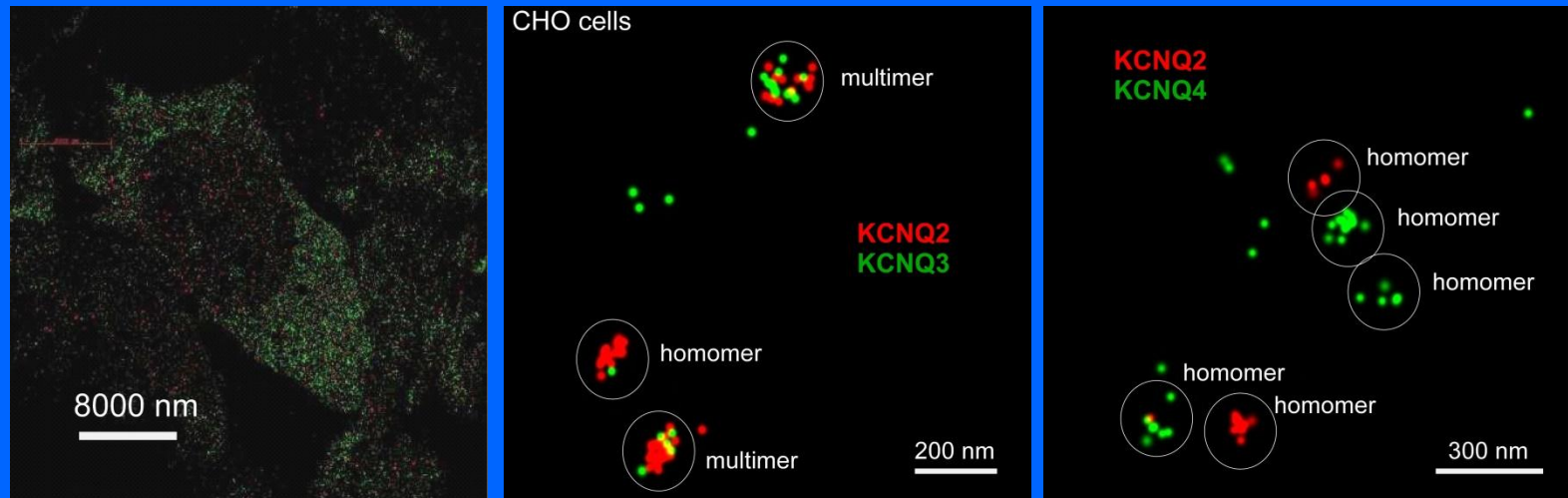
DRG: ANO1/panVGCC



# STORM microscopy reveals close proximity of ANO1 and the IP<sub>3</sub>R1 in DRG neurons

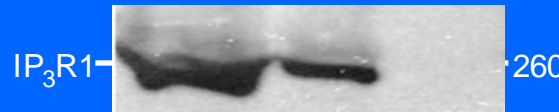
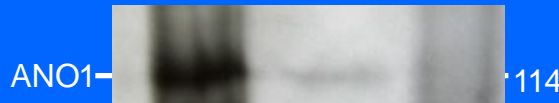


## Control experiments:

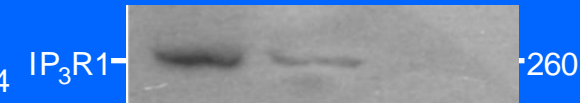


# ANO1 co-immunoprecipitates with the IP<sub>3</sub>R in DRG

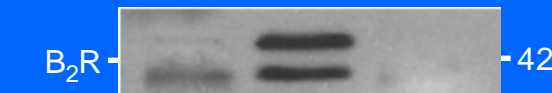
Sample:	co-IP	Lysate	co-IP
WB: IP:	IP <sub>3</sub> R1	—	IgG



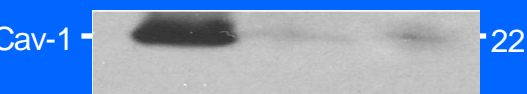
Sample:	co-IP	Lysate	co-IP
WB: IP:	ANO1	—	IgG



Sample:	co-IP	Lysate	co-IP
WB: IP:	IP <sub>3</sub> R1	—	IgG

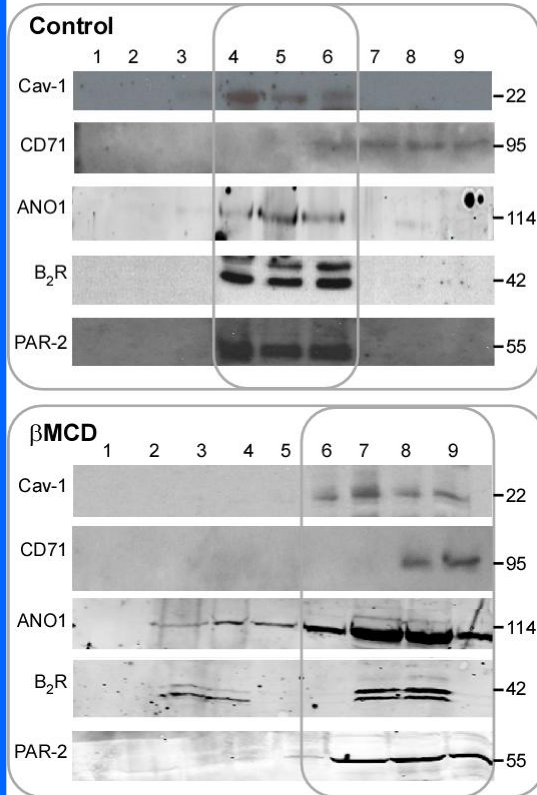


Sample:	co-IP	Lysate	co-IP
WB: IP:	Cav-1	—	IgG

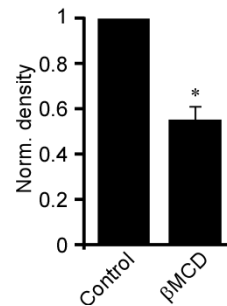
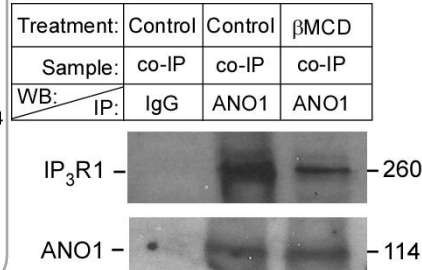


# ANO1-containing signaling complexes are localized to lipid rafts

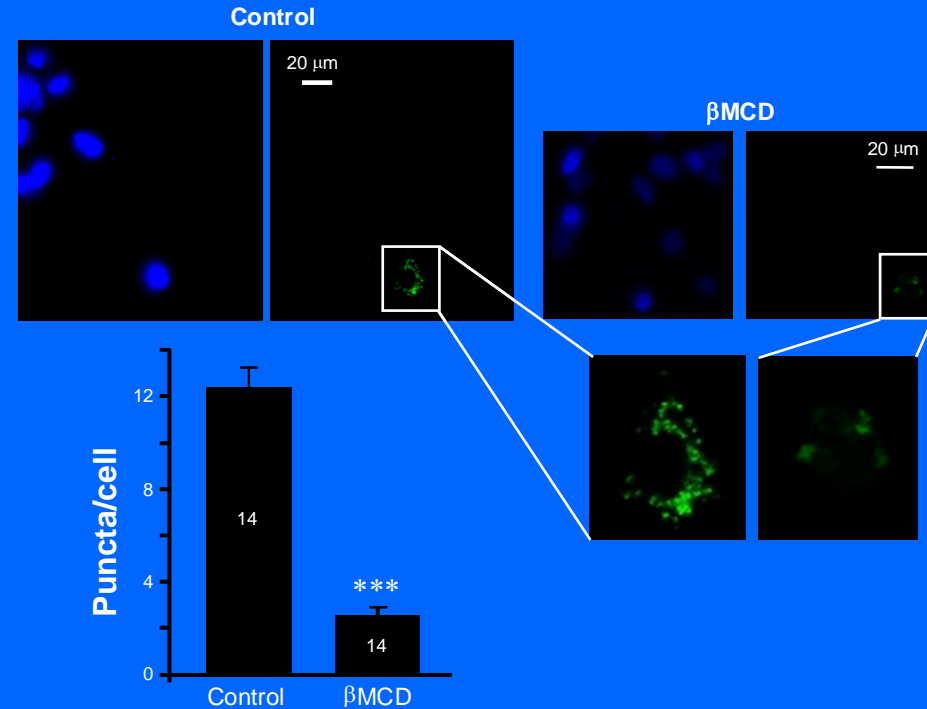
## Sucrose gradient centrifugation:



## Co-IP:



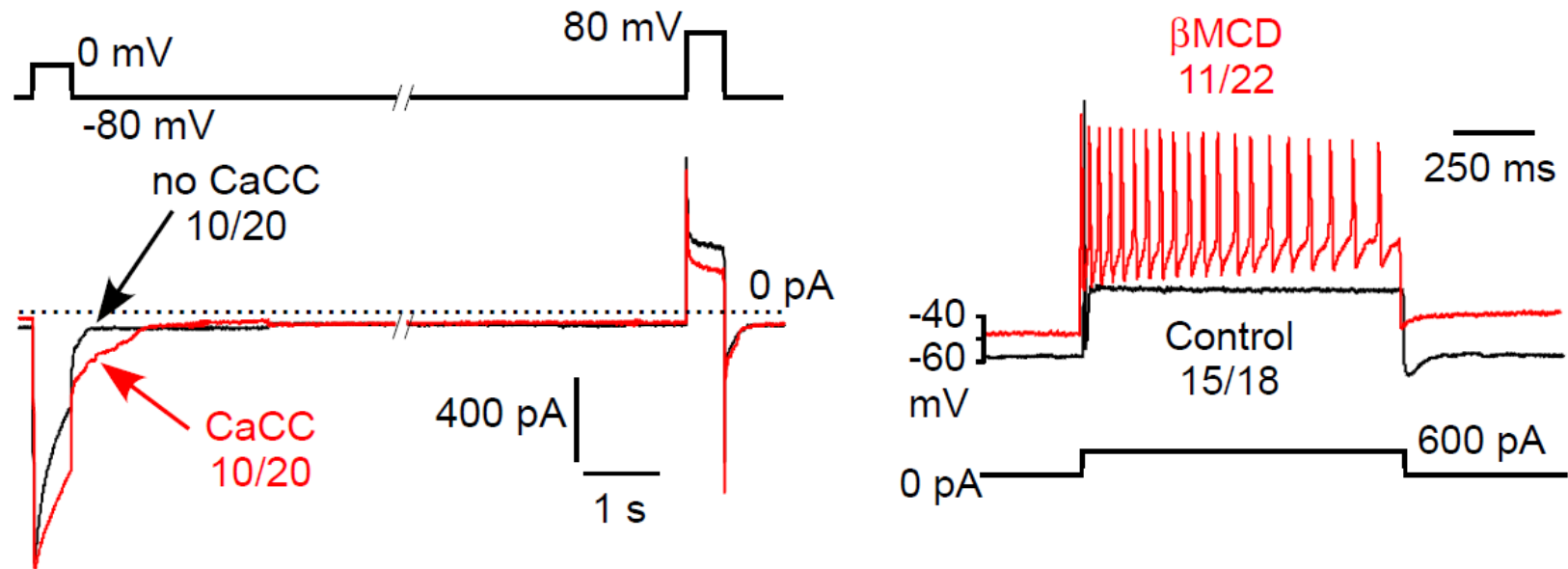
## PLA assay:



Cholesterol extraction (βMCD)  
destroys these complexes



# Disruption of the ANO1-containing signaling complex results in overexcitable neurons

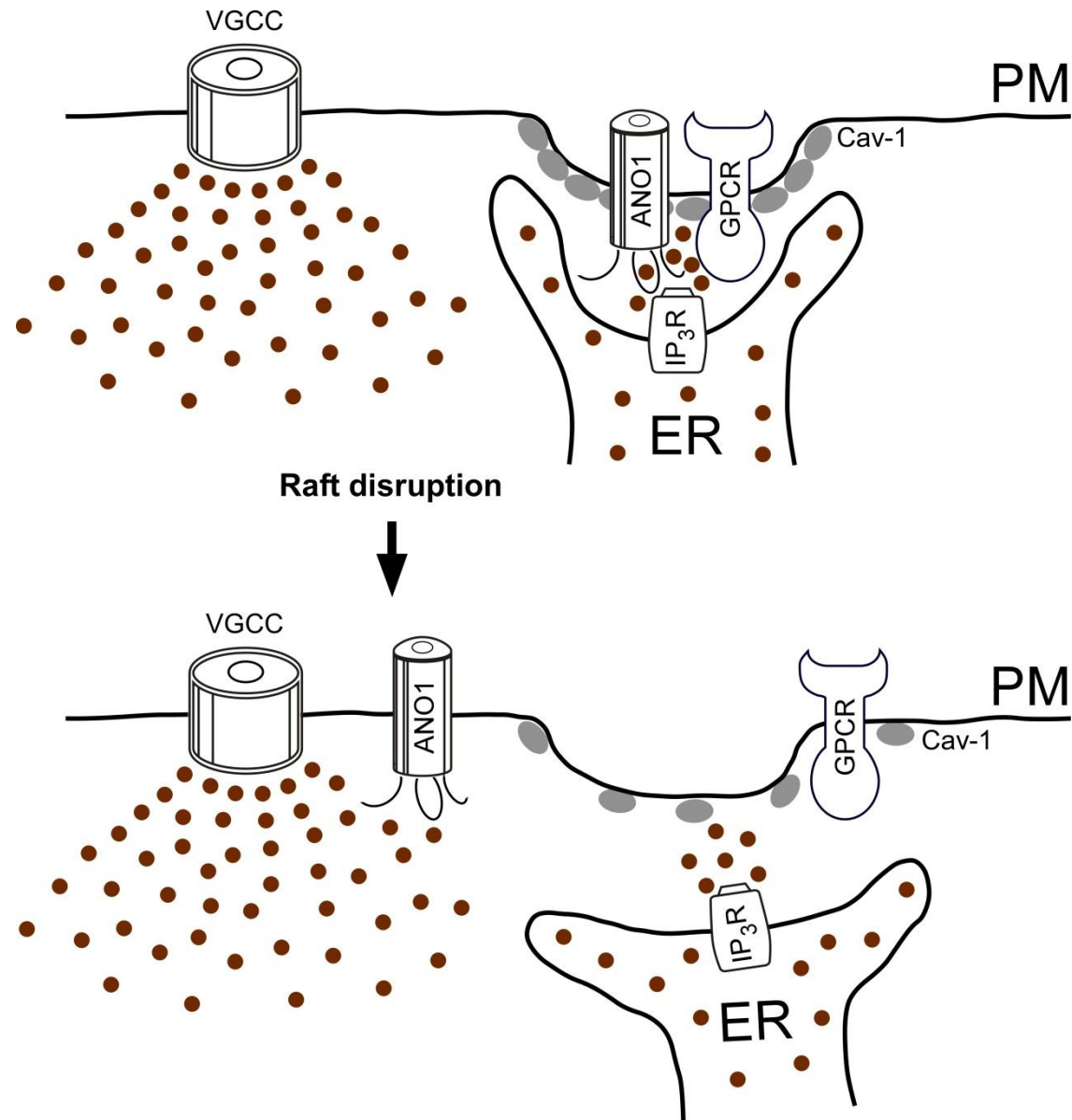


# Summary of the $\beta$ MCD experiments

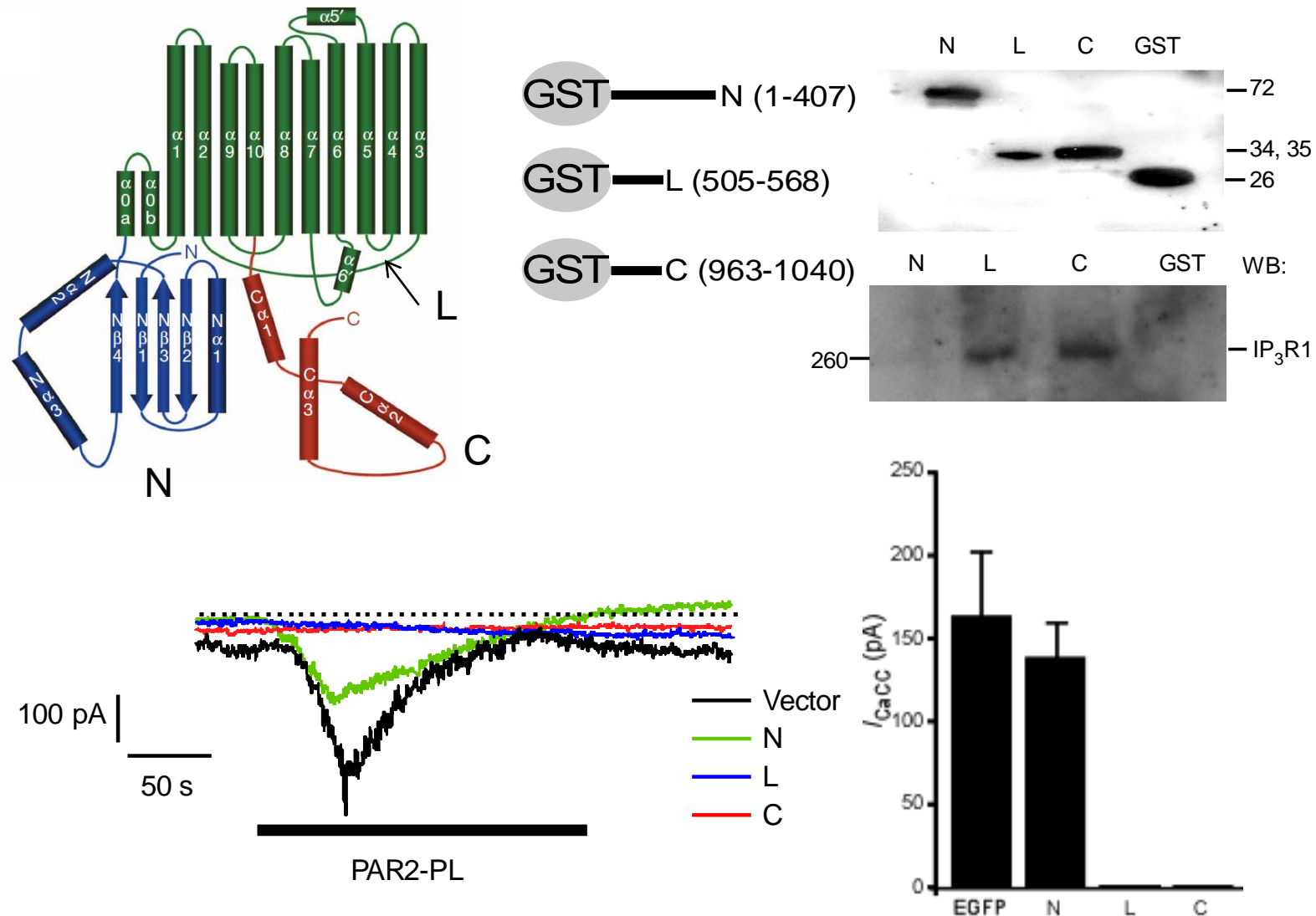
	Control	$\beta$ MCD	$\alpha$ CD
GPCR-induced cytosolic $\text{Ca}^{2+}$ transients.....	YES	YES	
GPCR-induced CaCC current.....	YES	NO	YES
CaCC tail current after VGCC activation.....	NO	YES	NO
'Overexcitable' neuron (multiple action potential firing).....	NO	YES	NO

But if no  $[\text{Cl}]_i$ , than NO

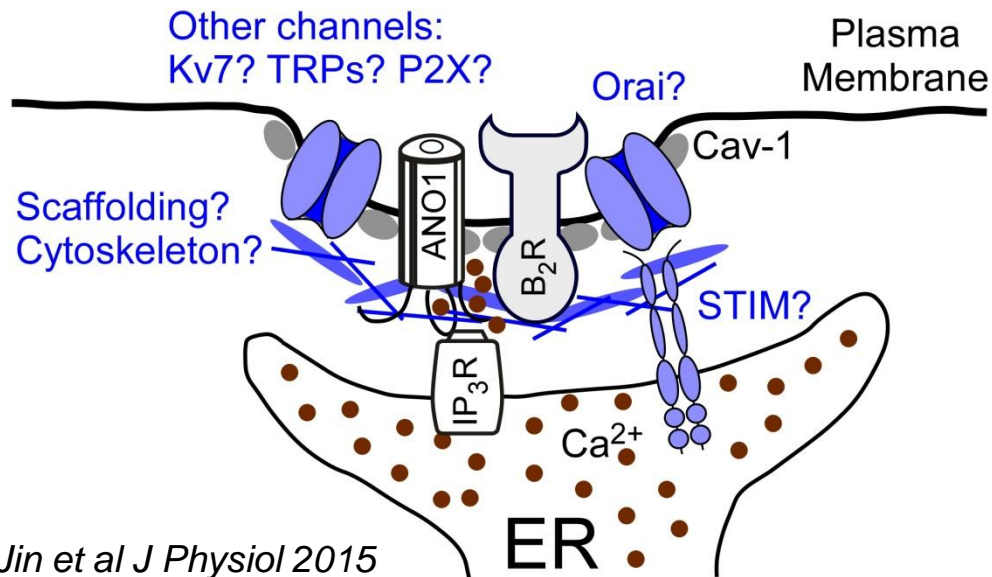
# Some ideas on possible mechanism



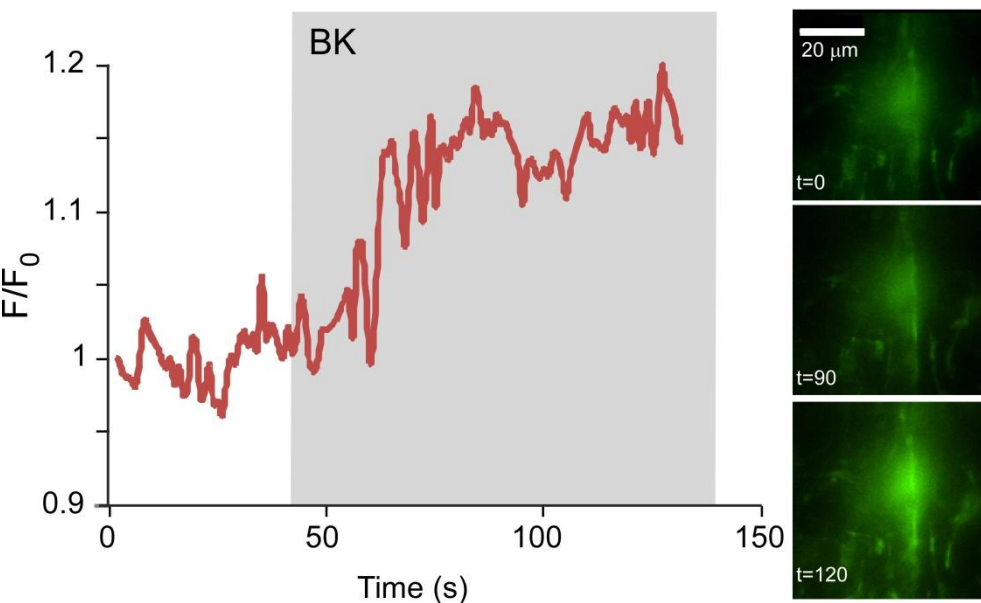
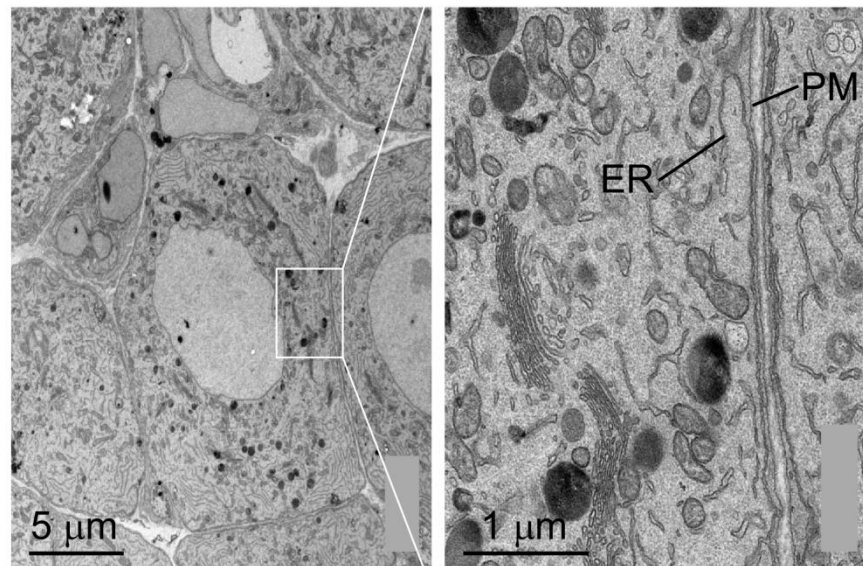
# Defining structural background of ANO1-IP<sub>3</sub>R1 interaction in DRG neurons



# Functional PM-ER microdomains: current concept & future questions



ER-PM junctions in DRG



- **What is the molecular composition of the junctional multiprotein complexes in sensory neurons?**
- **Are these complexes dynamics or static?**
- **What is a functional significance of the complex assembly (vs. its components functioning separately)?**



## **Gamper's lab (Leeds)**

**John Linley**

**Xin Jin**

**Lezanne Ooi**

**Shihab Shah**

**Kirsty Rose**

**Louisa Pettinger**

**Hannah Kirton**

**Katarzyna Marszalek**

**Ewa Jaworska**

**Aurelian Bolliat**

**Haixia Gao**

**Alexandra Gerghina**

## **UTHSCSA, San Antonio**

**Mark Shapiro**

**Jie Zhang**

## **Leeds collaborators:**

**Steve Baldwin**

**Rao Sivaprasadarao**

**Jon Lippiat**

**David Beech**

## **Hebei Medical University, China**

**Hailin Zhang**

**Boyi Liu**

**Liu Yani**

**Huiran Zhang**

**Dongyang Huang**

**Xiaona Du**

**Han Hao**

**Zhanfeng Jia**

# Pharmaceutical and Biopharmaceutical Innovation Hub



UNIVERSITY OF LEEDS

## Pharmaceuticals and Biopharmaceuticals Innovation Hub

[HOME](#) [FOR COMPANIES](#) [FOR ACADEMICS](#) [RESEARCH PRIORITIES](#) [PEOPLE](#)

You are here: University of Leeds > Pharma Hub > [Home](#)

### Search site

Search:

[News and events](#) ▶

[Who we work with](#) ▶

[Case studies](#) ▶

[Contact us](#) ▶

[Targeted Skin Therapeutics - Building Partnerships](#) ▶

[2015 Leeds Ion Channels workshop 6-11 Sept](#) ▶

### Useful links

- ▶ [The Astbury Centre](#)
- ▶ [Multidisciplinary Cardiovascular Research Centre](#)
- ▶ [Stratified Medicine hub](#)
- ▶ [Medicinal Chemistry and Chemical Biology group](#)
- ▶ [BioScreening group](#)
- ▶ [School of Chemistry](#)
- ▶ [Faculty of Biological Sciences](#)
- ▶ [School of Physics and Astronomy](#)

## Pharma Hub - your gateway to innovation



### Gold nanotubes able to detect and destroy cancer

Scientists have shown that gold nanotubes have many applications in fighting cancer: internal nanoprobes for high-resolution imaging; drug delivery vehicles; and agents for destroying cancer cells.



### Lead-oriented synthesis

Chemistry research at Leeds, funded by EPSRC and in collaboration with GSK, has developed a novel synthetic approach which generated compounds as starting materials for drug discovery which have the properties required by the pharmaceutical industry and could lead to new breakthroughs in drug discovery.

Despite huge investment and changes in practices over the years, the rate of drug discovery has not increased and the cost of bringing effective drugs to market remains very high.

The Pharmaceutical and Biopharmaceutical innovation hub brings together expertise in a range of physical and biological sciences to pioneer innovative approaches to drug discovery. We work with pharmaceutical and biopharmaceutical companies, as well as supporting industries, to help the sector discover safe new medicines faster.

The scale of the University's activities and state of the

### Tweets

[Follow](#)



**KTN Biosciences** @KTN\_Bioscience 22h  
£34m funding available from Industrial Biotechnology Catalyst - Round 4 [bit.ly/1defl1u](https://bit.ly/1defl1u)

Retweeted by Pharma & Biopharma

Forward



[www.pharmahub.leeds.ac.uk](http://www.pharmahub.leeds.ac.uk)



[pharmahub@leeds.ac.uk](mailto:pharmahub@leeds.ac.uk)



[@PharmaLeeds](#)



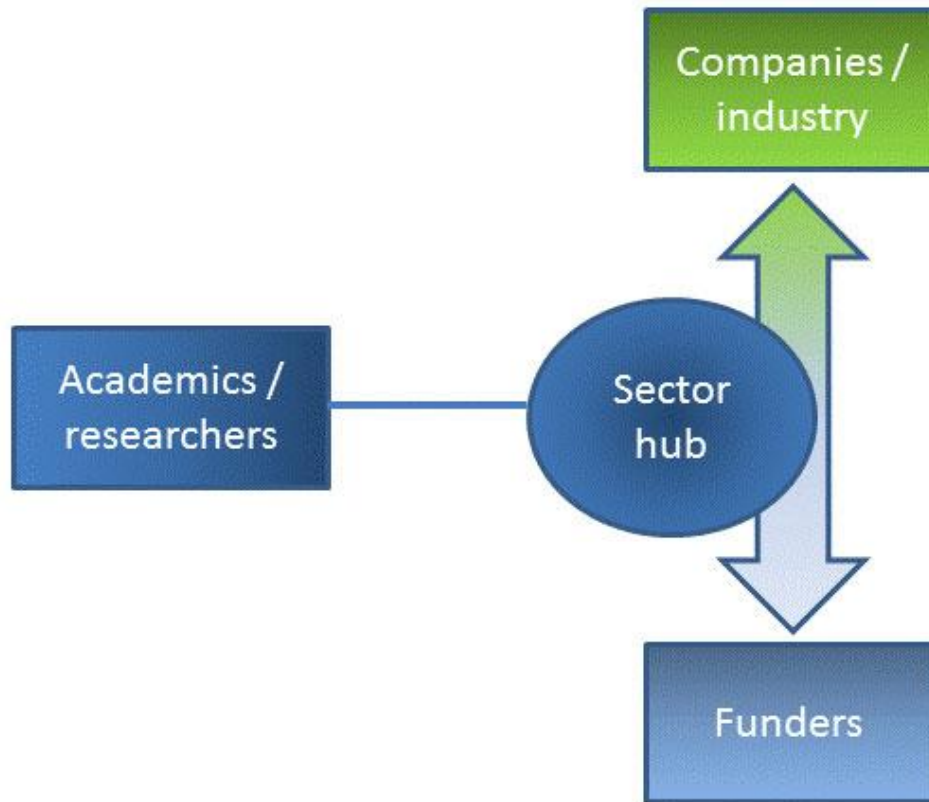
Pharmaceutical and Biopharmaceutical Innovation Hub Group

Director: Prof Adam Nelson  
Innovation Manager: Dr Kate Langton

# Pharmaceutical and Biopharmaceutical Innovation Hub



UNIVERSITY OF LEEDS



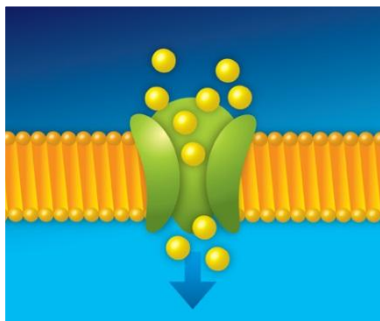
*Funding*

*Commercialisation  
strategy*

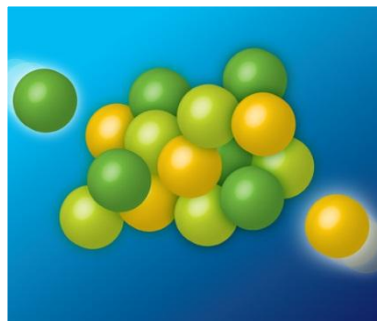
*Links to industry*

## Approach: Research Themes

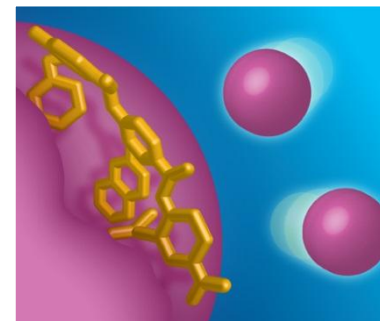
Ion Channels Research



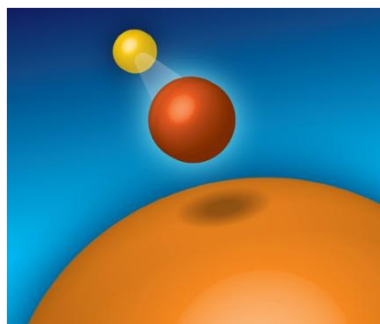
Protein Aggregation



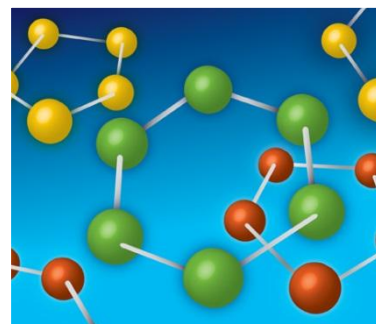
Protein-Protein Interactions



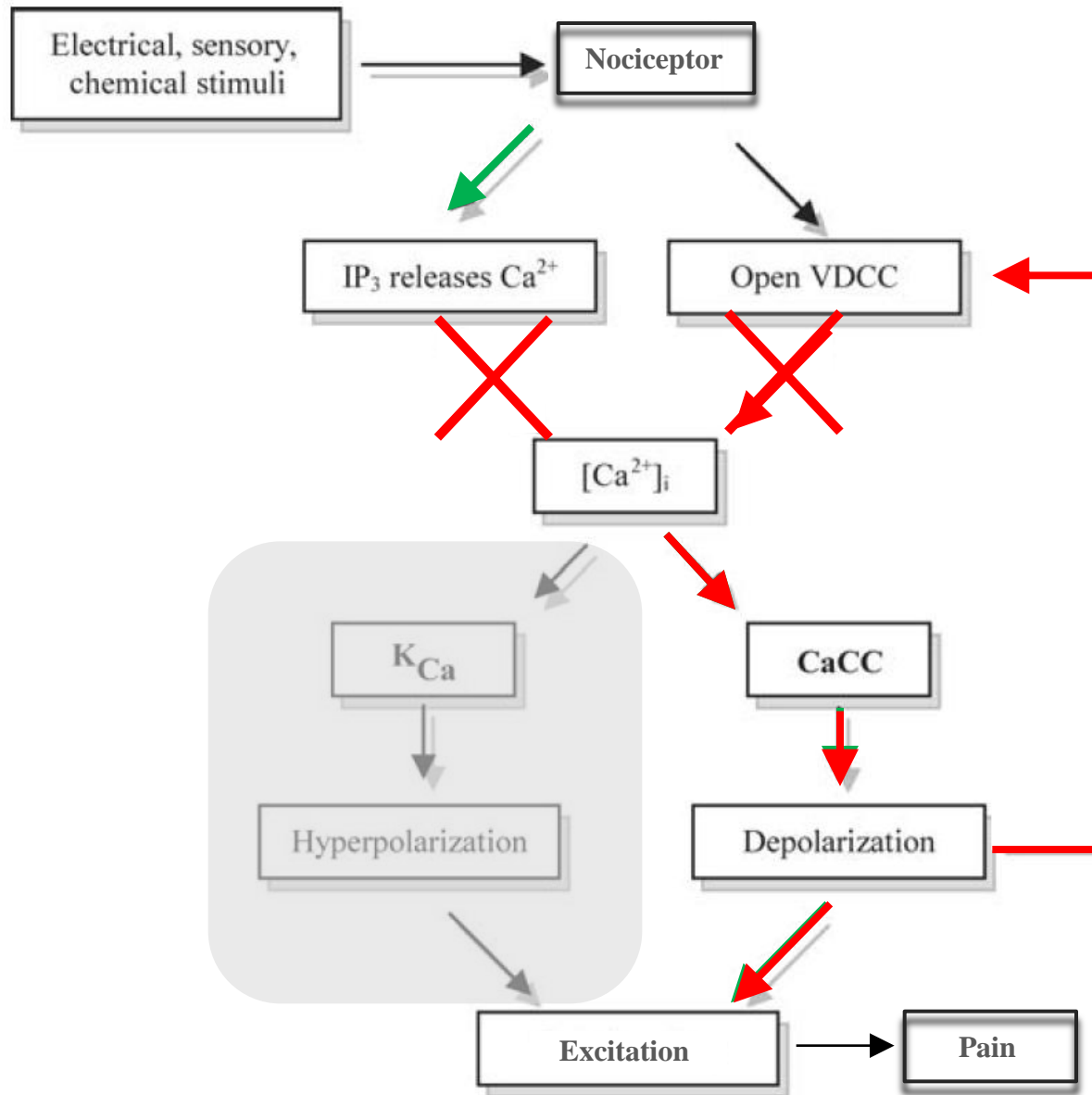
Targeted Drug Delivery



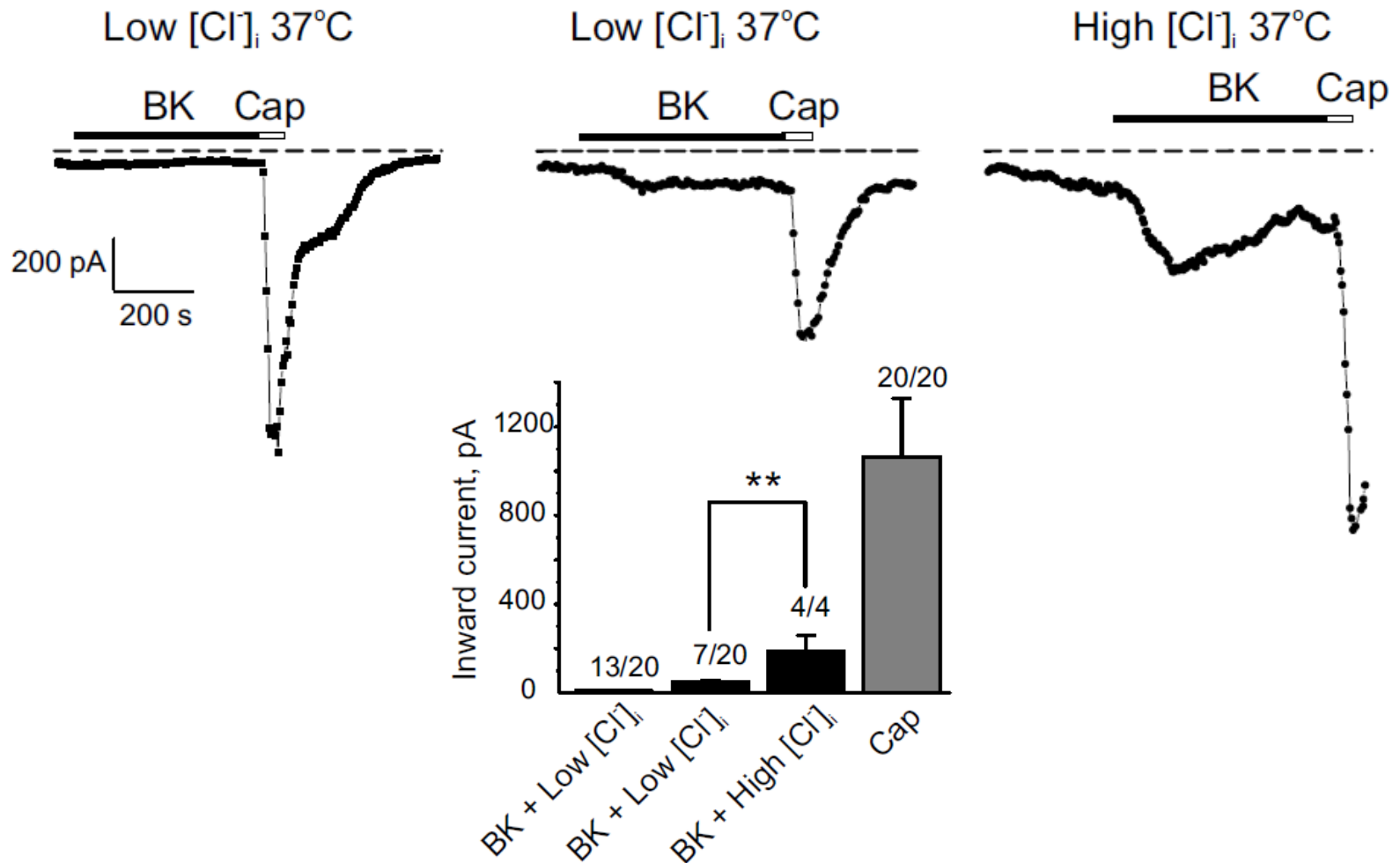
Translational Drug Discovery



# Reworked scheme of regulation of membrane potential by CaCC in sensory neurons



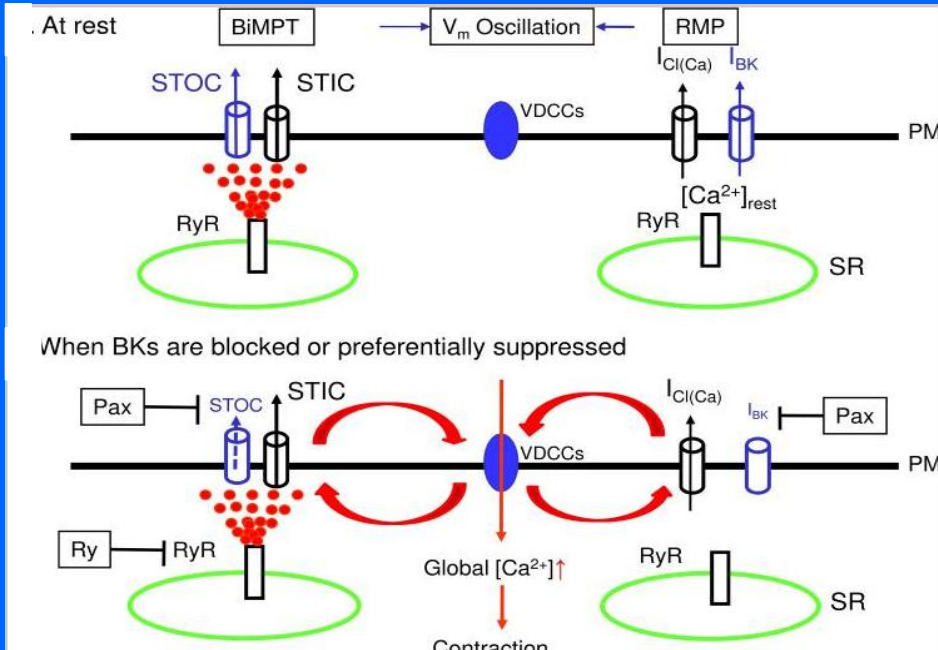
# CaCC is a dominant BK-induced inward current even at 37°C



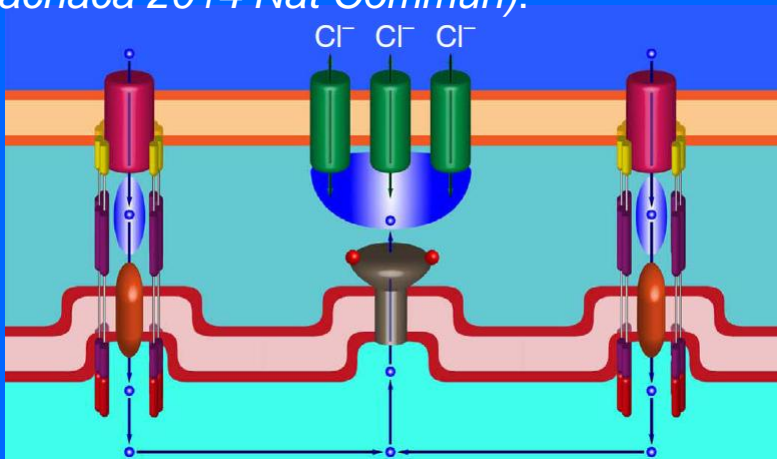


# Similar arrangements between ANO1/CaCC and intracellular $\text{Ca}^{2+}$ were proposed in other tissues

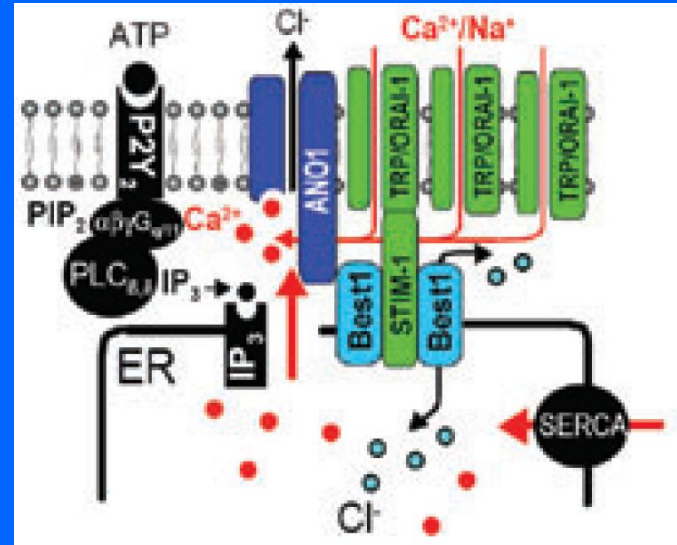
Smooth muscle (*Zhuge et al. 2010 JBC*):



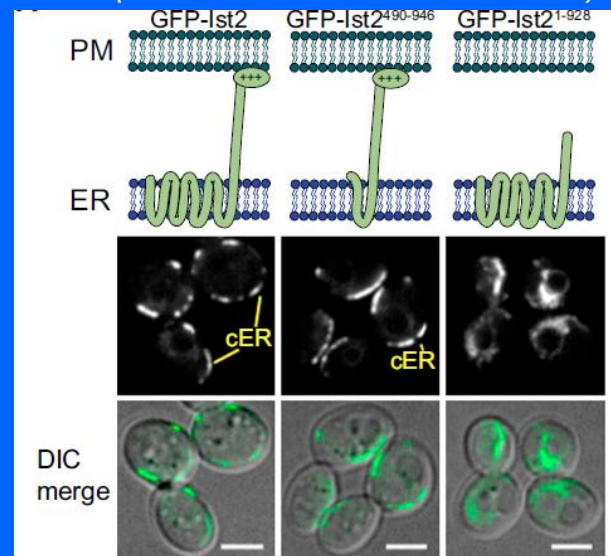
Xenopus oocytes (*Courjaret & Machaca 2014 Nat Commun*):



HEK293 cells (*Kunzelmann et al. 2011 Exp Physiol*):

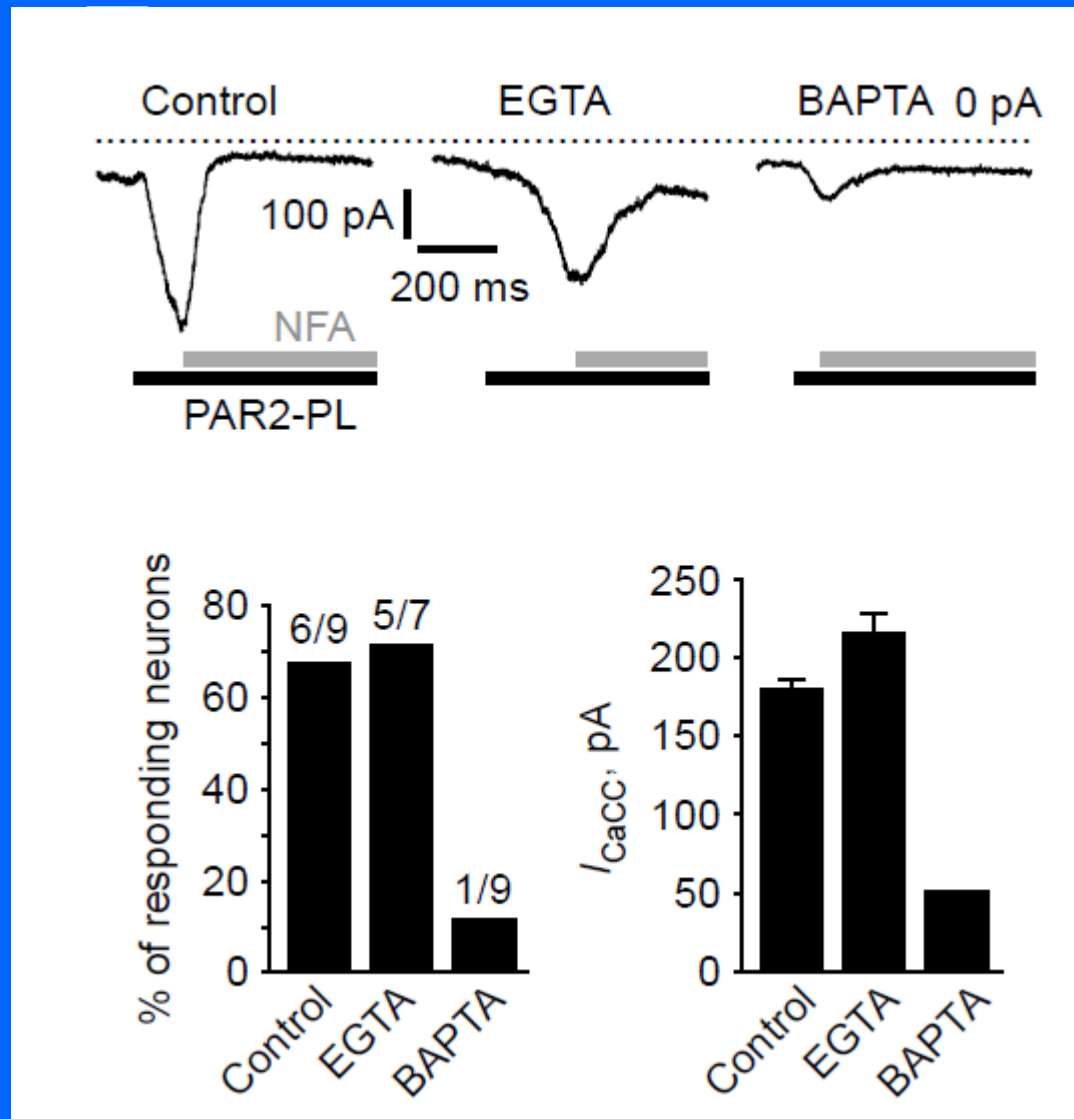


Yeast (*Manford et al 2012 Dev Cell*):





# CaCC are in close proximity to the IP<sub>3</sub> receptors



# TRP channels contribute little to the acute pain induced by BK

