School of Biomedical Sciences
FACULTY OF BIOLOGICAL SCIENCES
UNIVERSITY OF LEEDS

Ion Channel Retreat, Vancouver
July 9, 2015

Role of ANO1 (TMEM16A) channels in inflammatory pain

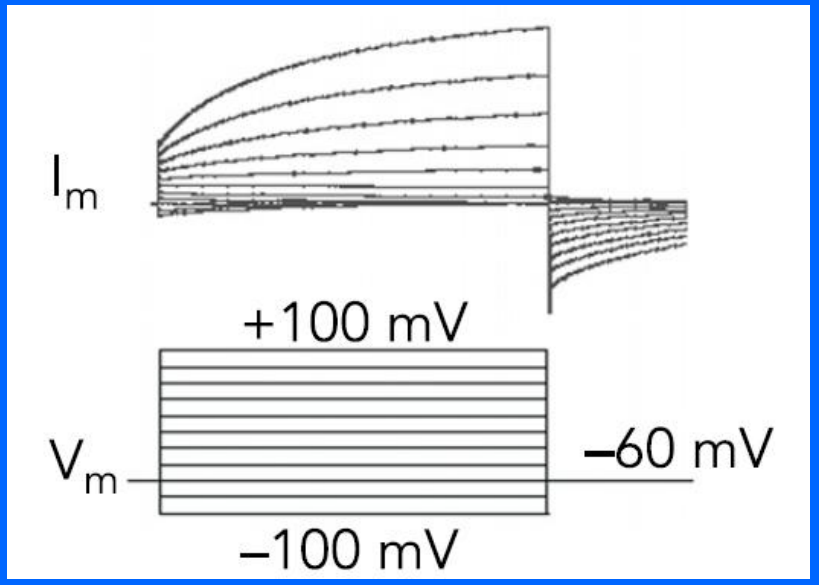
Nikita Gamper

Physiological roles of Ca^{2+}-activated 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 Ca^{2+}: low μM range
- $P_I > P_{Cl}$
- Voltage dependence with outward rectification
- Slow kinetics
- Sensitivity to NPPB, DIDS, NFA, FFA

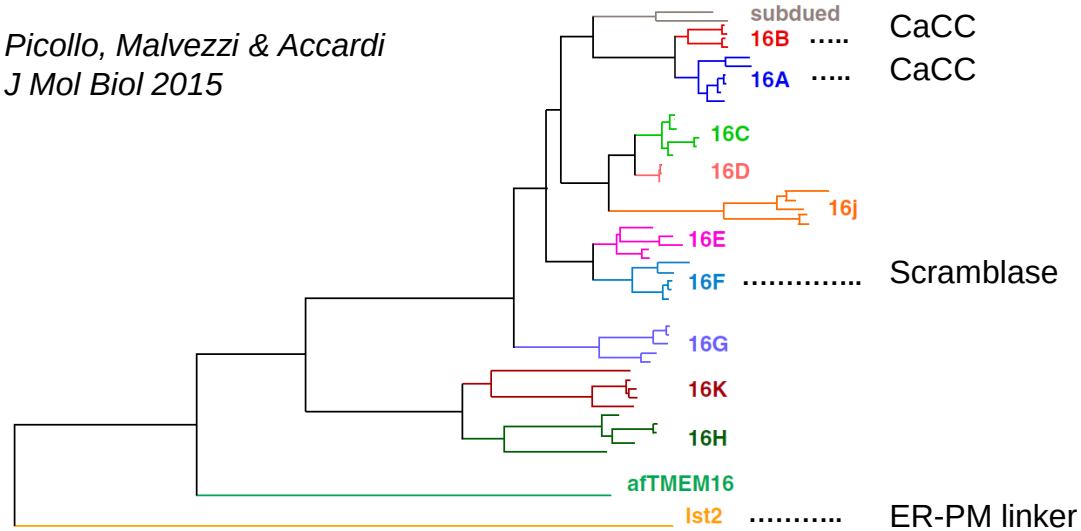


Molecular identity CaCC remained elusive for many years

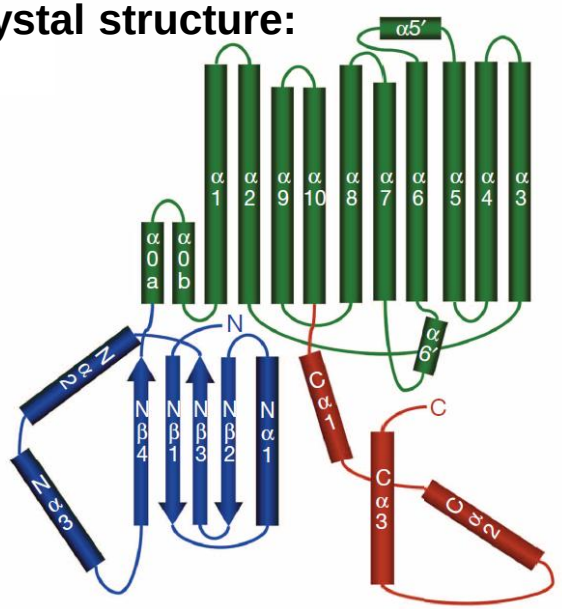
- CLCA?
- CLC3?
- Bestrophins?
- **ANO1/TMEM16A**

TMEM16 proteins in the spotlight

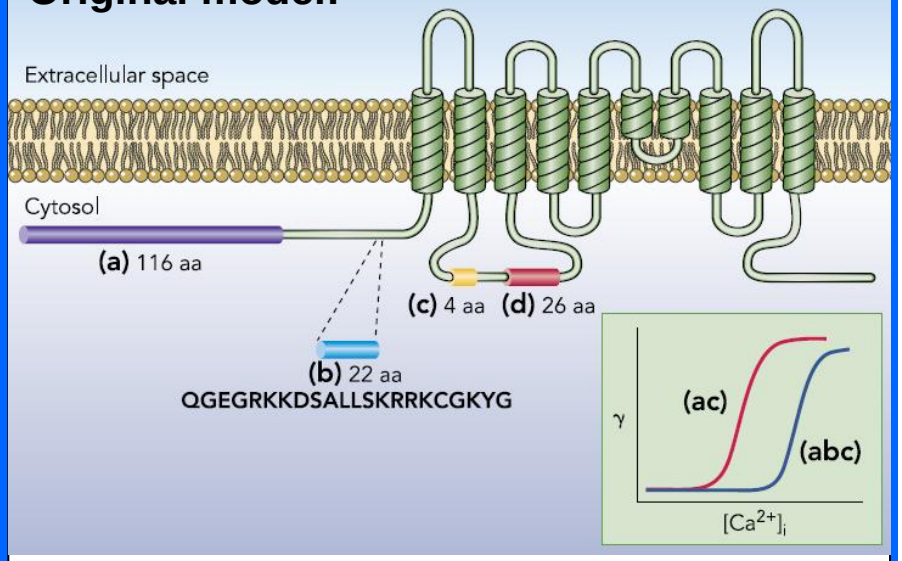
Picollo, Malvezzi & Accardi
J Mol Biol 2015



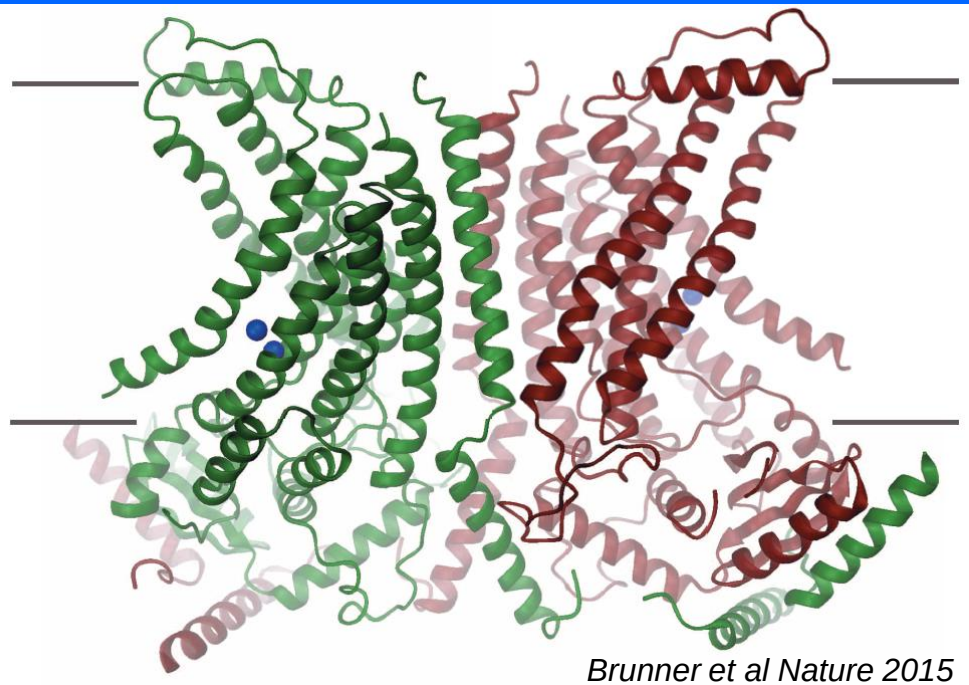
Crystal structure:



Original model:

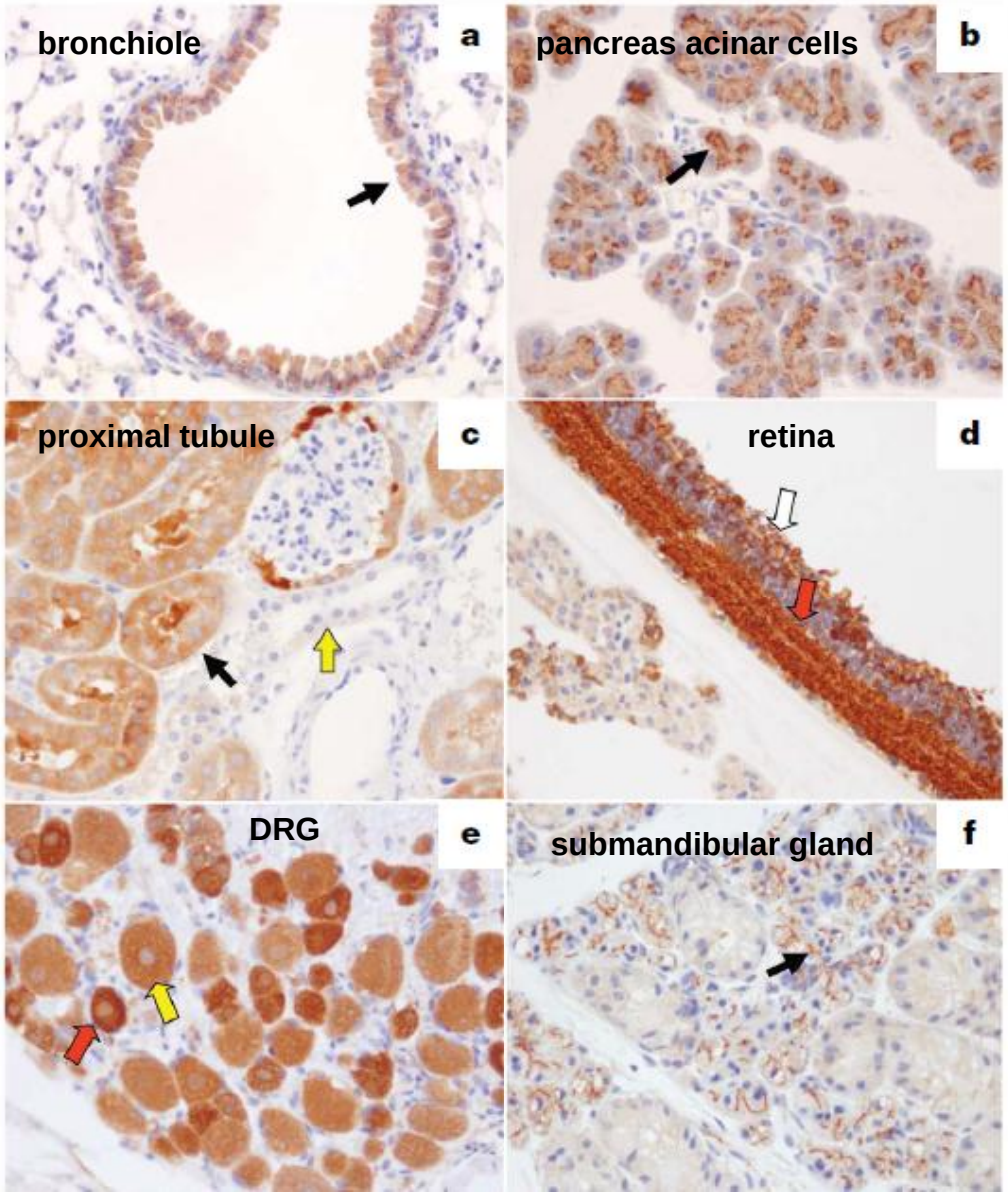


Ferrera et al. 2010 Physiology



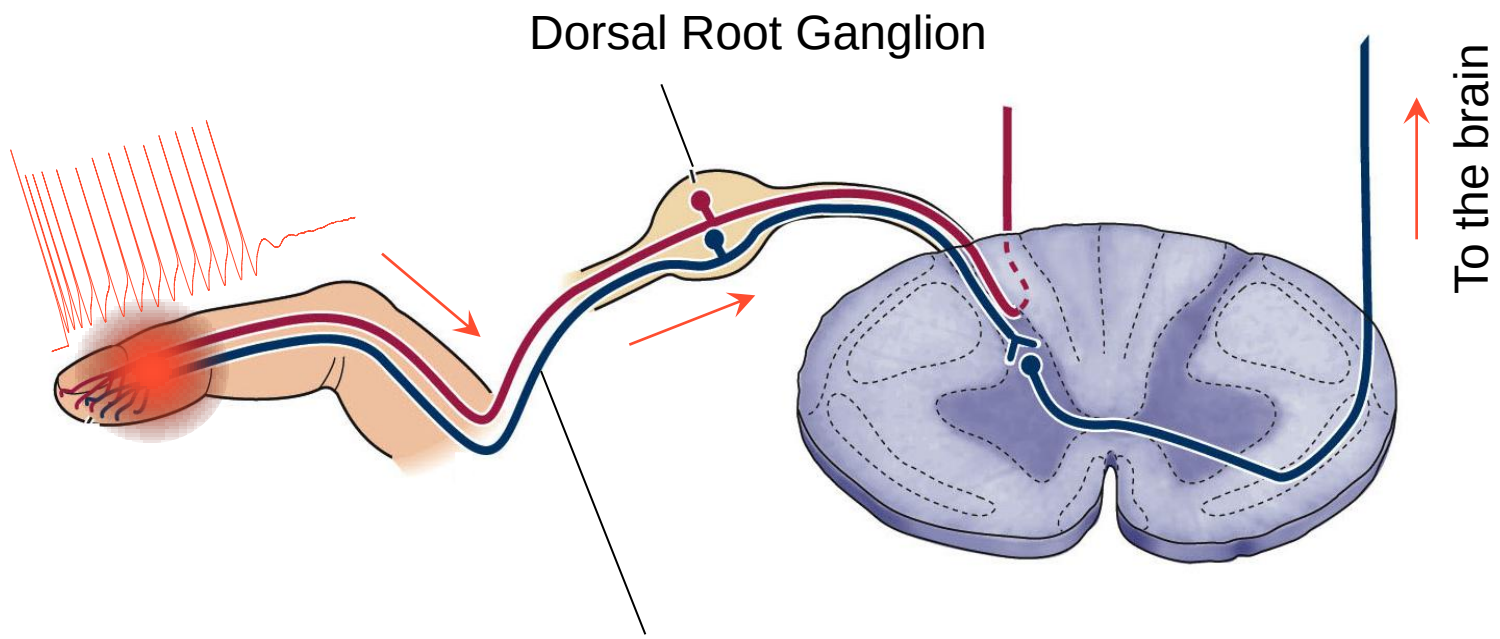
Brunner et al Nature 2015

Expression of TMEM16A various tissues

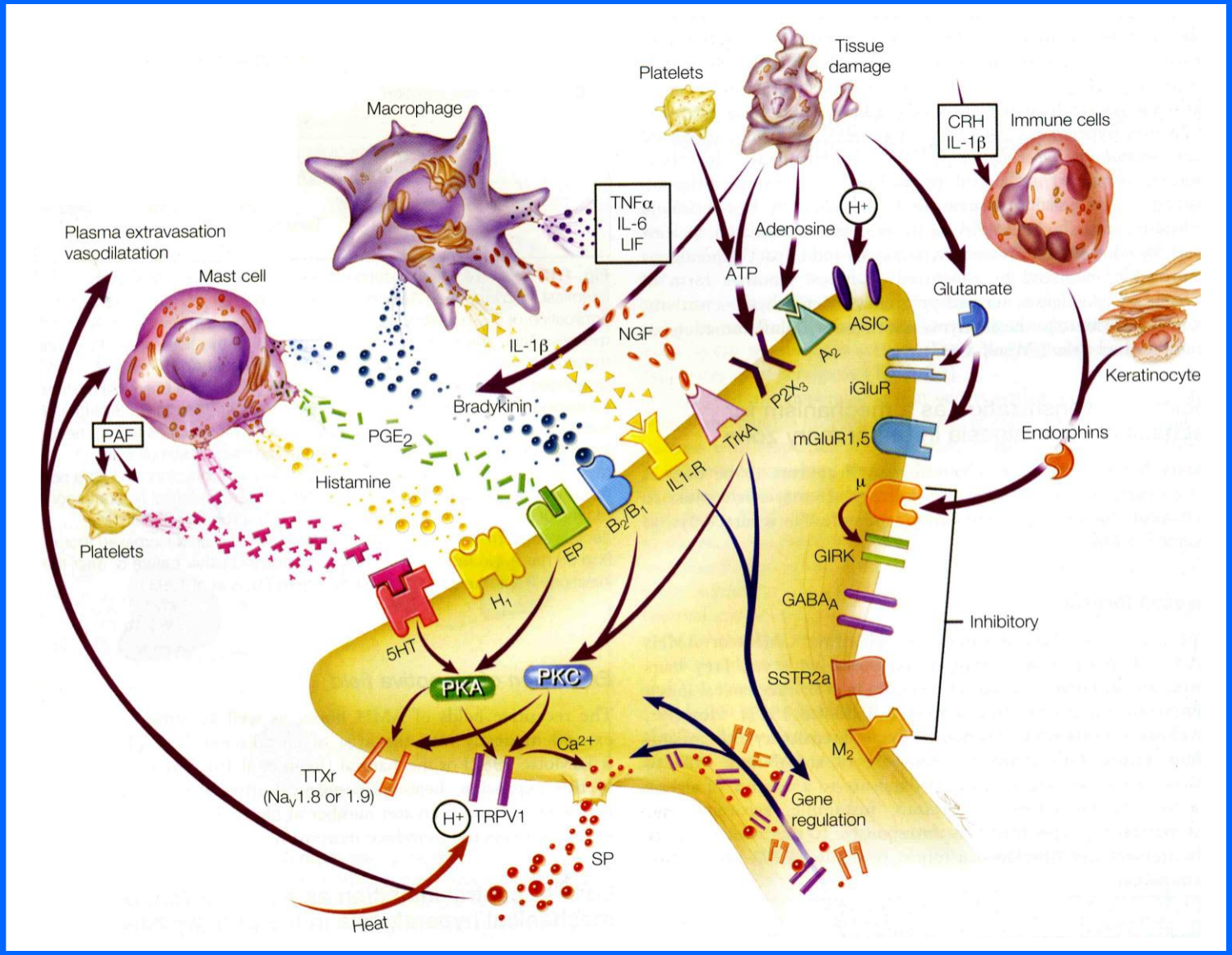


Yang et al 2008 Nature 455:1210-5

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

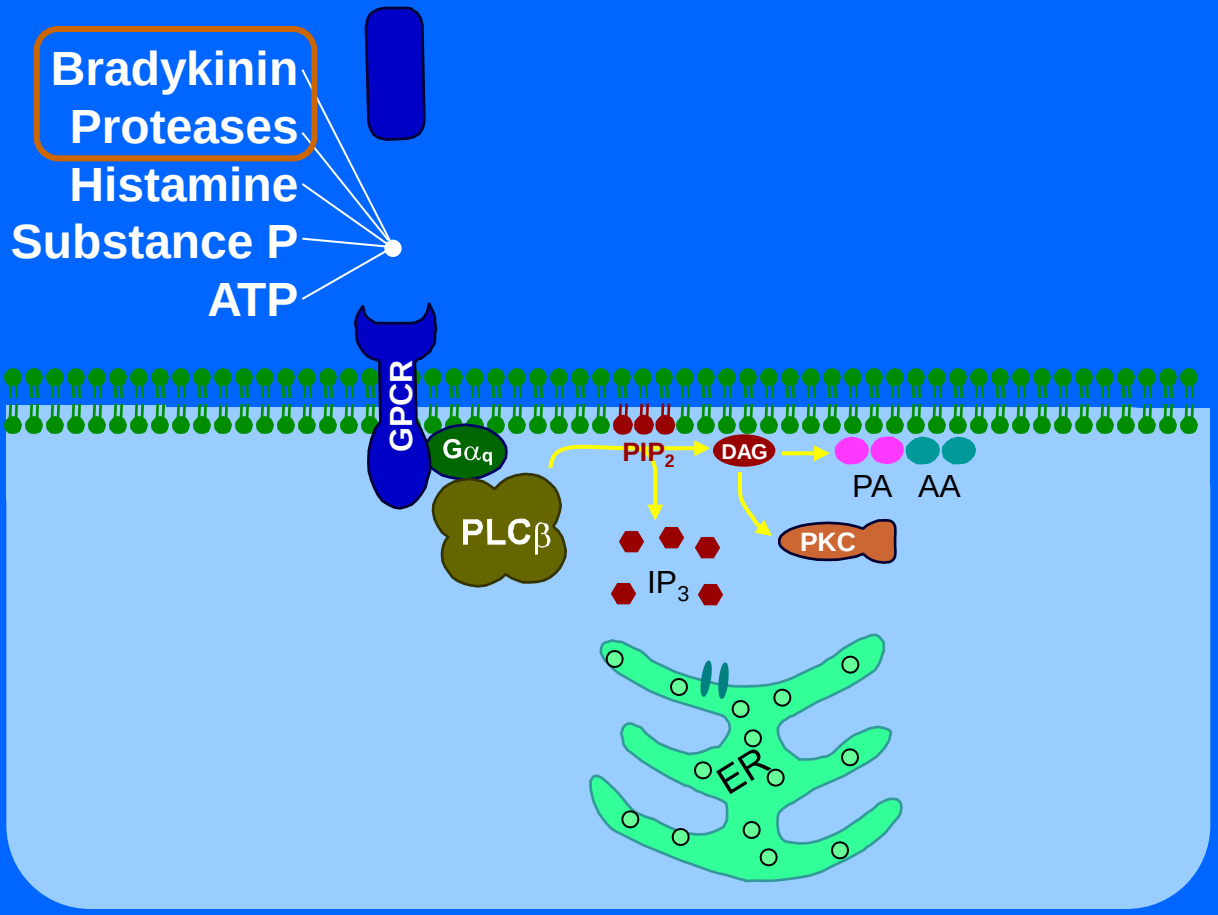


Many inflammatory mediators excite sensory neurons through G protein coupled receptors

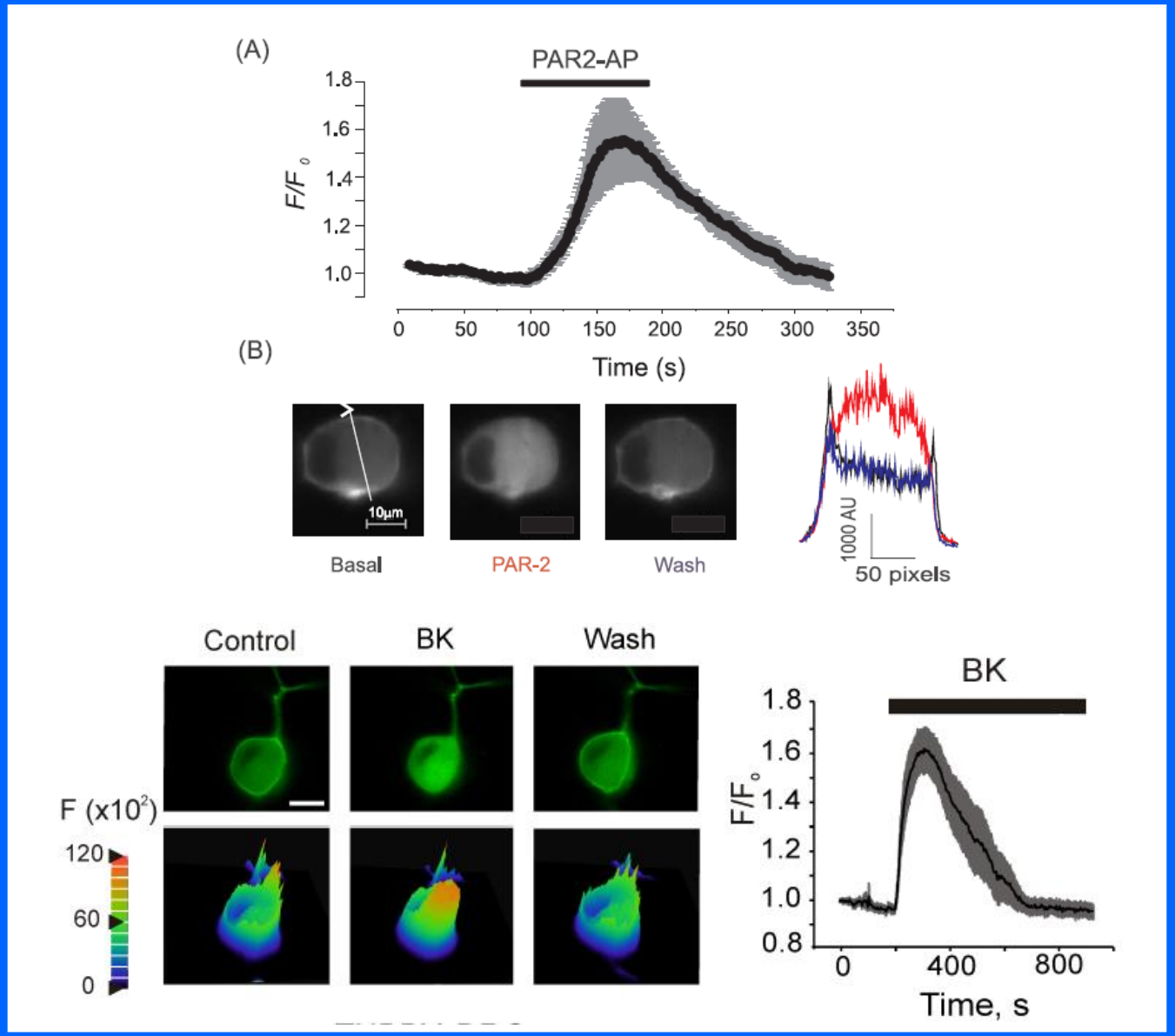


From Wall and Melzack's Textbook of Pain

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

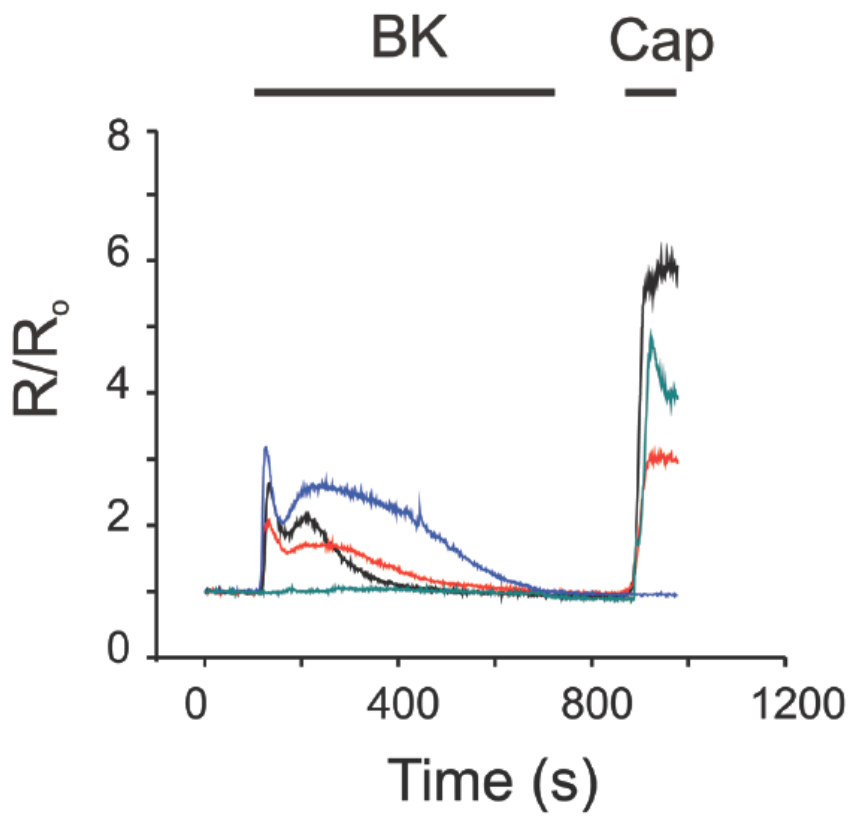


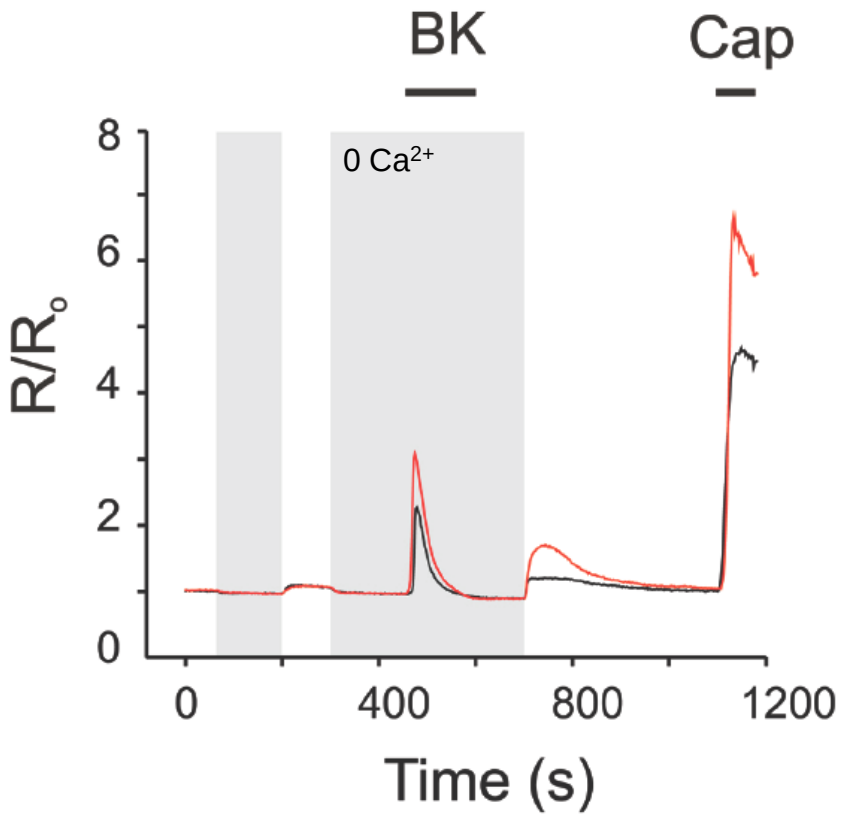
Both BK and PAR2-AP induces strong activation of PLC in DRG



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

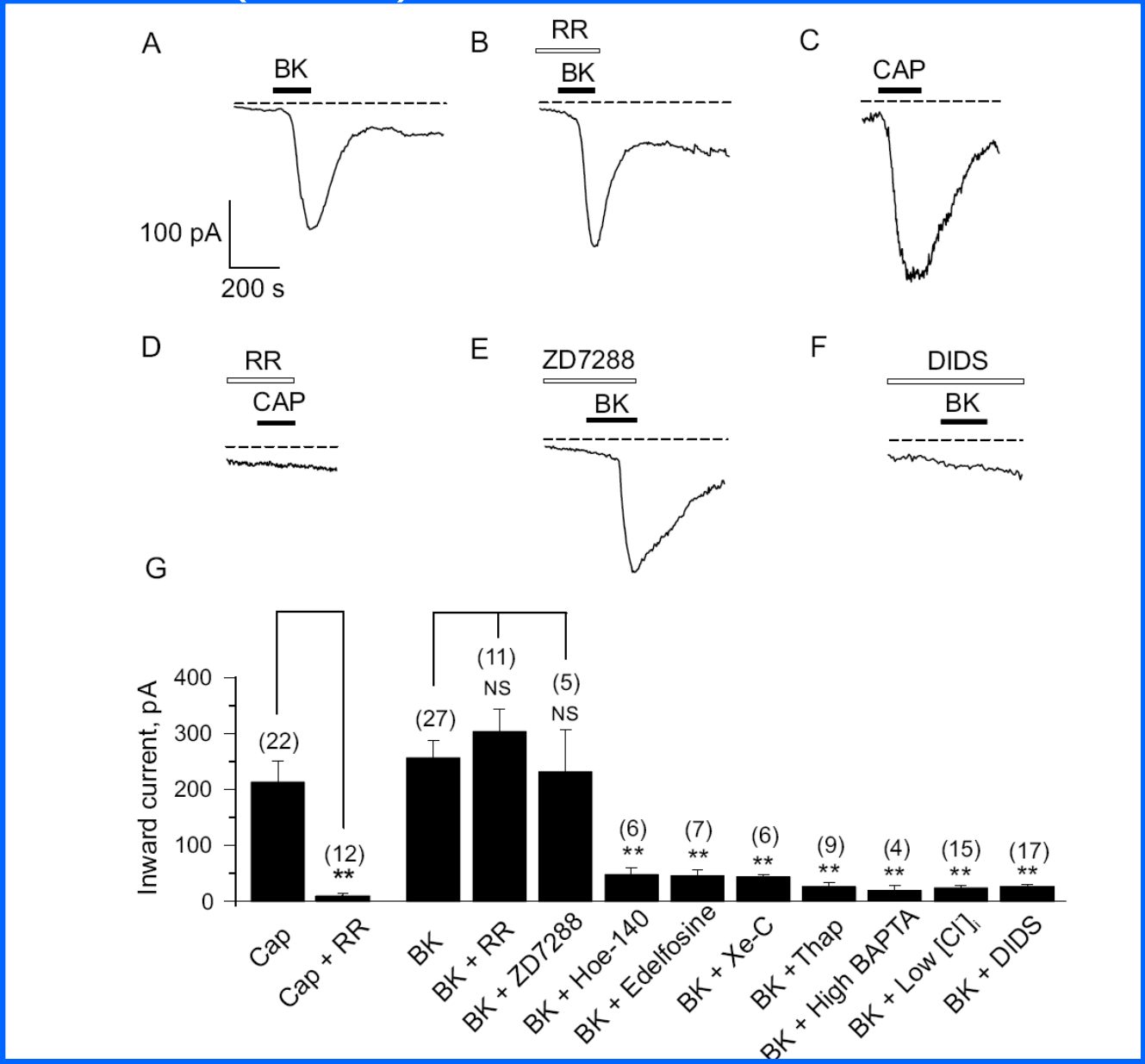
Inflammatory mediators induce Ca^{2+} release from the ER stores





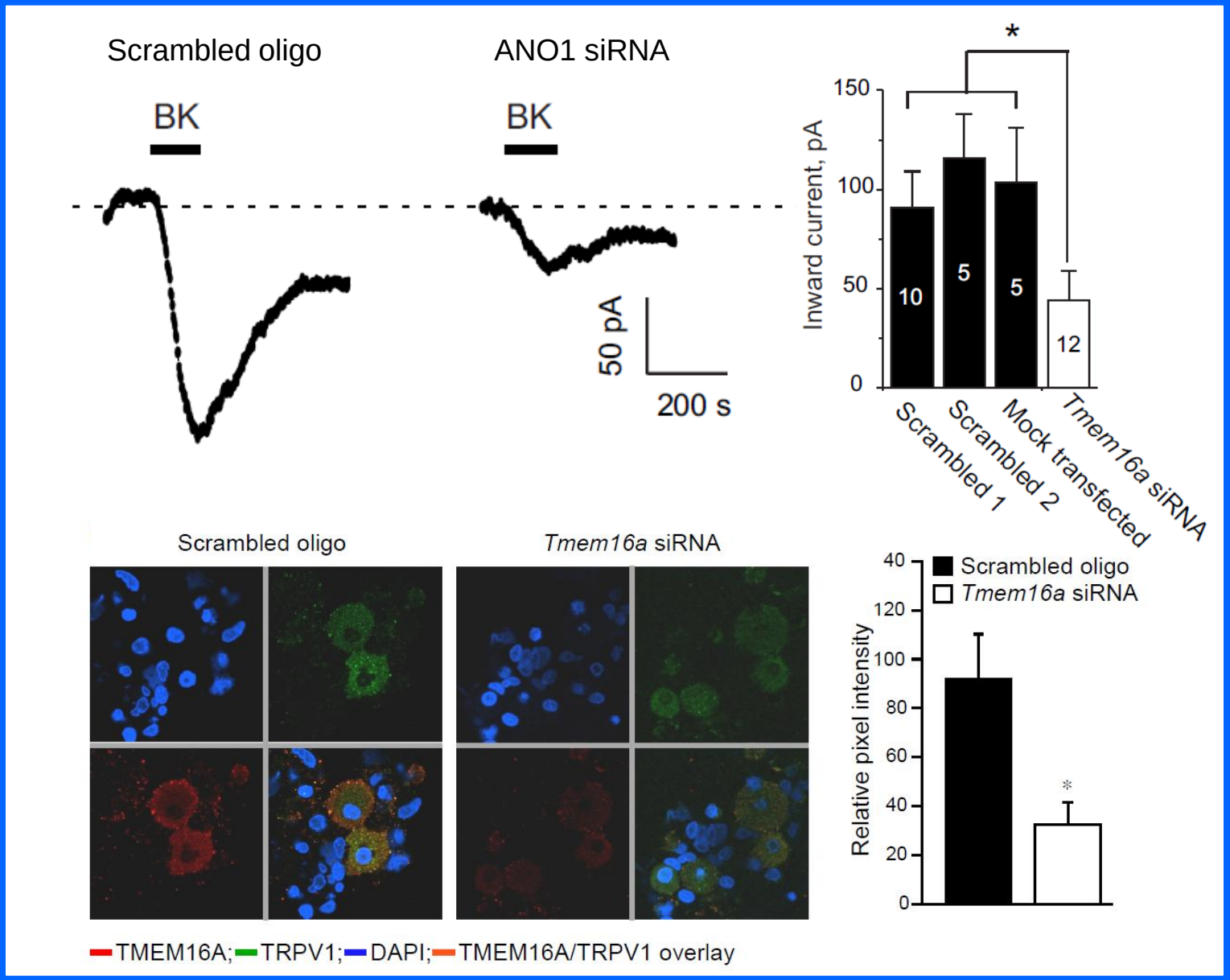
Linley et al. J Neurosci 2008
Liu et al. J Clin Invest 2010
Linley et al. PNAS 2012

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



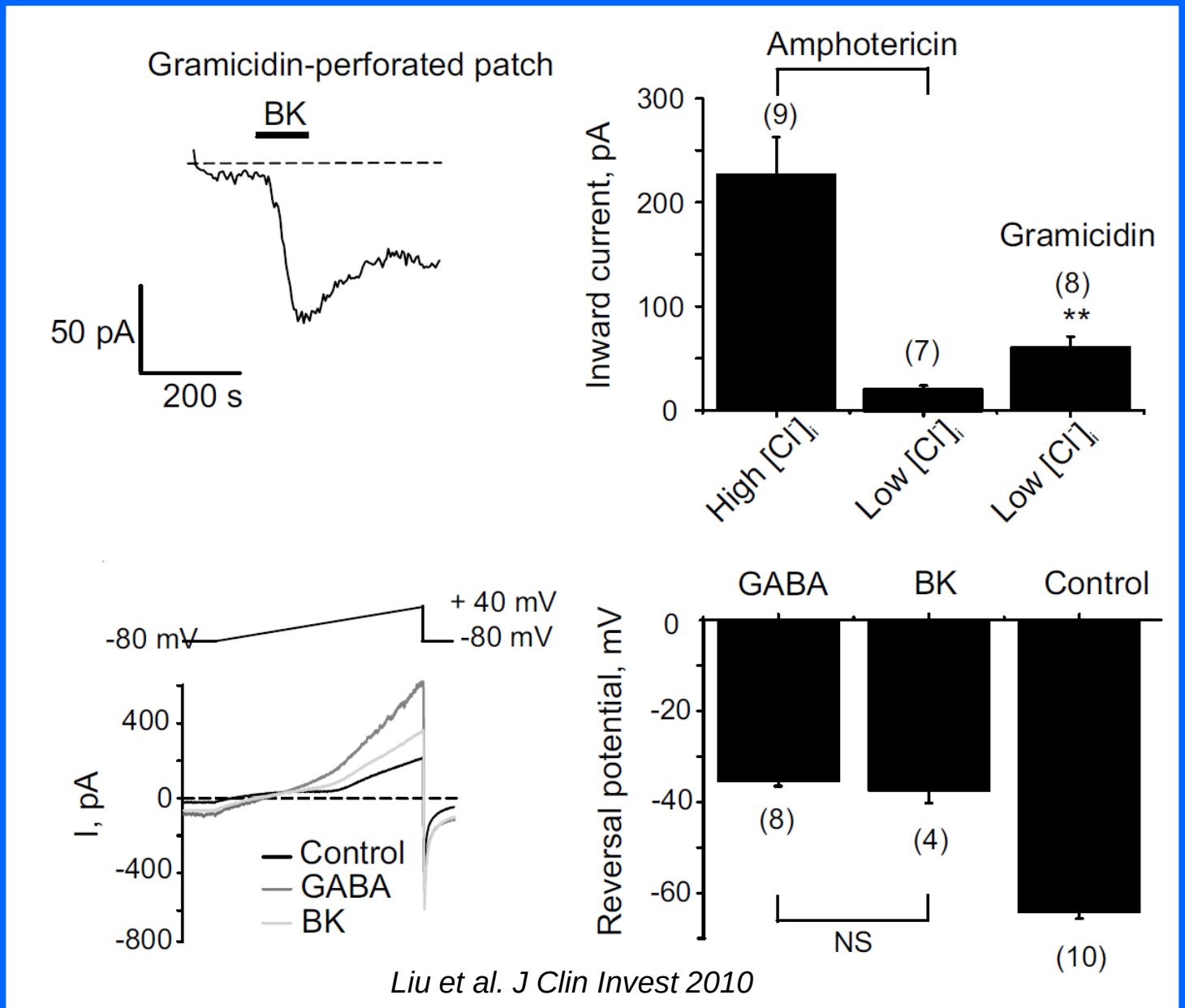
Liu et al. J Clin Invest 2010

siRNA ANO1 knock-down reduces BK-induced inward current



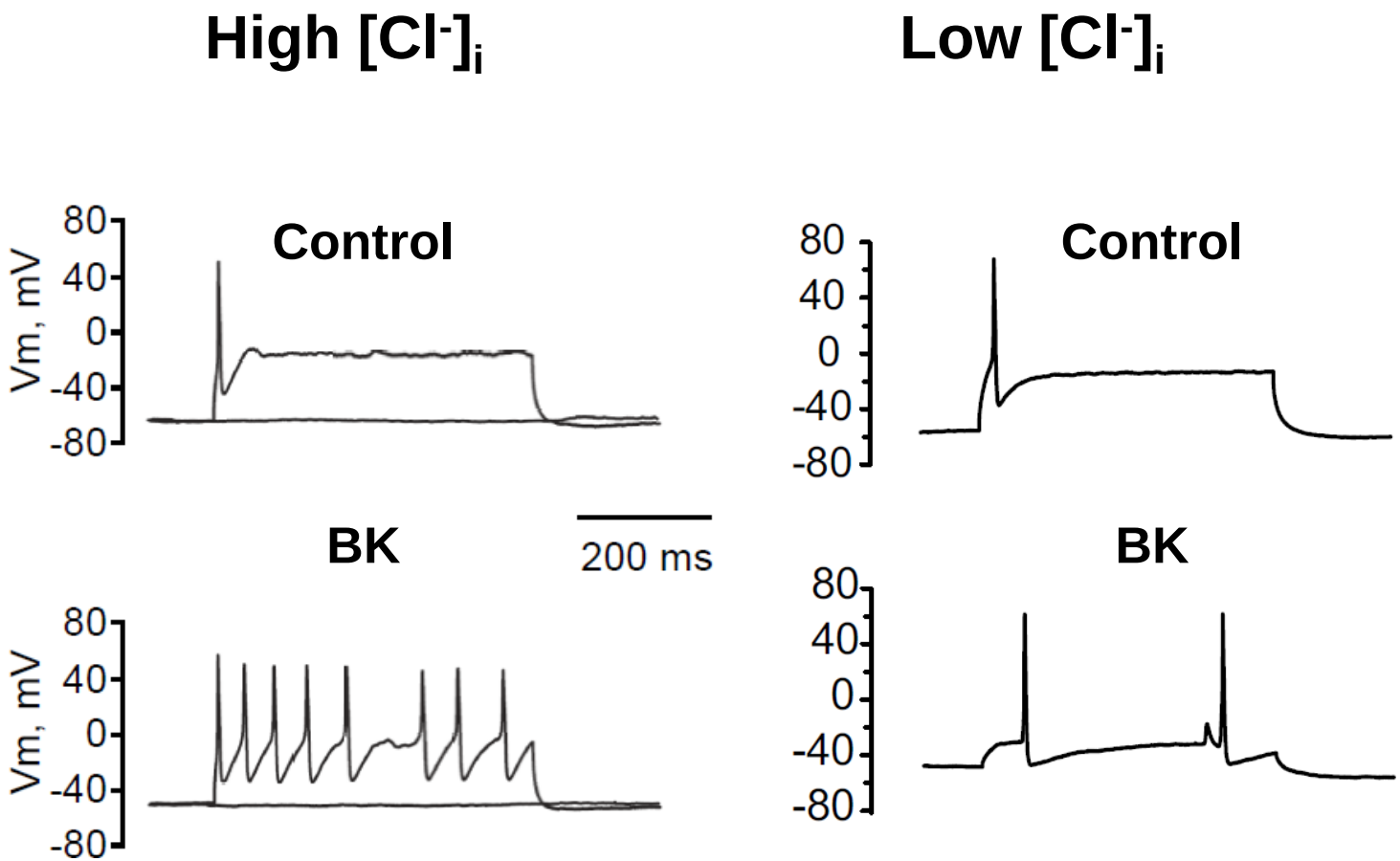
Liu et al. J Clin Invest 2010

The intracellular Cl^- concentration in DRG neurons provides driving force for depolarizing Cl^- currents through Cl^- channels.



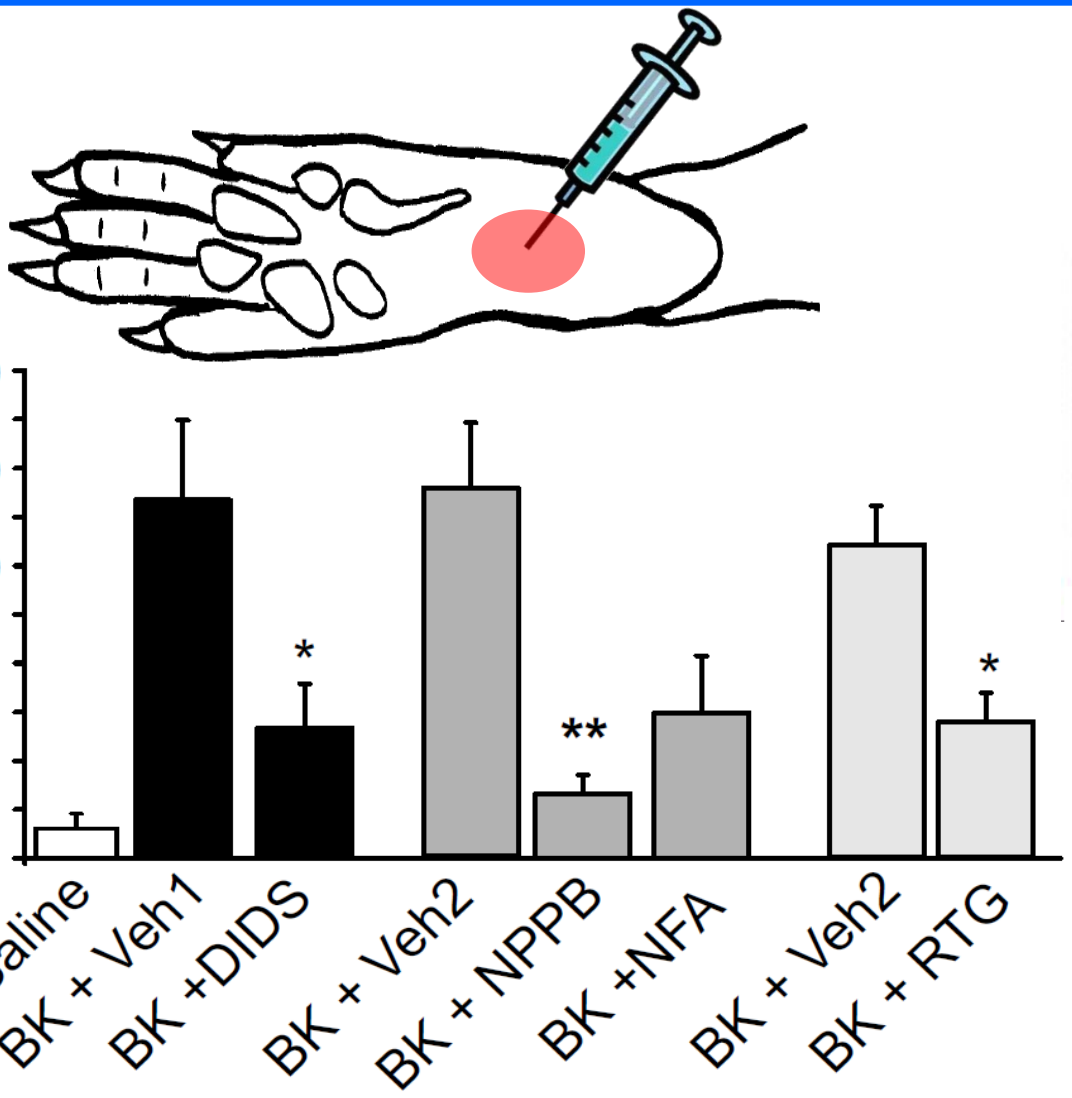
Liu et al. J Clin Invest 2010

CaCC contributes to the BK-induced excitation of DRG

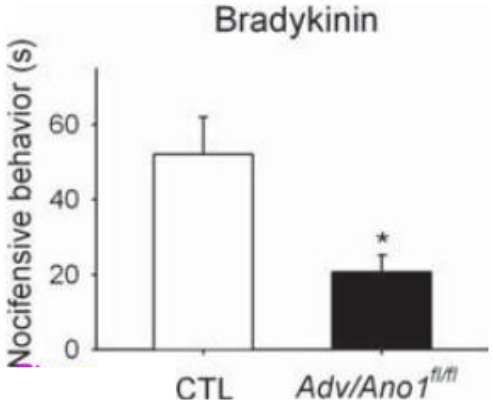


Liu et al. J Clin Invest 2010

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



Liu et al. J Clin Invest 2010



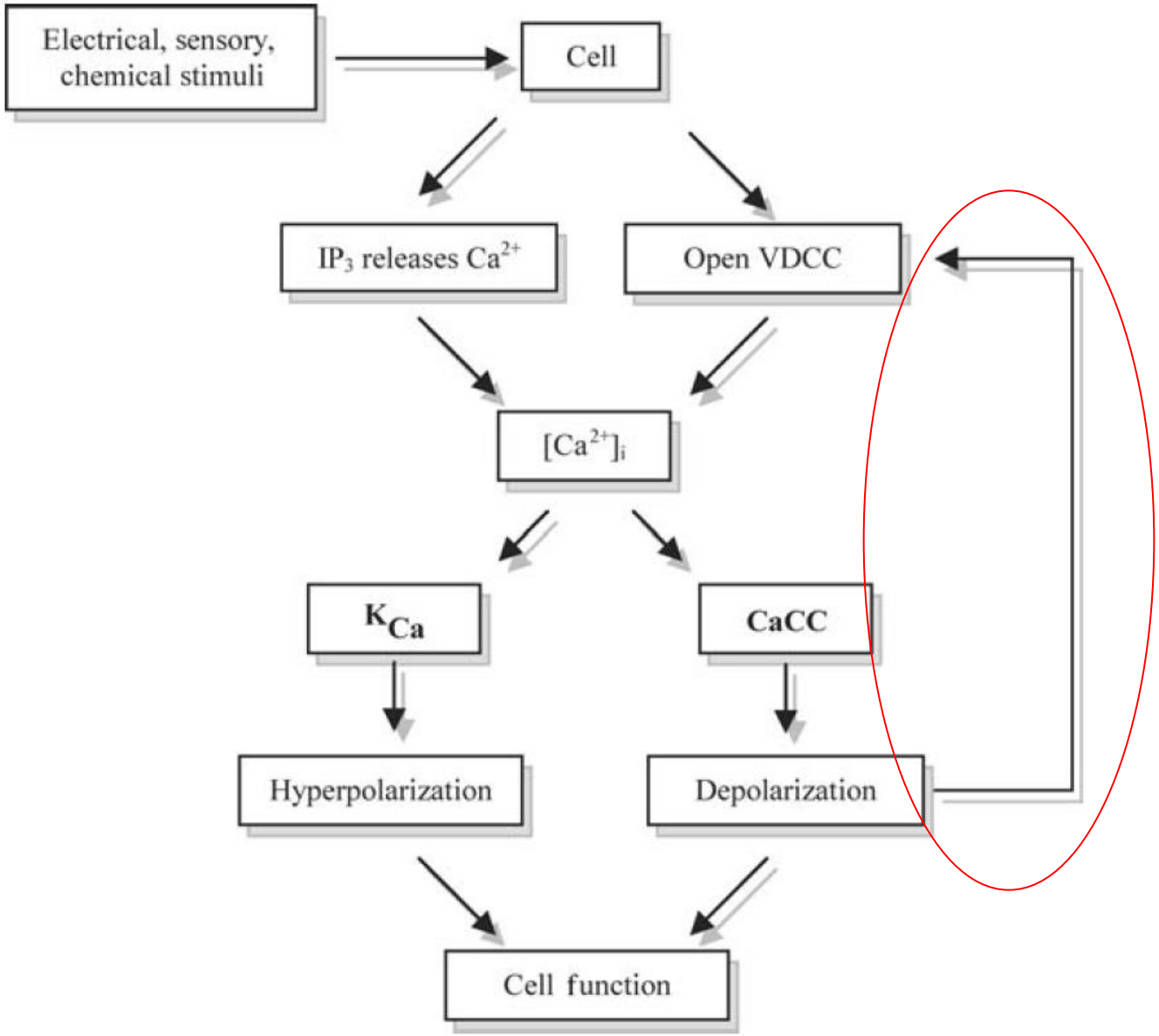
Lee et al Mol Pain 2014

Conclusions thus far:

- ANO1 underlies CaCCs in small DRG neurons

- This channel is activated by Ca^{2+} release from the ER and this activation has an excitatory effect which may result in pain

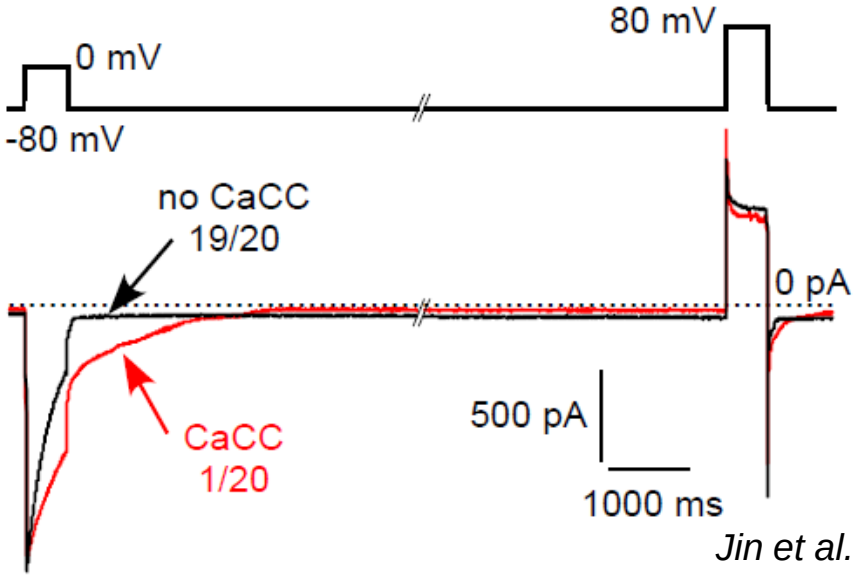
Does it matter where Ca^{2+} is coming from?



Hartzell et al. 2005 Annu Rev Physiol.67:719-58

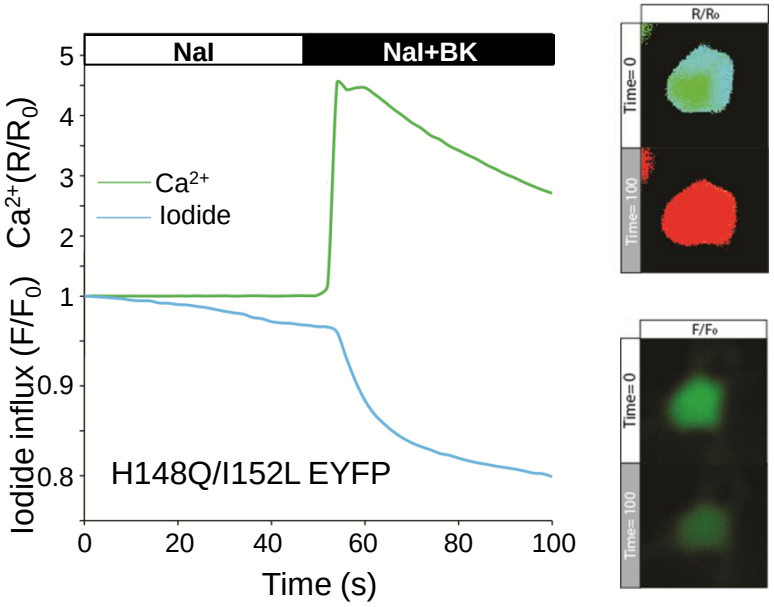
CaCC couple **poorly** to VGCC in small DRG neurons

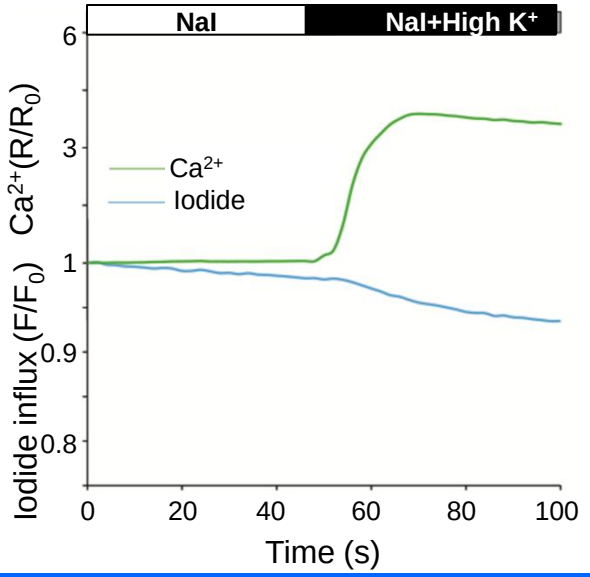
VGCC-coupled 'tail' CaCC is only seen in a minor proportion of small DRG neurons



Jin et al. Science Signaling 2013

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

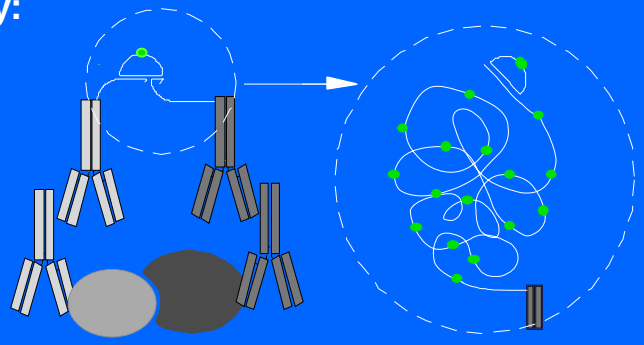




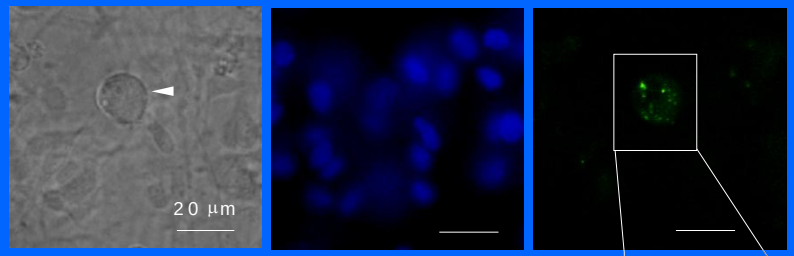
Shab and Gamper, unpublished

ANO1 is in close proximity to the IP_3 receptors

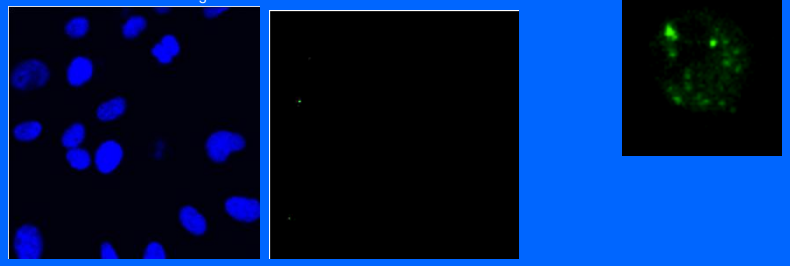
Proximity ligation assay:



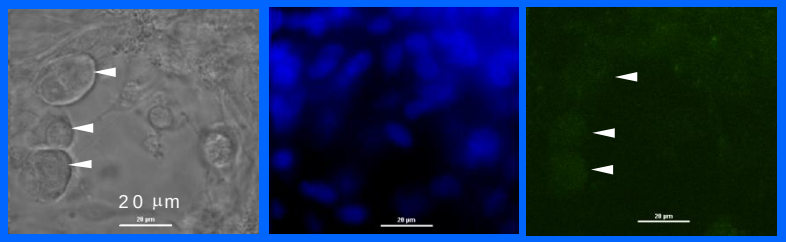
DRG: ANO1/IP_3R1



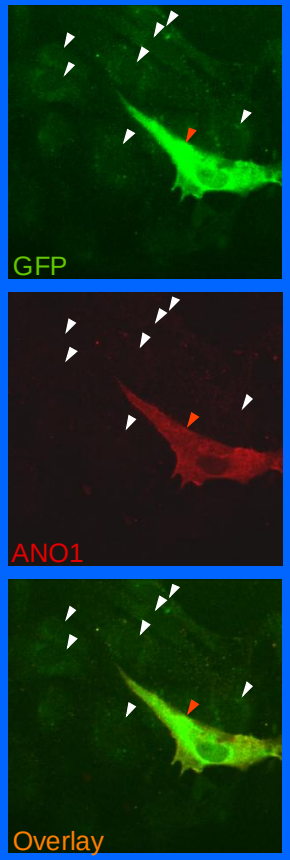
HUVEC: ANO1/IP_3R1



DRG: ANO1/panVGCC

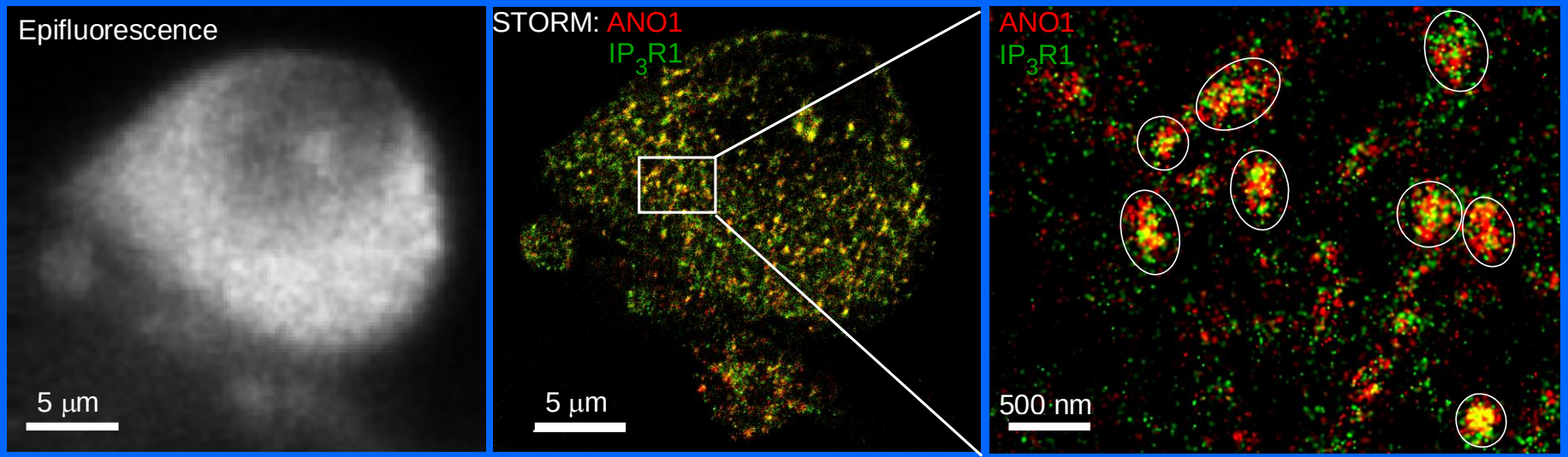


HUVECs transfected With ANO1 and GFP:

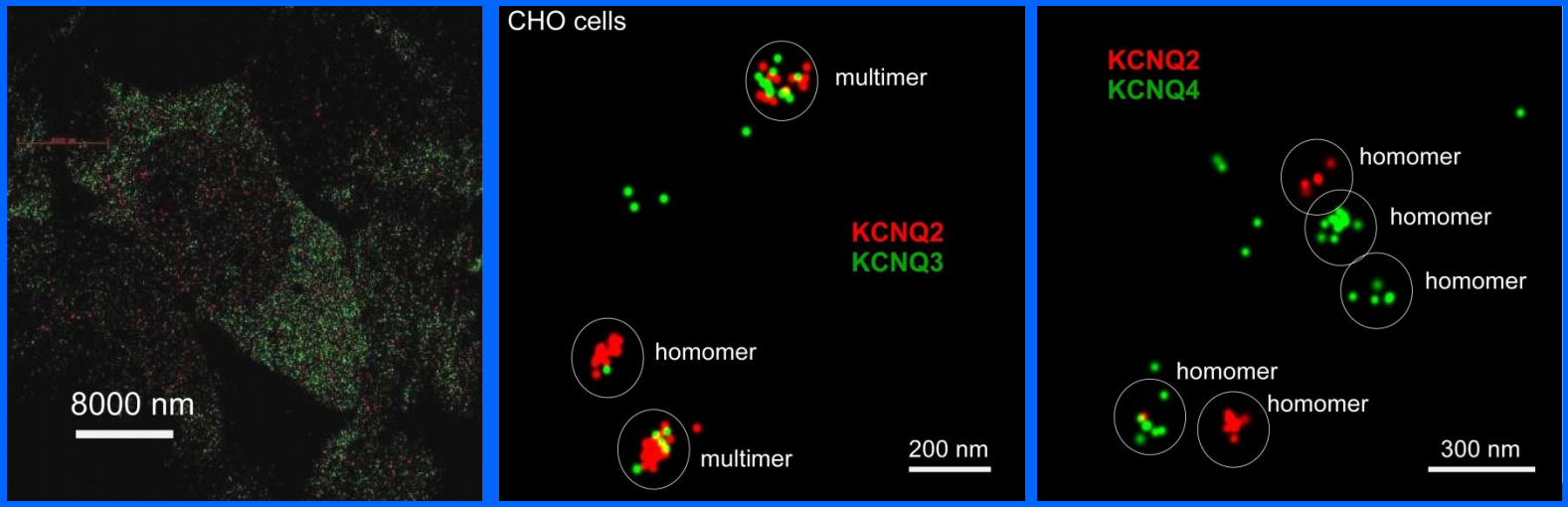


Jin et al. Science Signaling 2013

STORM microscopy reveals close proximity of ANO1 and the IP_3R1 in DRG neurons

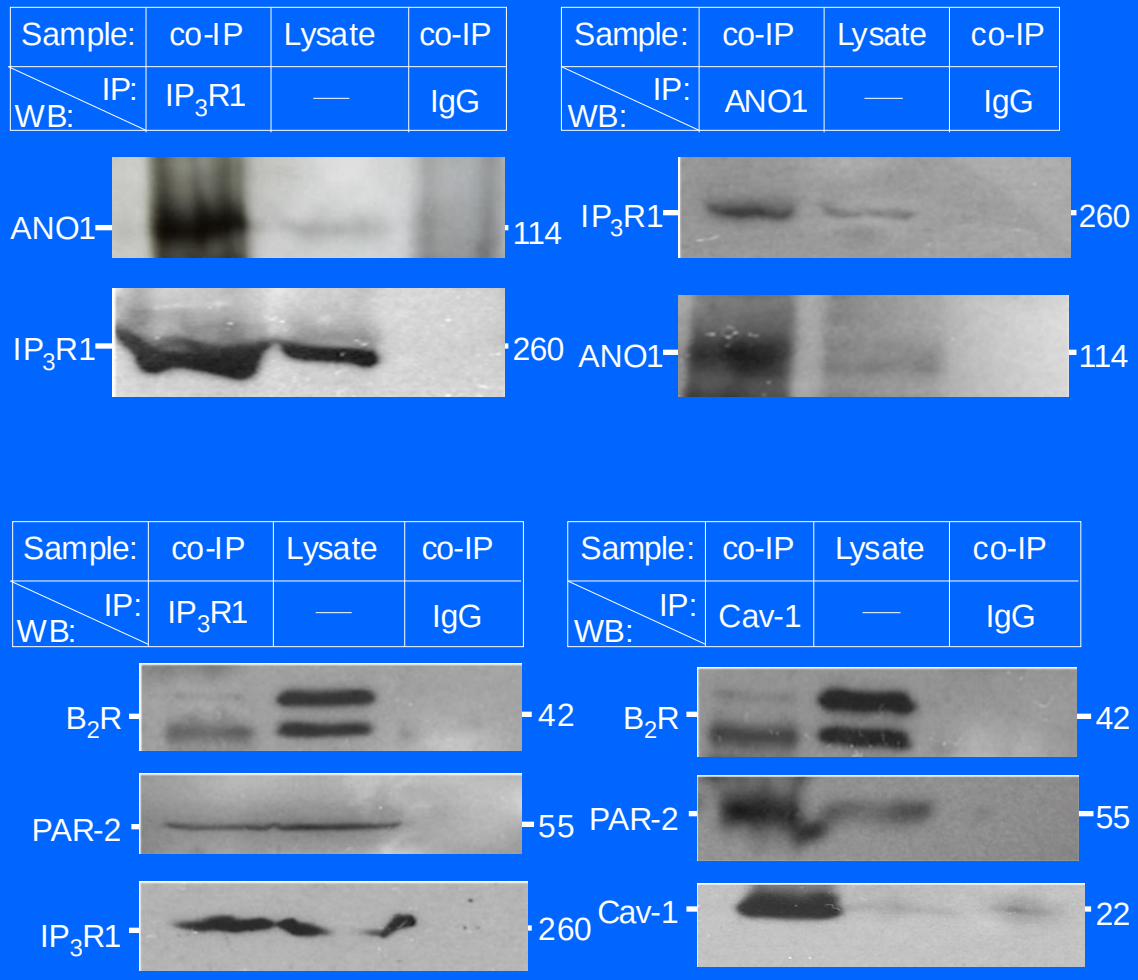


Control experiments:



Zhang, Shapiro & Gamper, unpublished

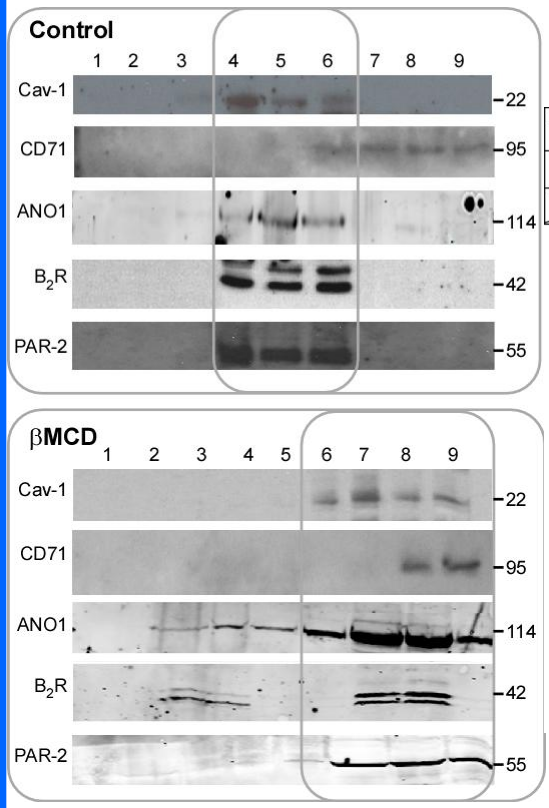
ANO1 co-immunoprecipitates with the IP_3R in DRG



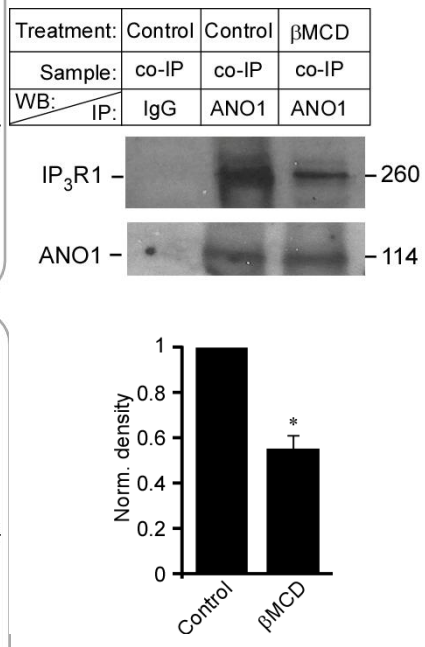
Jin et al. Science Signaling 2013

ANO1-containing signaling complexes are localized to lipid rafts

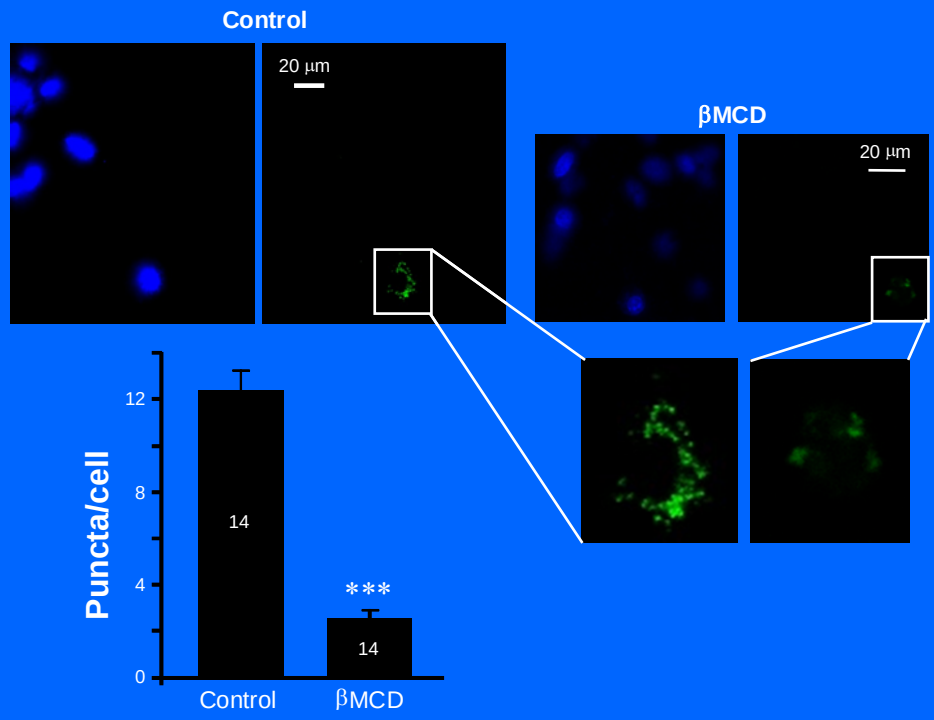
Sucrose gradient centrifugation:



Co-IP:



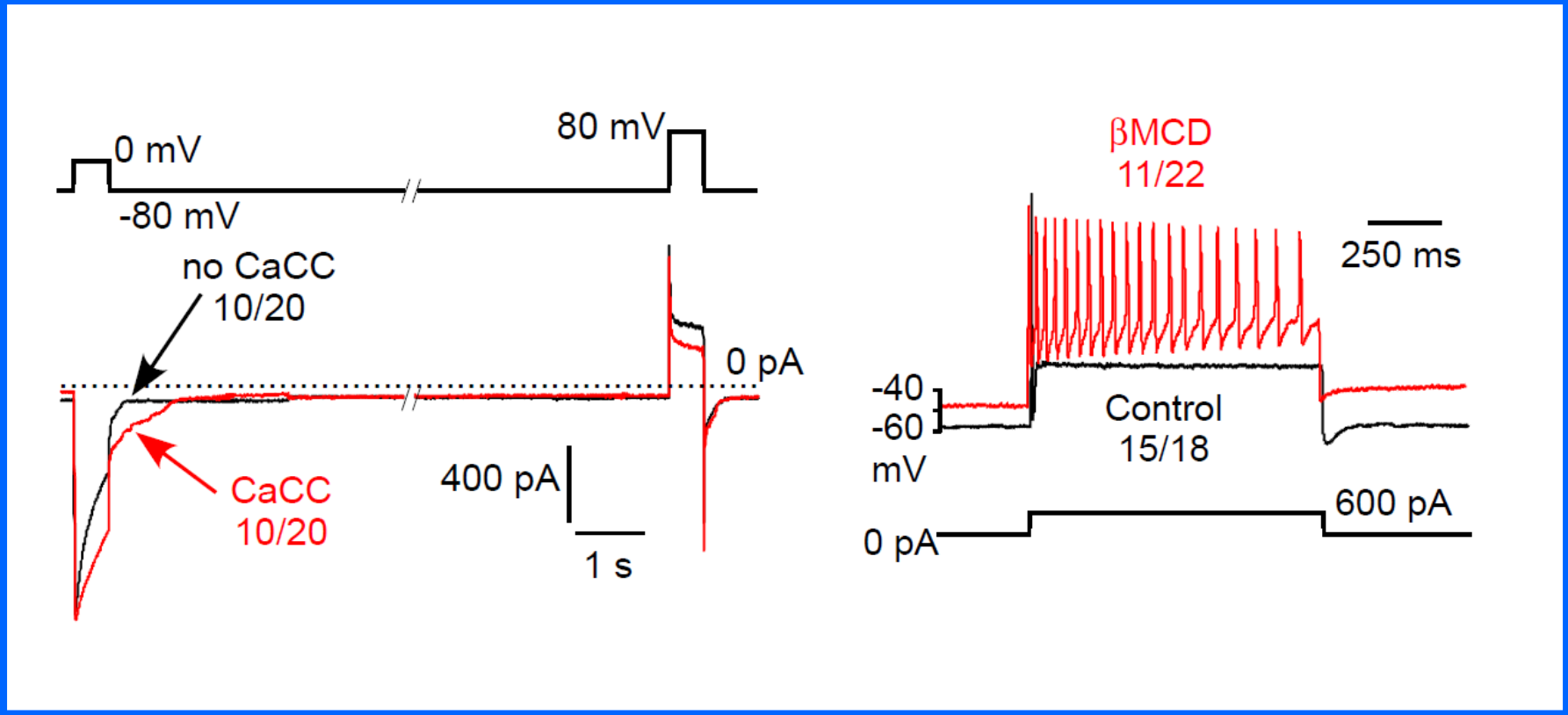
PLA assay:



Cholesterol extraction (βMCD) destroys these complexes

Jin et al. Science Signaling 2013

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

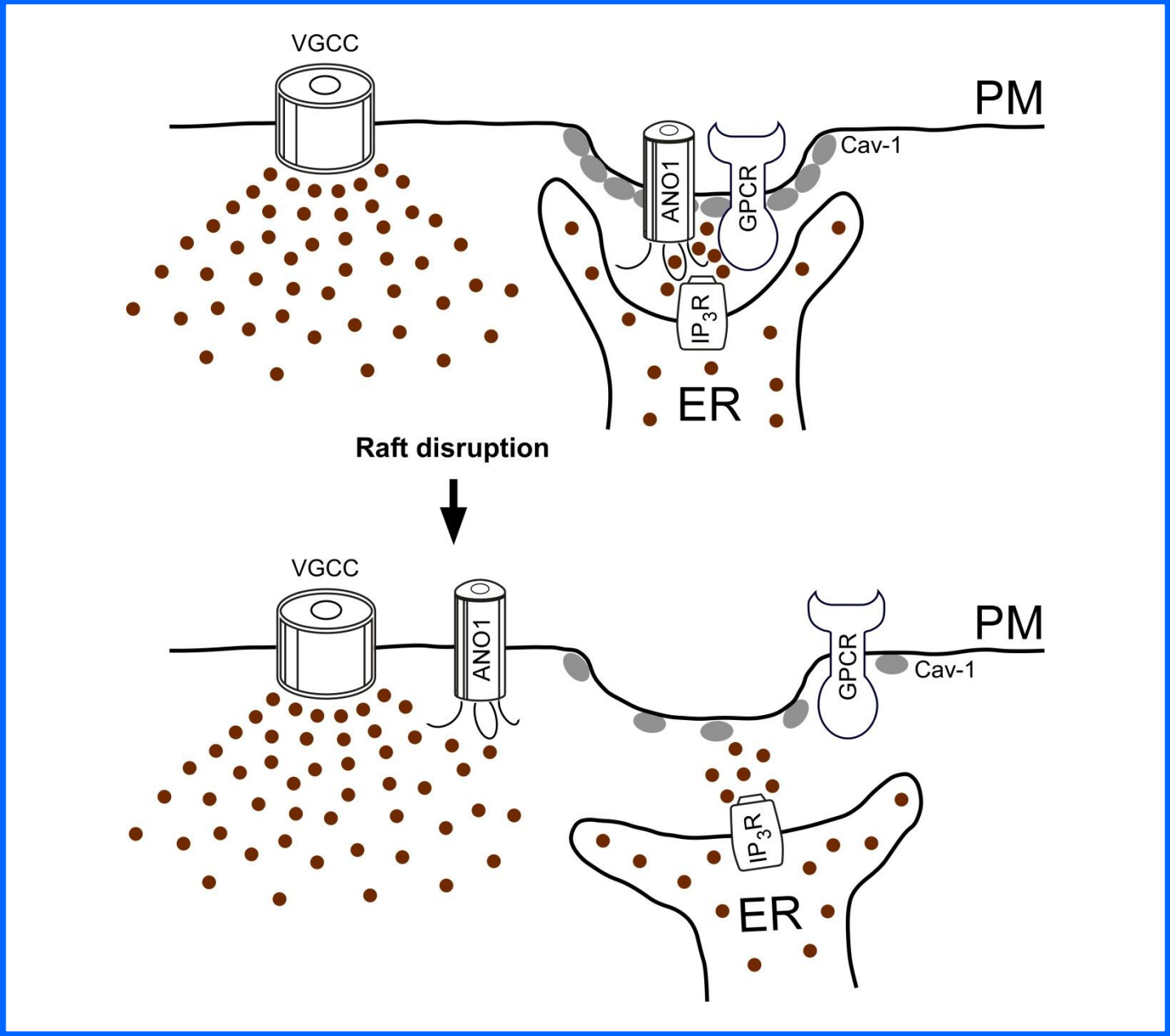


Jin et al. Science Signaling 2013

Summary of the βMCD experiments

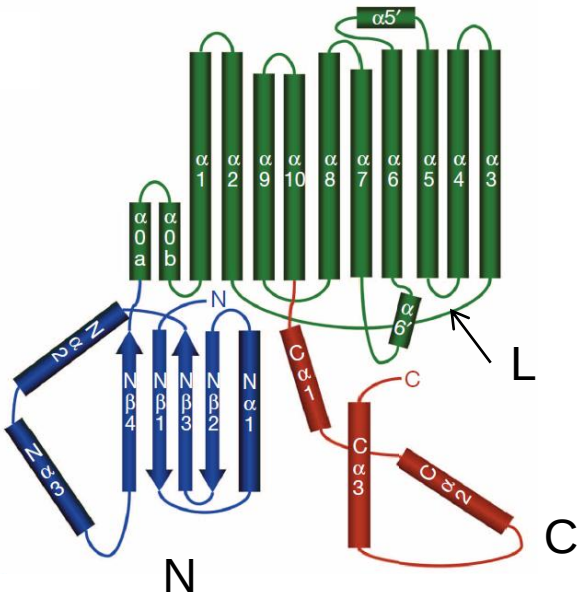
	Control	βMCD	αCD
GPCR-induced cytosolic Ca^{2+} transients……	YES	YES	
GPCR-induced CaCC current……	YES	NO	YES
CaCC tail current after VGCC activation……	NO	YES	NO
'Overexcitable' neuron (multiple action potential firing)……..	NO	YES But if no $[Cl^-]_i$ than NO	NO

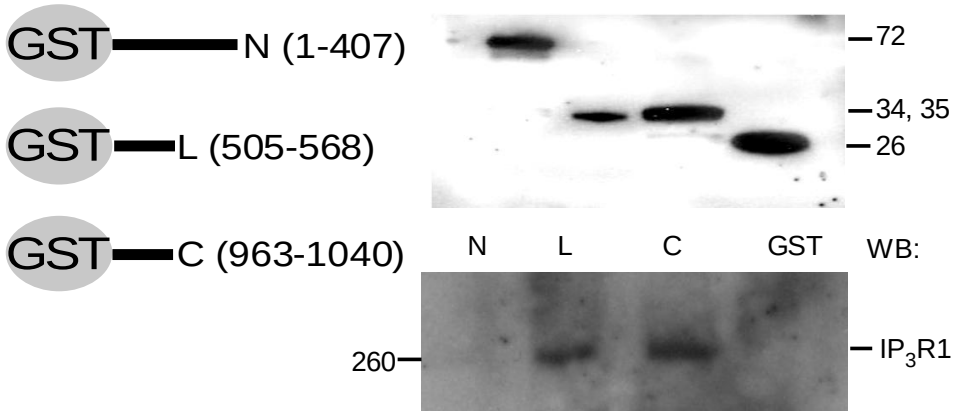
Some ideas on possible mechanism

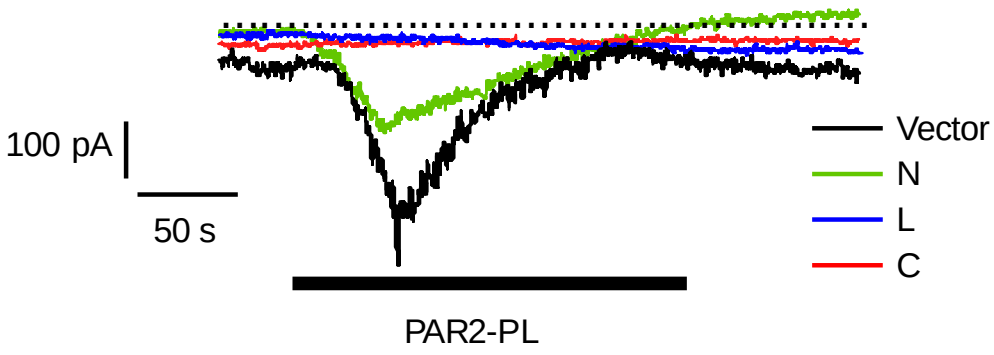


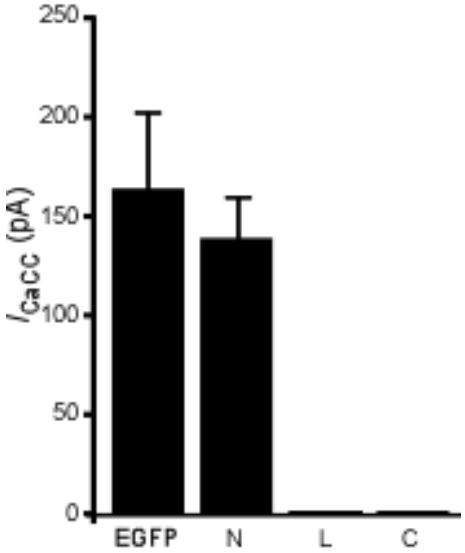
Jin et al. Journal of Physiology 2015

Defining structural background of ANO1-IP$_3$R1 interaction in DRG neurons



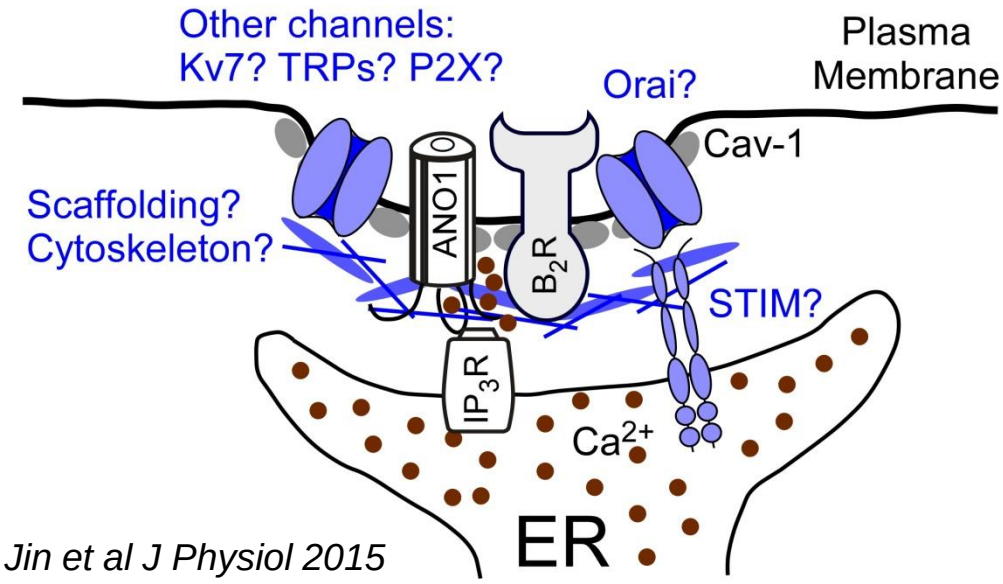






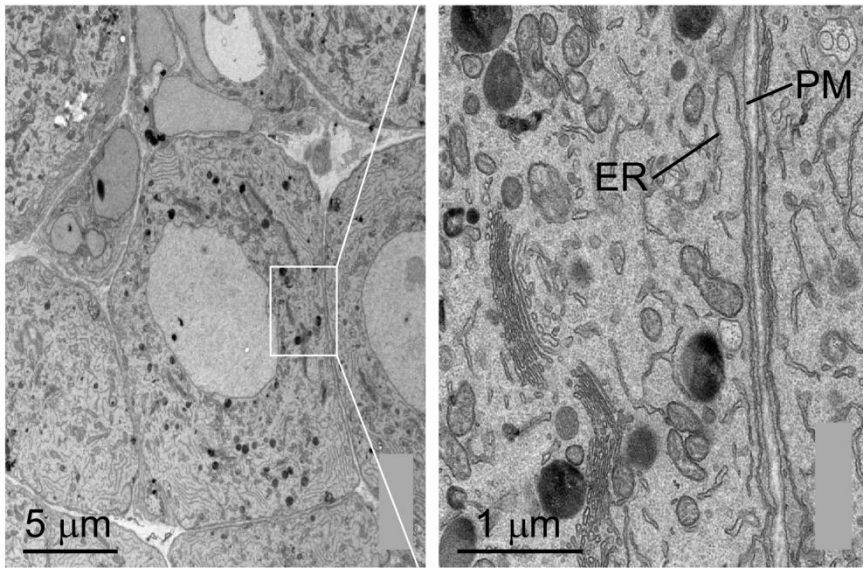
Jin et al. Science Signaling 2013

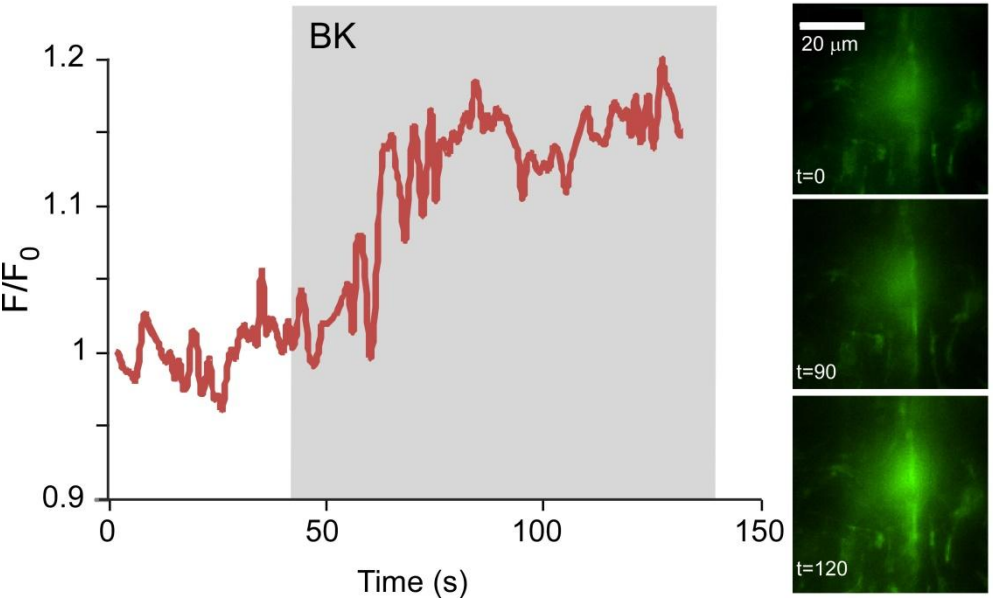
Junctional PM-ER microdomains: current concept & future questions



Jin et al J Physiol 2015

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)

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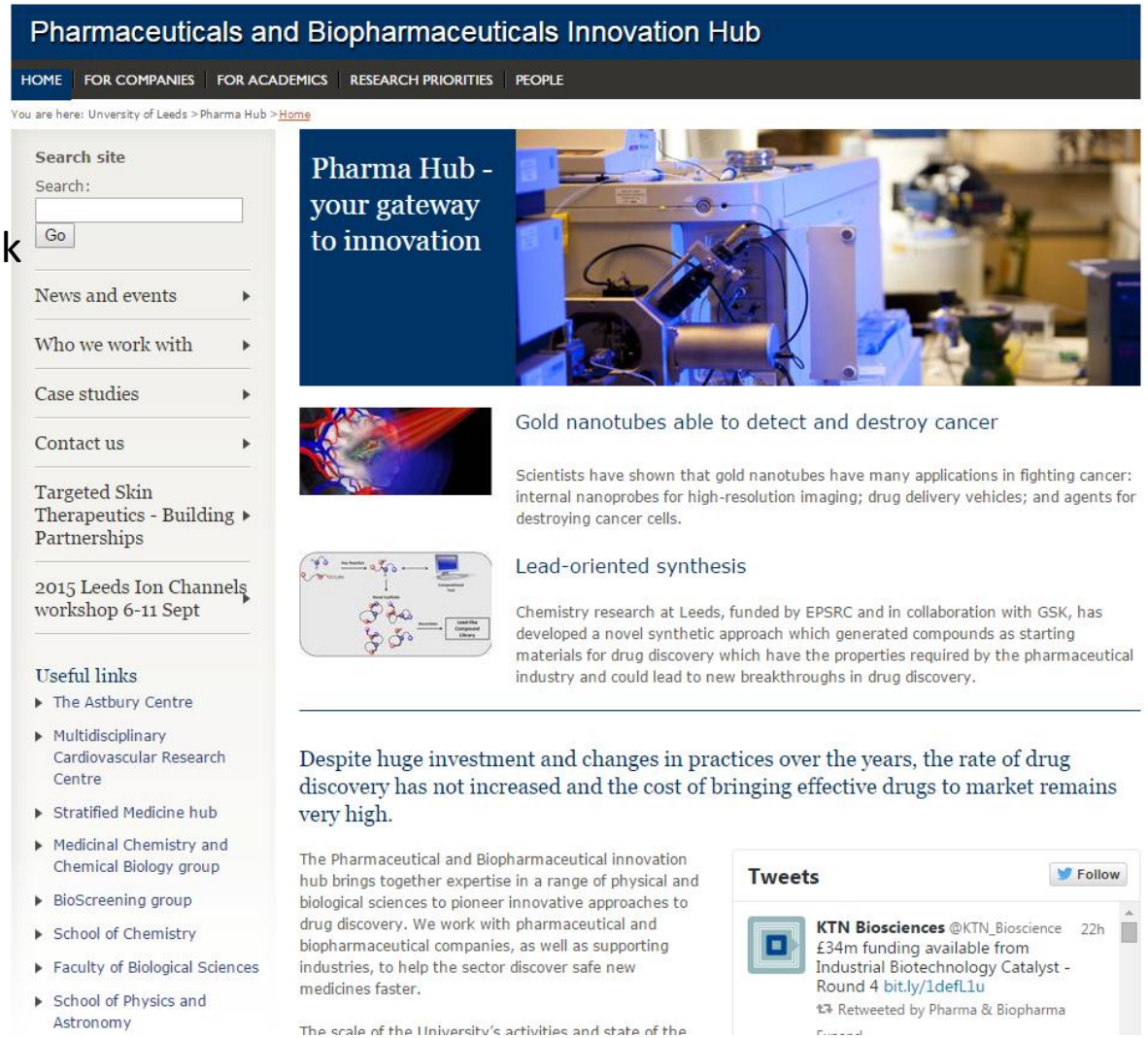
Pharmaceutical and Biopharmaceutical Innovation Hub Group



Director: Prof Adam Nelson

Innovation Manager: Dr Kate Langton

Pharmaceutical and Biopharmaceutical Innovation Hub

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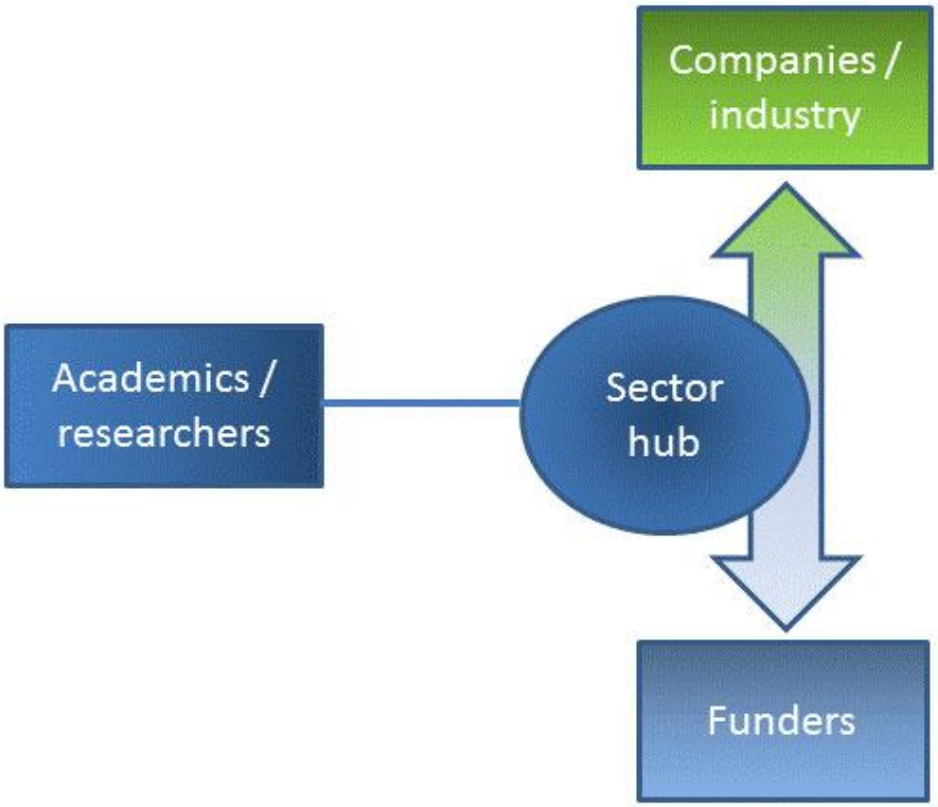


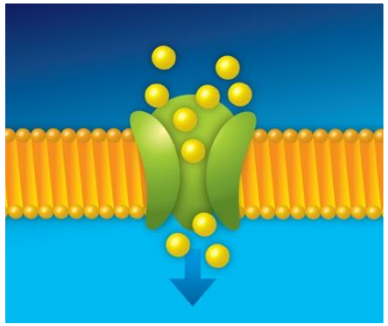
Funding

Commercialisation strategy

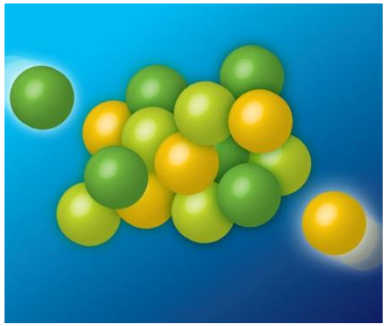
Links to industry

Approach: Research Themes

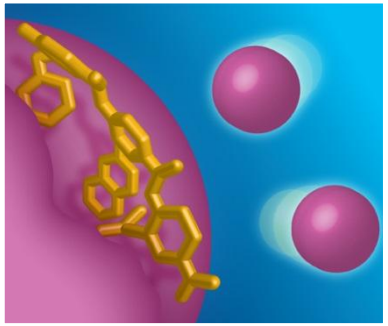
Ion Channels Research



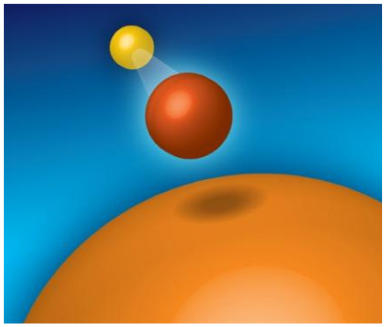
Protein Aggregation



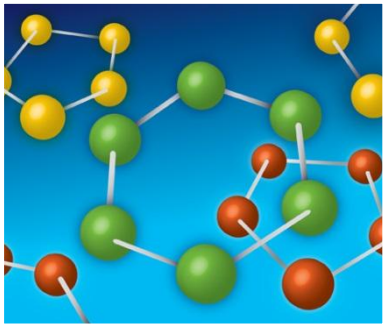
Protein-Protein Interactions



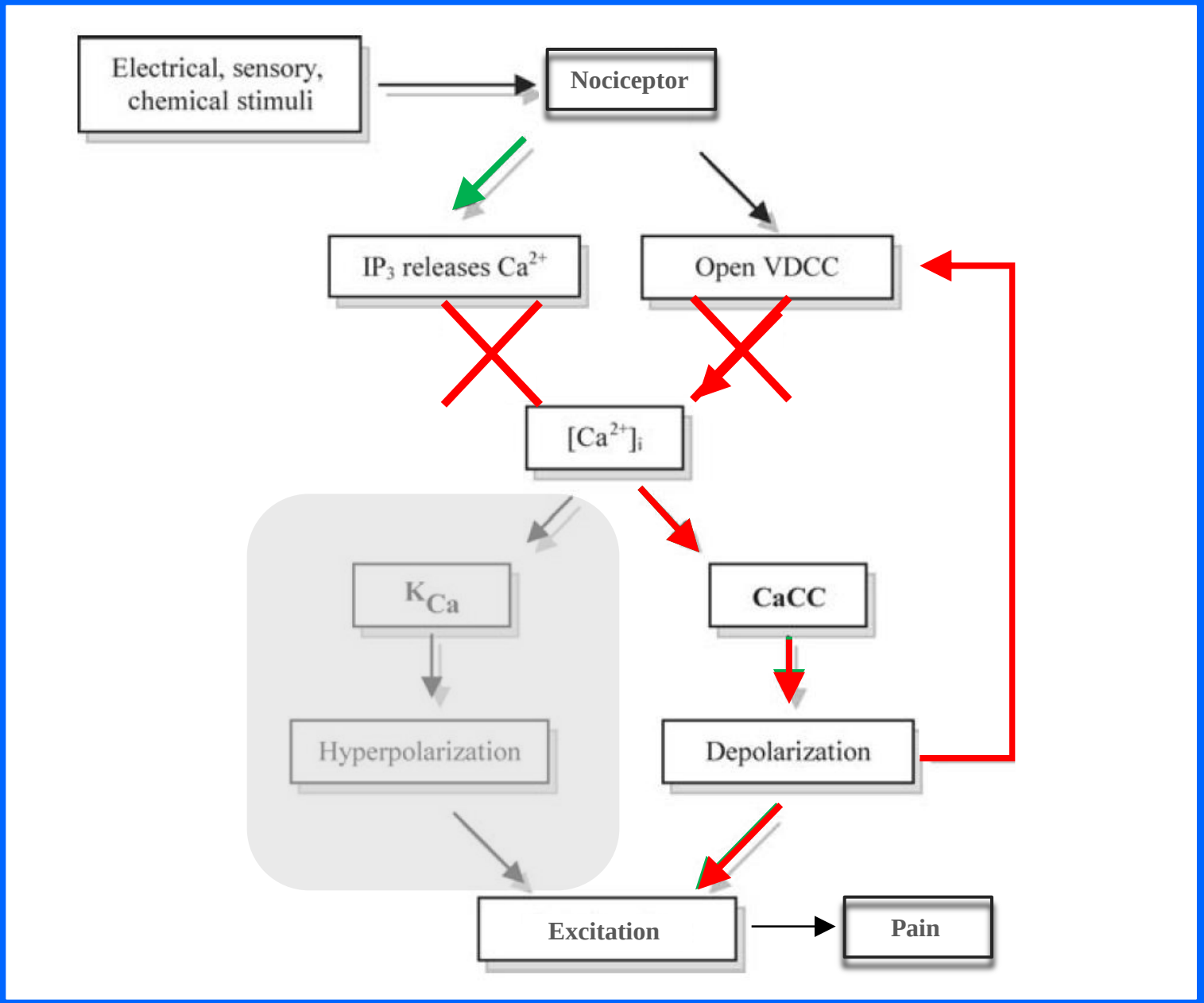
Targeted Drug Delivery



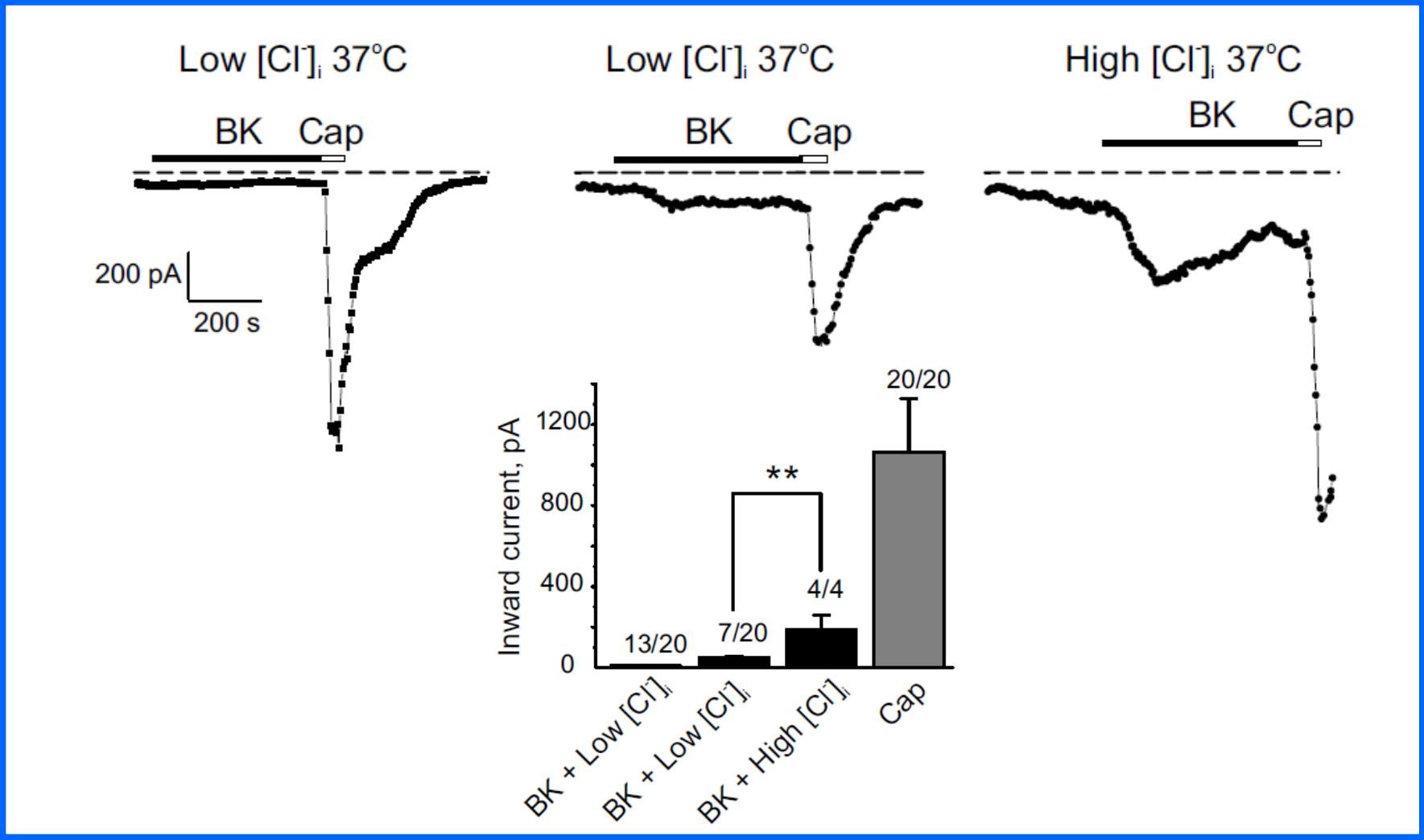
Translational Drug Discovery



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

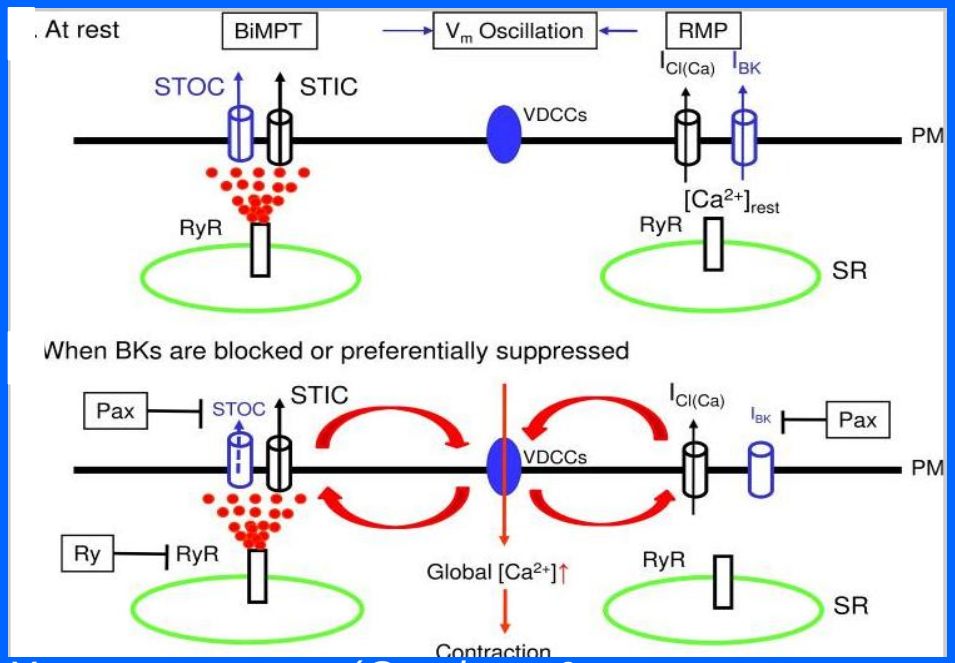


CaCC is a dominant BK-induced inward current even at 37ºC

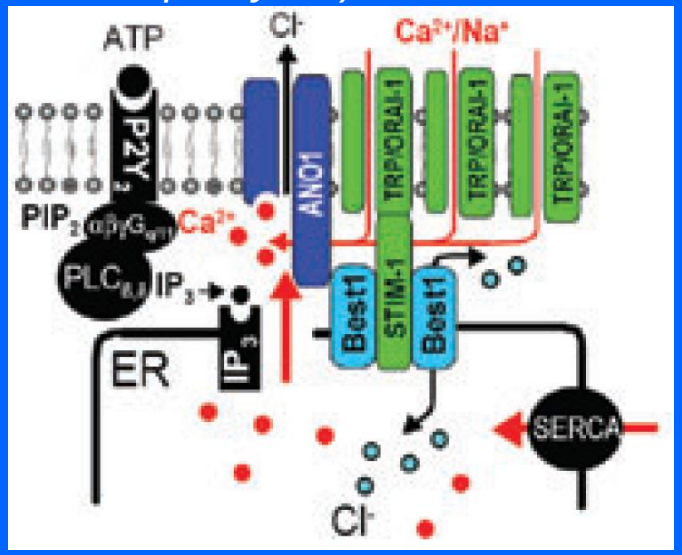


Similar arrangements between ANO1/CaCC and intracellular Ca^{2+} were proposed in other tissues

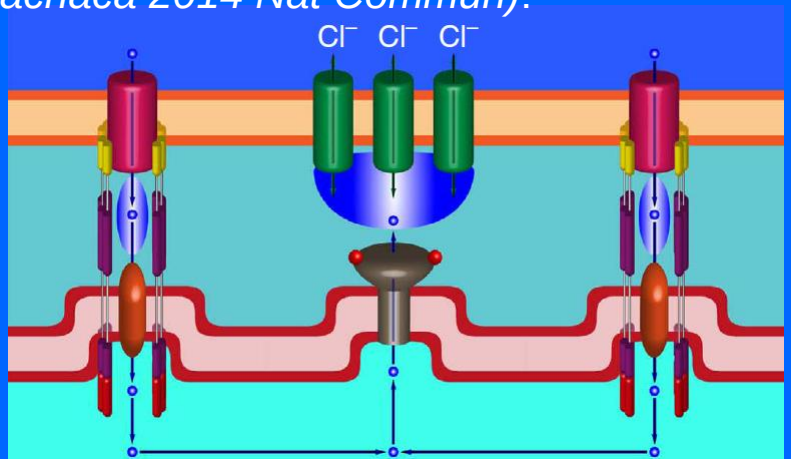
Smooth muscle (*Zhuge et al. 2010 JBC):*



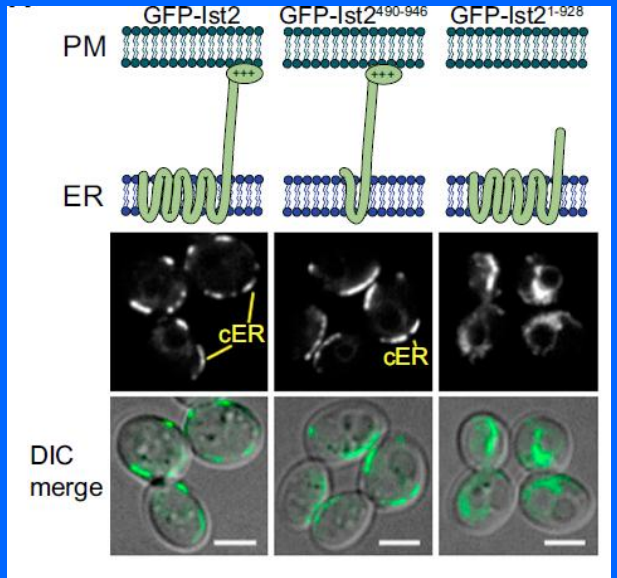
HEK293 cells (*Kunzelmann et al. 2011 Exp Physiol*):



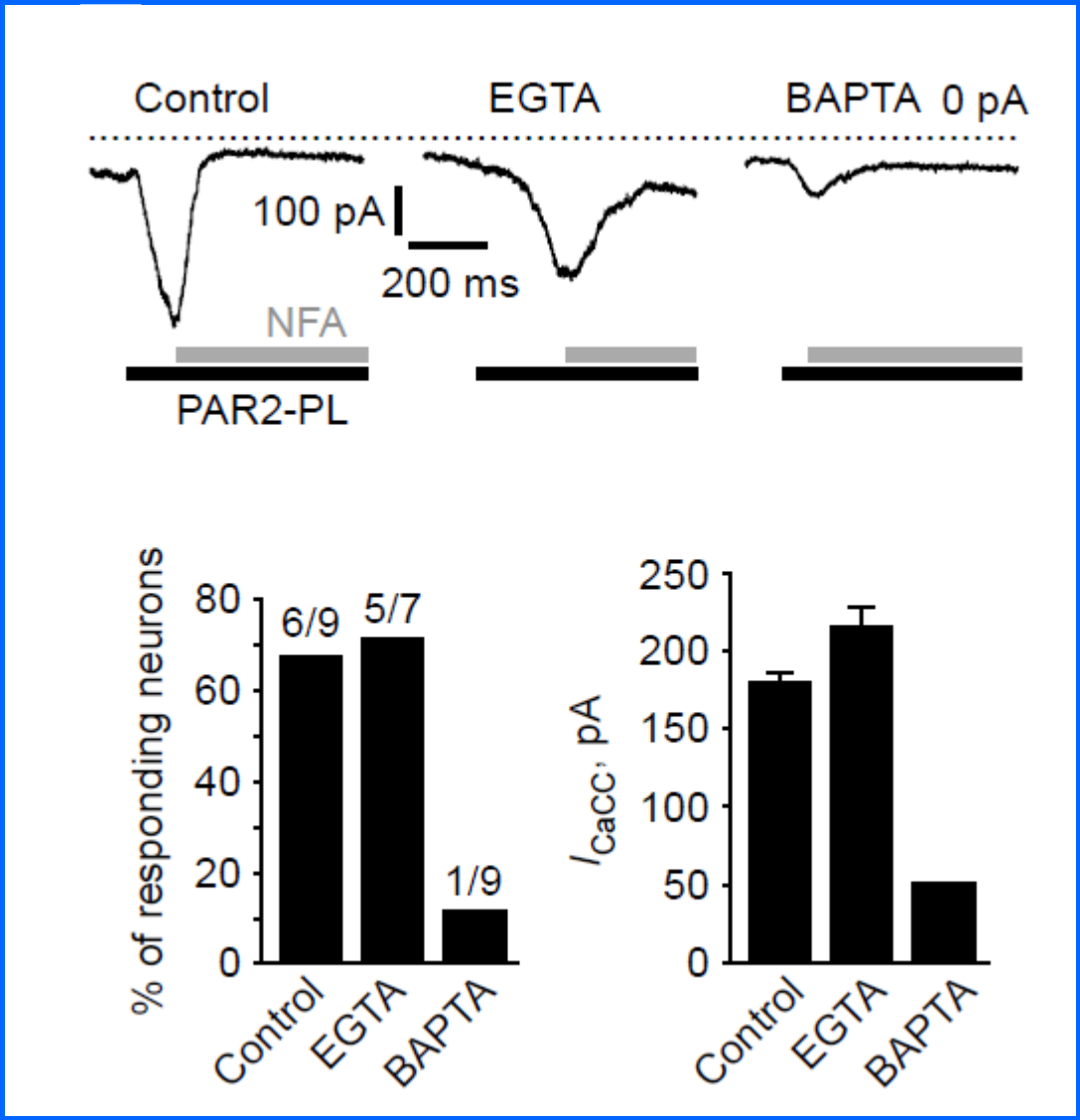
Xenopus oocytes (*Courjaret & Machaca 2014 Nat Commun*):



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



CaCC are in close proximity to the IP_3 receptors



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TRP channels contribute little to the acute pain induced by BK