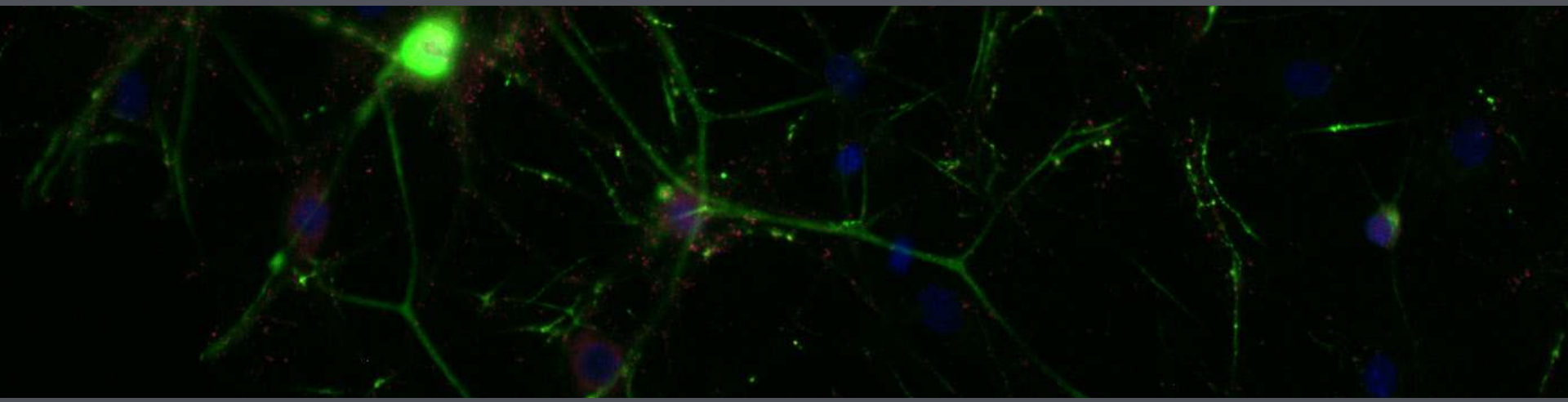
Pharmacology Group, Reading
School of Pharmacy

CINN Centre for Integrative Neuroscience and Neurodynamics

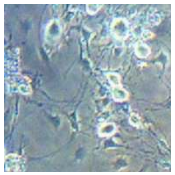
University of Reading

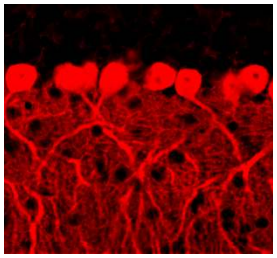
MODULATION OF VOLTAGE-GATED CALCIUM CHANNELS IN DISEASE



Gary Stephens
Room 204 Hopkins Building
g.j.stephens@reading.ac.uk

Electrophysiology at RSOP

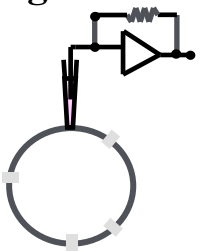




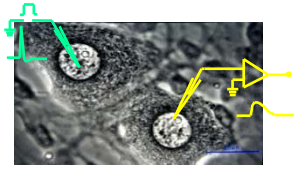
Intracellular (patch/'sharp')

Extracellular

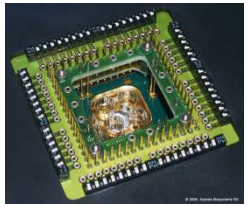
Single electrode

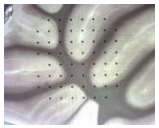


Multi-electrode

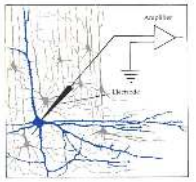


Multi-electrode





Single electrode



Gary Stephens
Mark Dallas (K^+, TRP channels)

Gary Stephens
(Sumiko Mochida)

Ben Whalley
Angela Bithell
(human stem cells)

Alister McNeish
(BK channel)
Ben Whalley

University of Reading

Gary Stephens research focus

***in vitro* electrophysiology**; manual and automated patch clamp ion channel and receptor pharmacology: voltage-gated Ca^{2+} channels ($Ca_V2.2$ and $Ca_V3.1$), TRP channels, CB_1 and $GABA_B$ receptors;

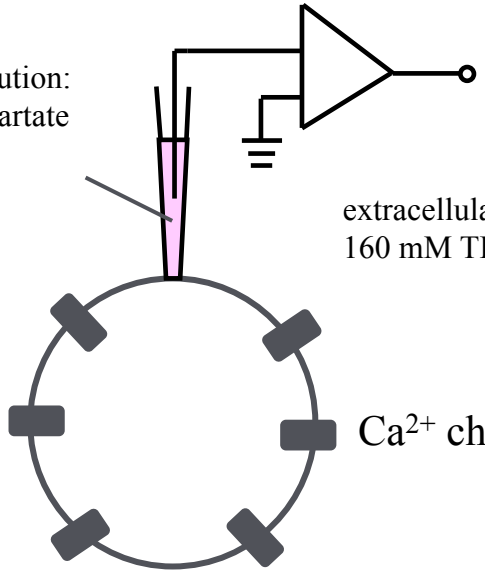
- modulation of recombinant VGCCs
- phytocannabinoids
- cerebellar brain slices
- animal models of disease (ataxia, epilepsy (with Ben Whalley) and pain)

- Molecular Biology, Western blotting, immunohistochemisty (with Graeme Cottrell)

- Novel VGCC subunits (with Pfizer Neusentis)
- antibodies as therapeutic agents (with UCB Pharma)

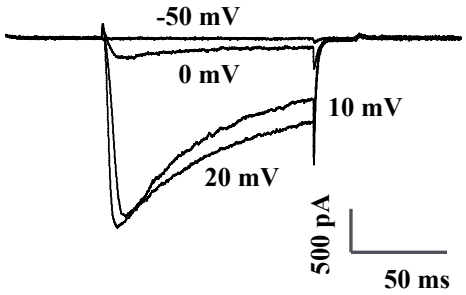
Patch clamp recording of Ca^{2+} currents

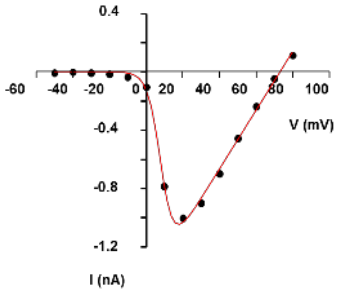
intracellular solution:
140 mM CsAspartate



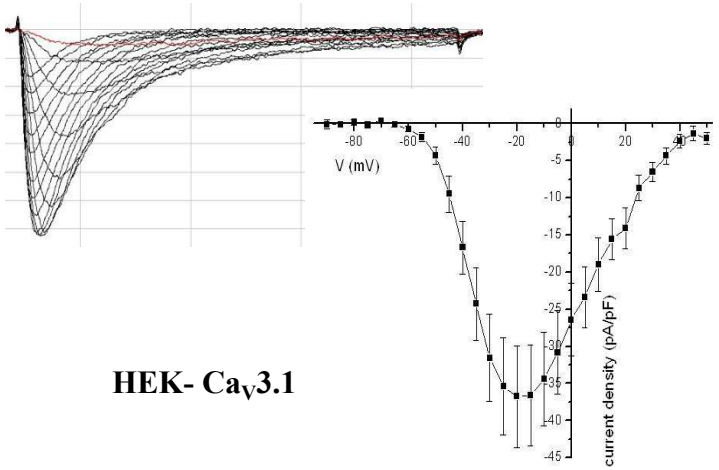
extracellular solution:
160 mM TEABr + 1 mM Ba^{2+}

Ca^{2+} channel





SGN neuron



HEK- $Ca_V3.1$

Transiently or stably transfected HEK cell/DRG/SCG neuron/brain slice neuron

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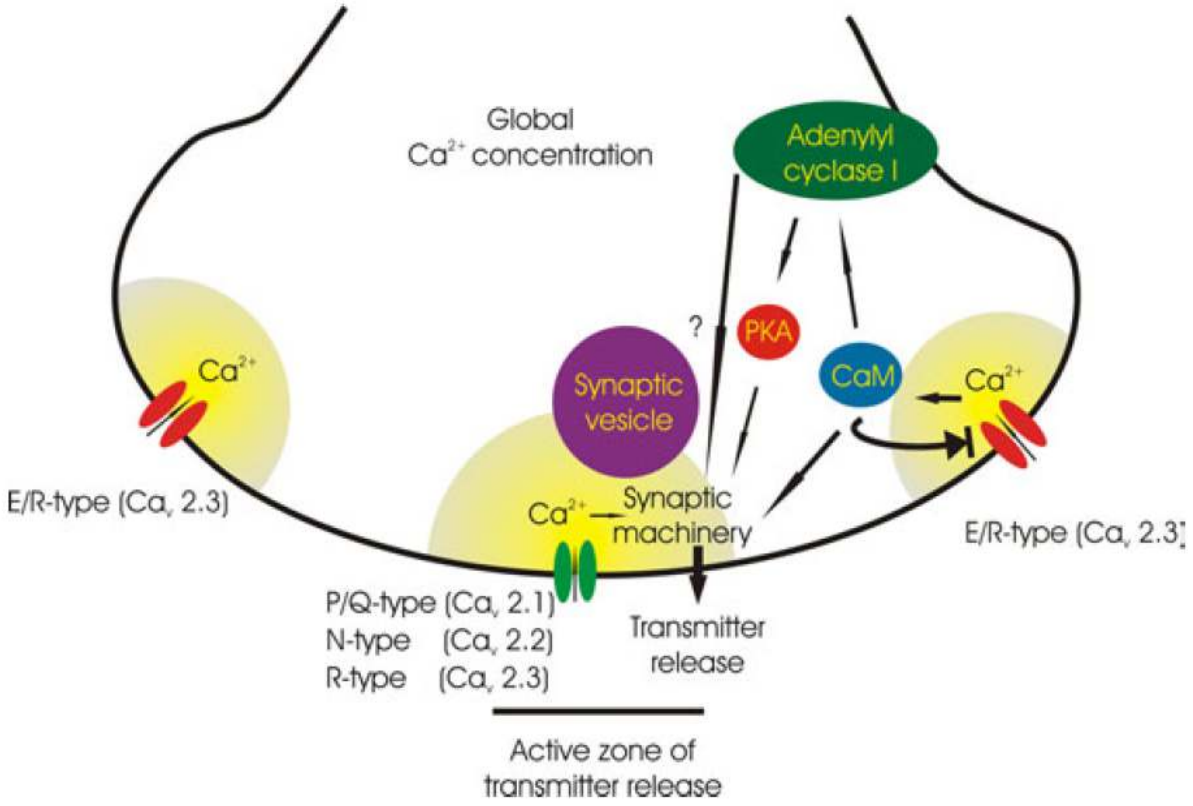
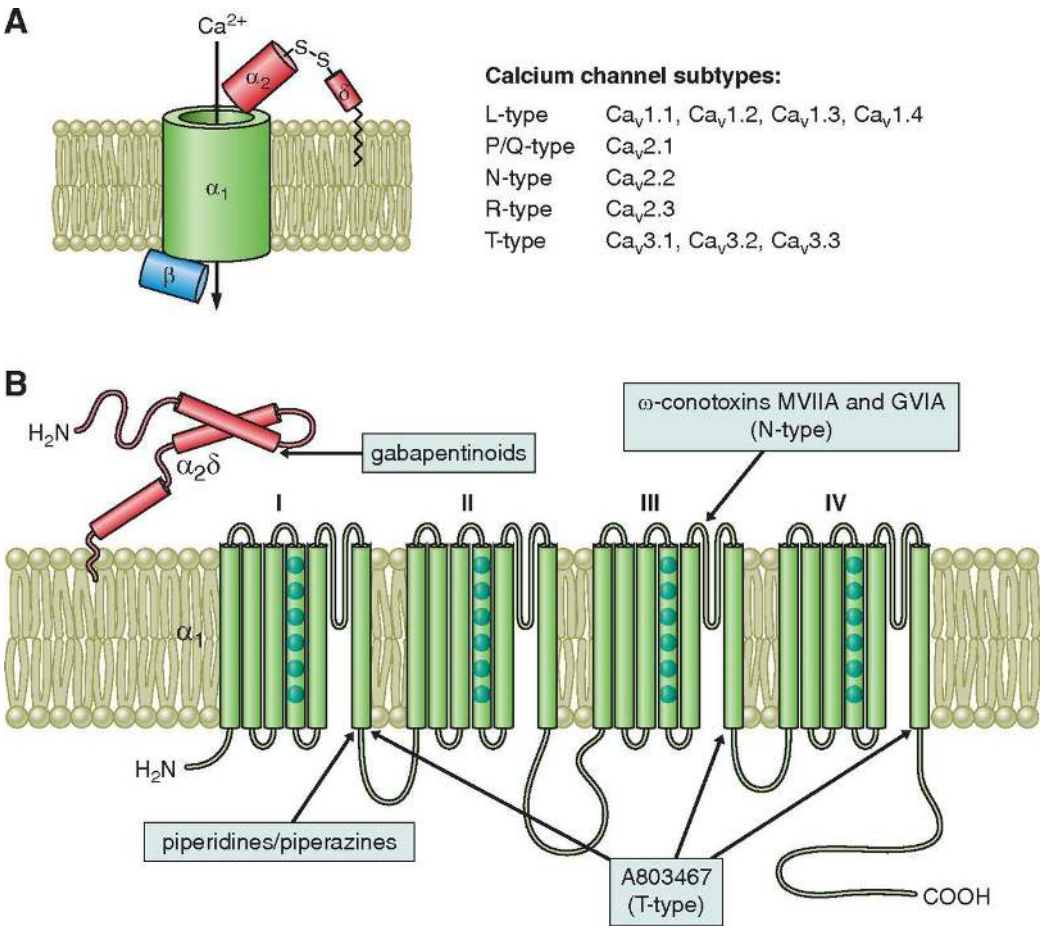
Voltage-gated Ca^{2+} channel synaptic function



Kamp et al. EJN 2005; 21, 1617-1625

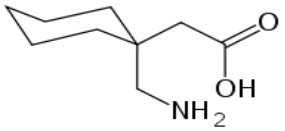
Voltage-gated Ca^{2+} channel structure



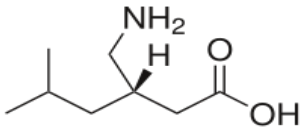
Bourinet et al. Physiol Rev 2014;94:81-140

Modulation of voltage-gated calcium channels to treat epilepsy and chronic pain

- **Gabapentin** and **pregabalin** are used to treat focal seizure in epilepsy, neuropathic pain and neuralgia.
- Both bind to the $\alpha_2\delta$ subunit of VGCC and prevent the anterograde trafficking of VGCC (Tran-Van-Minh, 2010).

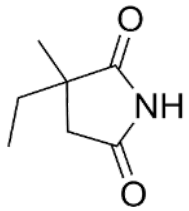


gabapentin



pregabalin

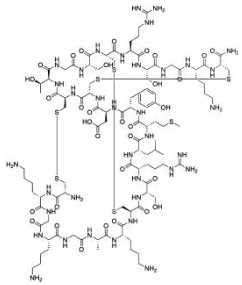
- **Ethosuxamide** Ca_V3 (T-type) VGCC blocker is also an anti-epileptic treating absence seizure.



ethosuxamide

- **Ziconotide** $Ca_V2.2$ (N-type) VGCC blocker (synthetic peptide toxin)

ziconotide

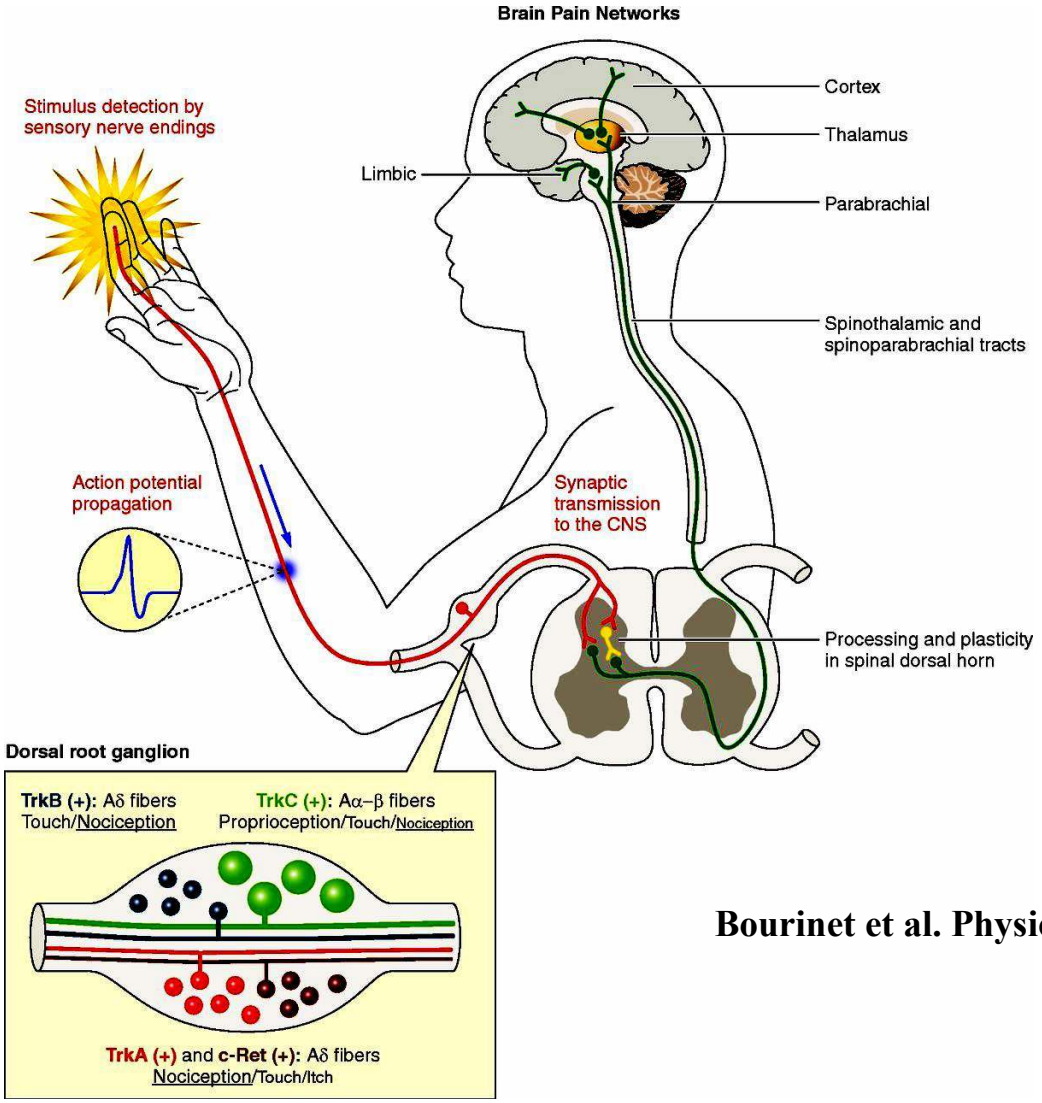


"DIVINE IS THE TASK TO RELIEVE PAIN"……

The World Health Organisation define pain as "an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage"

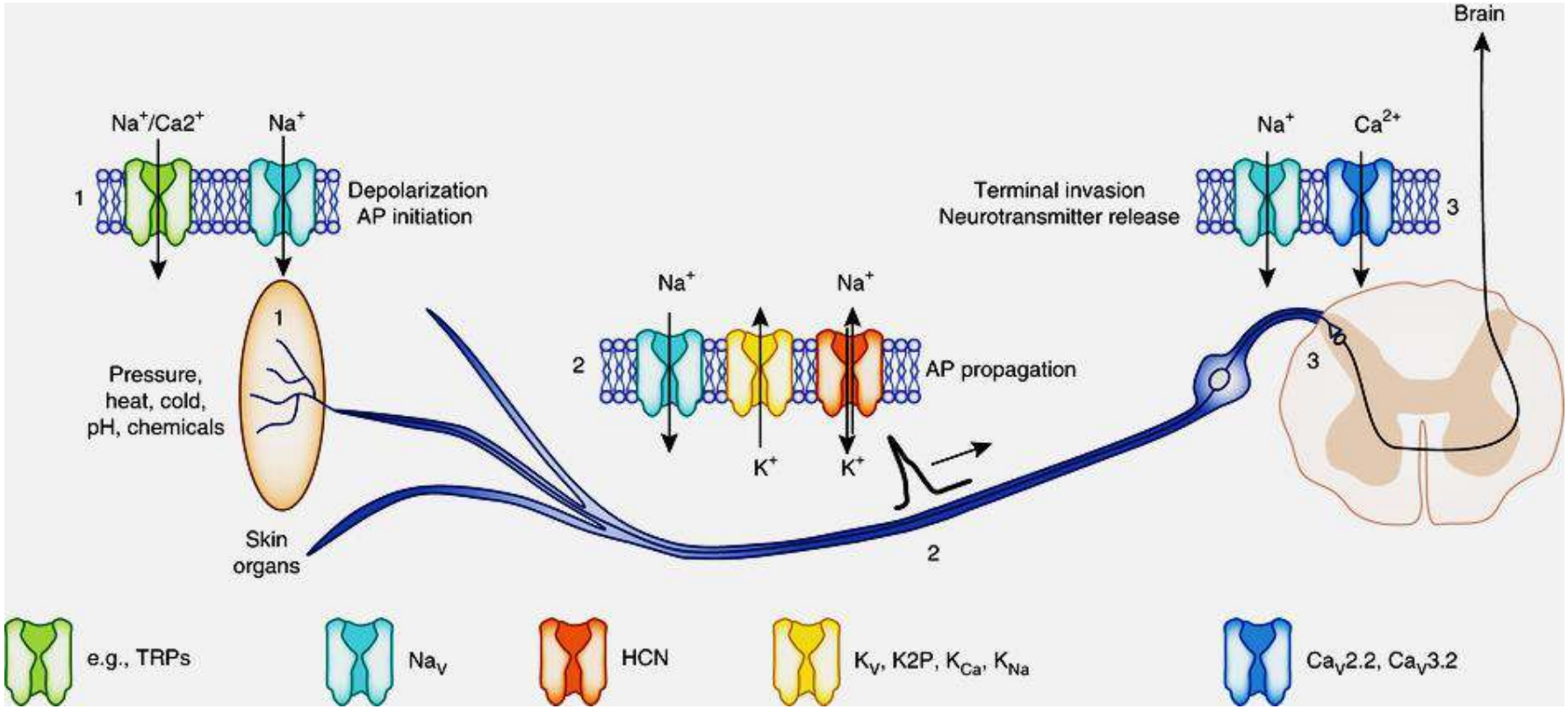
- Chronic pain affects ~1.5 billion people globally; neuropathic pain affects up to 4.5% of the world population
- In the USA alone, at least 116 million adults suffer from chronic pain; the associated costs exceed $500 billion/year
- The pharmaceutical industry estimates that the global market in pain therapeutics will reach $600 billion this year

NOCICEPTIVE PATHWAYS



Bourinet et al. Physiol Rev 2014;94:81-140

ION CHANNEL TARGETS



Waxman & Zamponi Nature Neuroscience 2014;17:153-163

ION CHANNEL TARGETS

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Channel	Gene	Current type	Compound	Status
Na_V1		Na^+ current family	CNV1014802 (Convergence) TV-45070 (Teva/Xenon) DSP 2230 (Dainippon Sumitomo) Tetrodotoxin (Wex)	Phase II Phase II Phase I Phase III
$Na_V1.7$	*SCN9A*	TTX-sensitive rapidly inactivating Na^+ current	PF-05089771 (Pfizer) AZD3161 (AstraZeneca) GDC-0276 (Genentech/Xenon) GDC-0310 (Genentech/Xenon)	Phase I Phase II Phase I Phase I
$Na_V1.8$	*SCN10A*	TTX-resistant slowly inactivating Na^+ current	VX-150 (Vertex)	Phase I
$Na_V1.9$	*SCN11A*	TTX-resistant persistent Na^+ current		
$Na_V1.1$	*SCN1A*	TTX-sensitive rapidly inactivating Na^+ current		
$Ca_V2.2$	*CACNA1B*	N-type Ca^{2+} current	Ziconotide CNV2197944 (Convergence) Z160 (Epirus) TROX-1 (Grünenthal) Gabapentin (acts via $Ca_V\alpha2\delta$ subunit) Pregabalin (acts via $Ca_V\alpha2\delta$ subunit)	Approved Phase II Failed Phase II Preclinical Approved Approved
$Ca_V3.2$	*CACNA1H*	T-type Ca^{2+} current	Ethosuximide Z944 (Epirus) ABT-639 (AbbVie) TTA-A2/TTA-P2	Approved Phase II Phase II Preclinical

ION CHANNEL TARGETS

University of Reading

Channel	Gene	Current type	Compound	Status
$K_V1.2$	*KCNA2*	Non-inactivating K^+ current		
$K_V1.4$	*KCNA4*	A-type K^+ current		
$K_V3.4$	*KCNC4*	A-type K^+ current		
$K_V4.2$	*KCND2*	A-type K^+ current		
$K_V4.3$	*KCND3*	A-type K^+ current		
$K_V7.2$	*KCNQ2*	M-type K^+ current	Retigabine Flupirtine	Approved/Phase II for pain Phase II for pain
$K_V7.3$	*KCNQ3*	M-type K^+ current	Retigabine Flupirtine	Approved/Phase II for pain Phase II for pain
$K_V9.1$	*KCNS1*	Does not support currents, but regulates $K_V2.1$ delayed rectifier K^+ current		

ION CHANNEL TARGETS

University of Reading

Channel	Gene	Current type	Compound	Status
TRPV1	*TRPV1*	Non-selective cation current	Capsaicin cream (NGX-4010) or local patch (Qutenza) (antagonists have issues with hyperthermia)	Approved
TRPA1	*TRPA1*	Non-selective cation current	HX-100 (Hydra Biosciences)	Phase I
HCN1	*HCN1*	Hyperpolarization-activated cation current		
HCN2	*HCN2*	Hyperpolarization-activated cation current		
Ano1	*ANO1*	Ca^{2+}-activated Cl^- current		
BK	*KCNMA1*	Ca^{2+}-activated K^+ current		
SK1	*KCNN1*	Ca^{2+}-activated K^+ current		
IK	*KCNN4*	Ca^{2+}-activated K^+ current		
TREK-1	*KCNK2*	Leak K^+ current		
TASK-2	*KCNK3*	Leak K^+ current		
TRESK	*KCNK18*	Leak K^+ current		

Modulation of voltage-gated Ca^{2+} channels by Small Ubiquitin-like Modifier (SUMO) protein

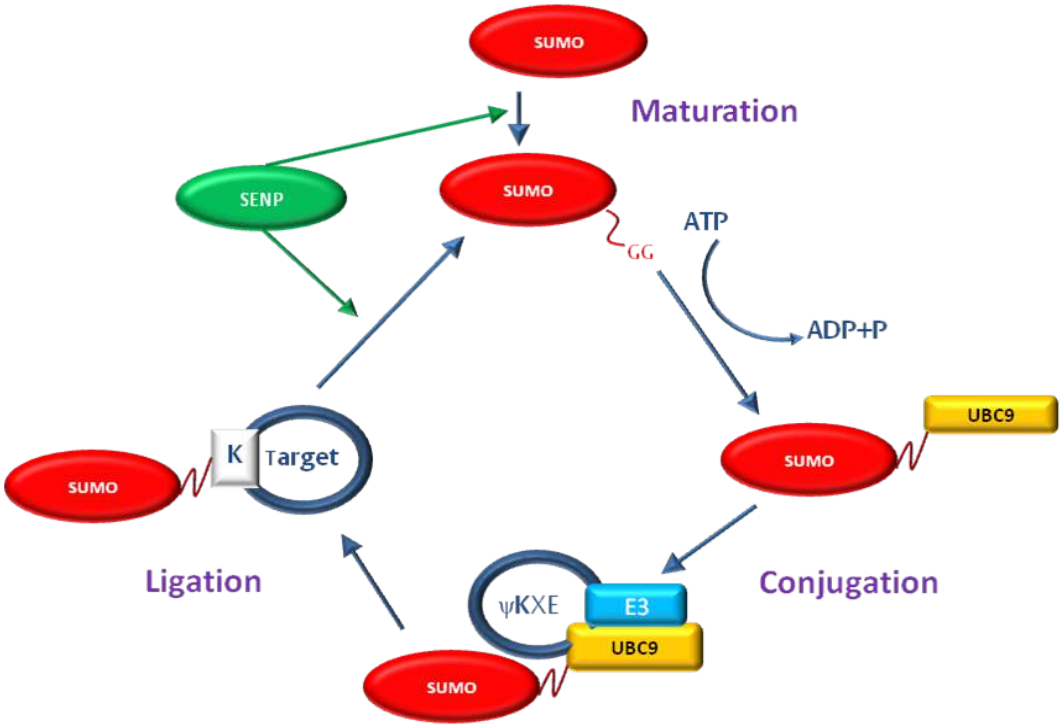
$Ca_V2.2$ (N-type) voltage-dependent calcium channels are targets for SUMOylation

Vasco C. Silveirinha[1], Hong Lin[1], Graeme S. Cottrell[1], Sumiko Mochida[2], Helena Cimarosti[1,3] and Gary J. Stephens[1]

[1]School of Pharmacy, University of Reading
[2]Dept of Physiology, Tokyo Medical University, Japan
[3]Universidade Federal de Santa Catarina, Florianópolis, Brazil

University of Reading

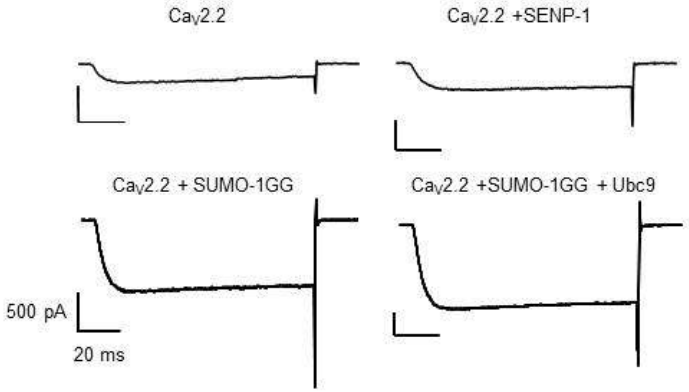
Modulation of voltage-gated Ca^{2+} channels by SUMOylation



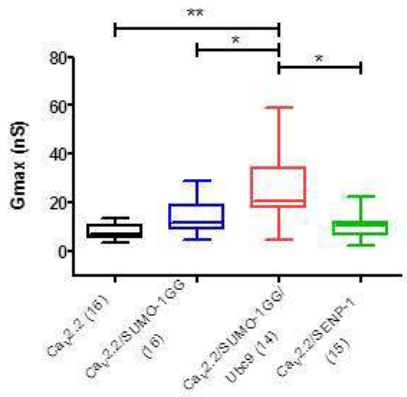
The SUMO-specific family of proteases, sentrin-specific proteases SENP, maturate SUMO proteins revealing a double-glycine motif, which allows for SUMO to interact with target proteins. The conjugating enzyme Ubc9 facilitates this conjugation before the SENP family of proteases terminates the ligation.

SUMO-1 increases $Ca_V2.2$ Ca^{2+} current density

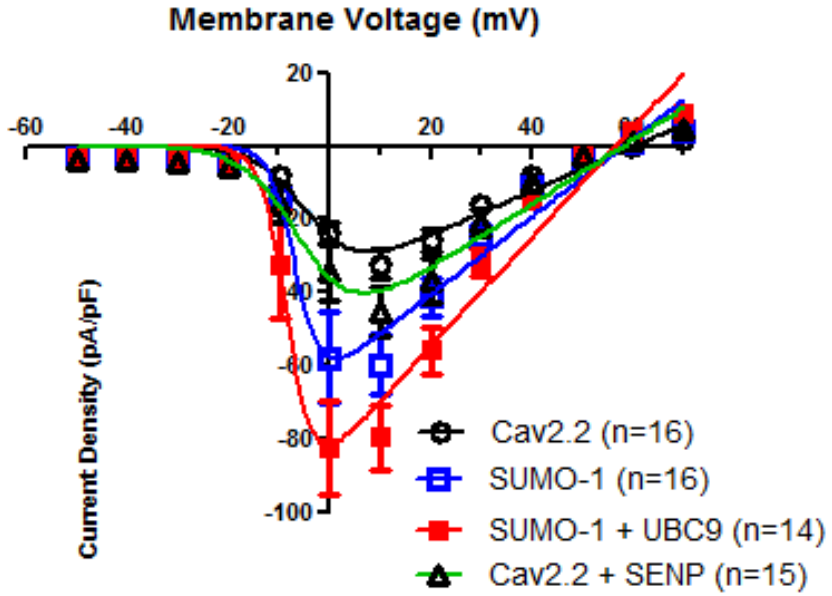
A



B

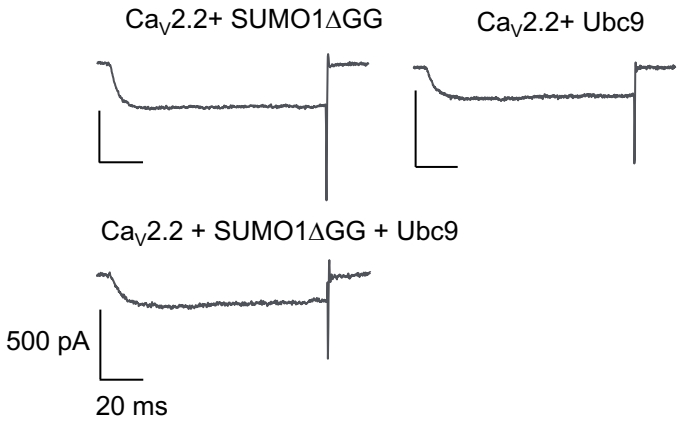


C



SUMO-1ΔGG has no effect on $Ca_V2.2$ Ca^{2+} current density

A



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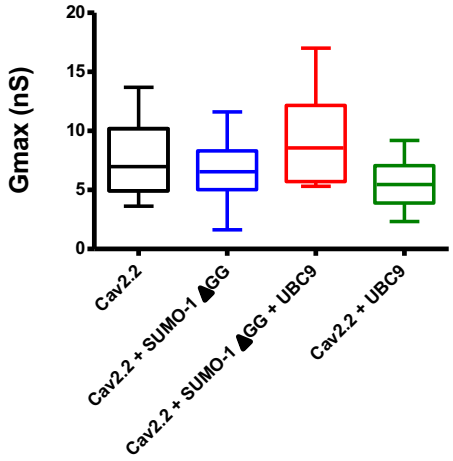
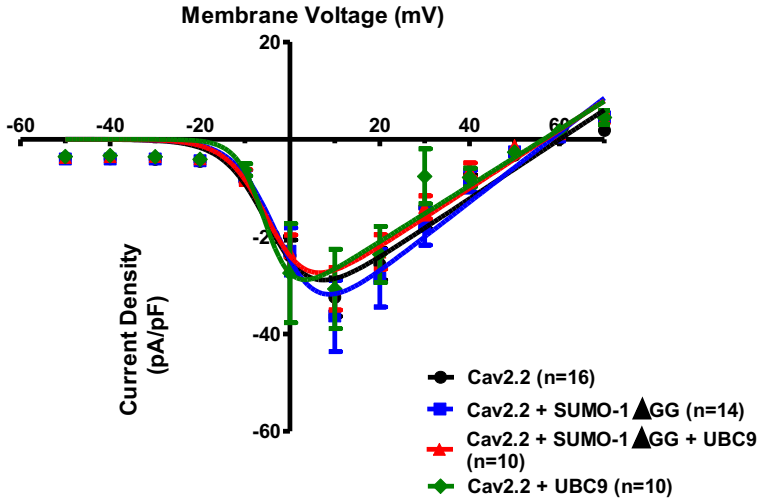
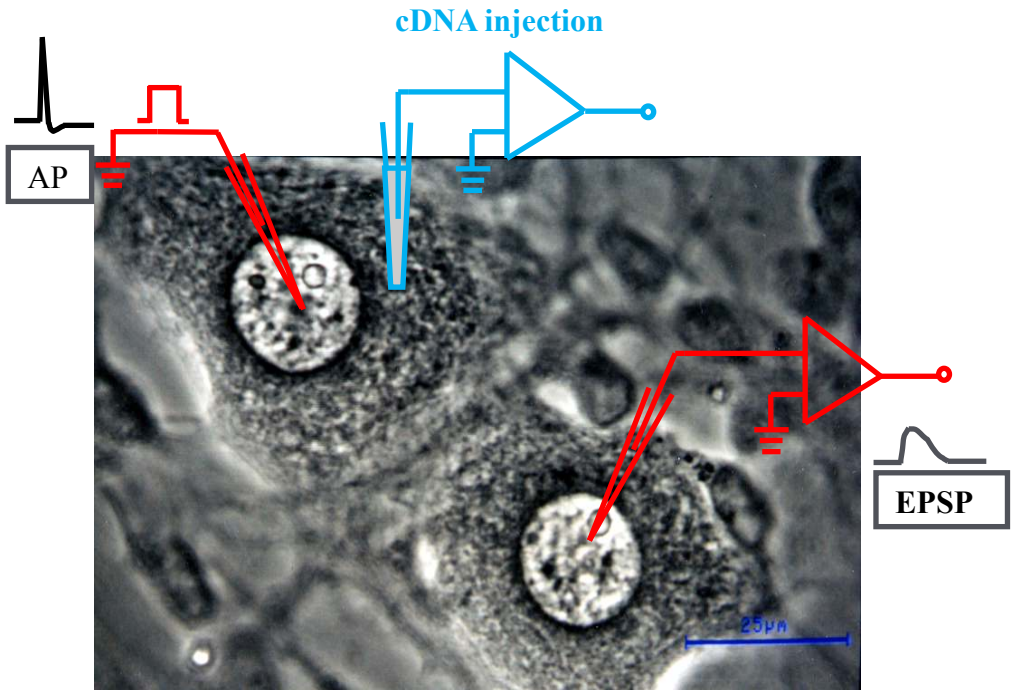


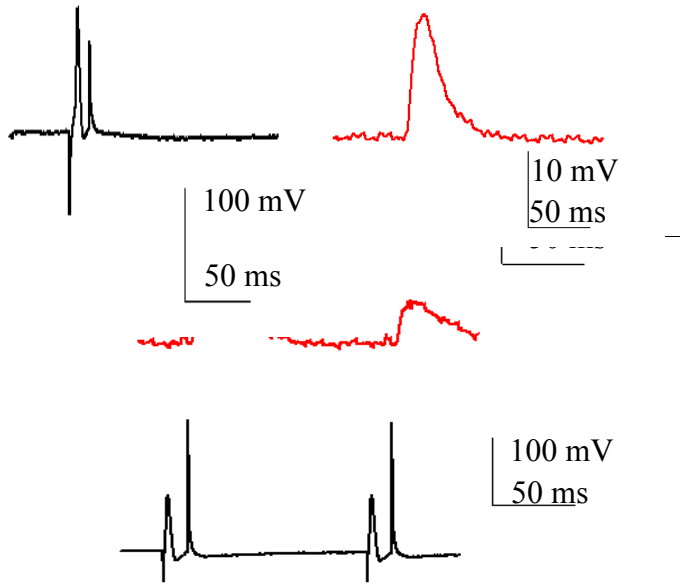
C



SUMOylation effects on synaptic transmission/plasticity in superior cervical ganglion (SCG) neurons

Japan Society for the Promotion of Science Short Term Fellowship Award (April 2016) for research sabbatical to visit Professor Sumiko Mochida, Tokyo Medical University





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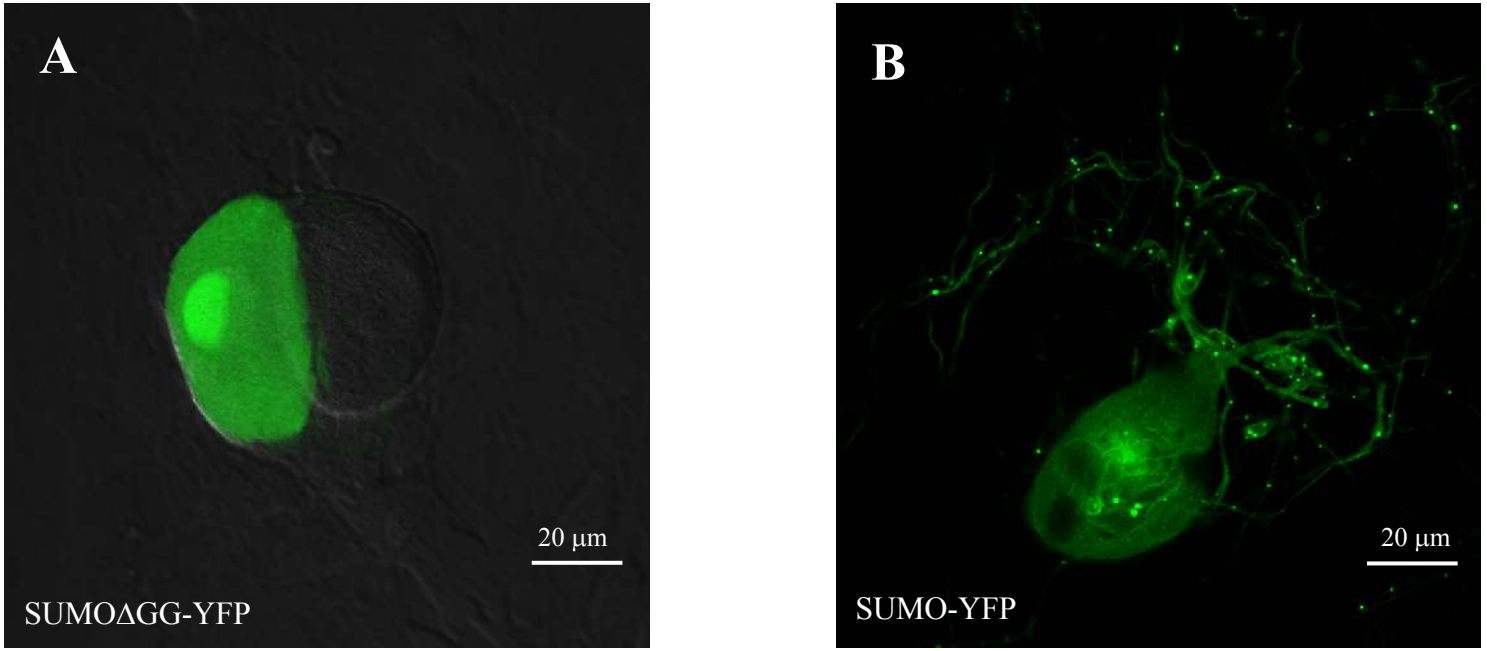
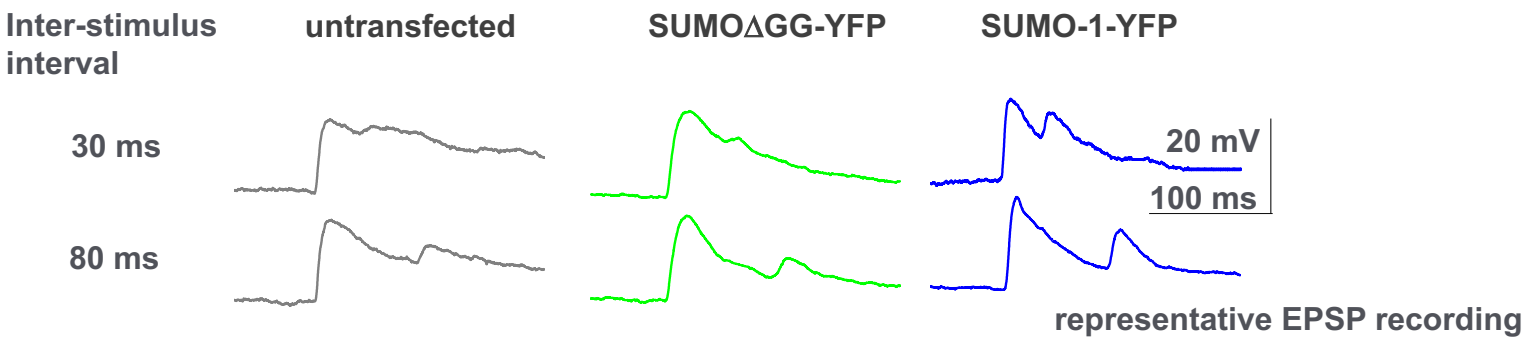
SUMOylation effects on synaptic transmission/plasticity

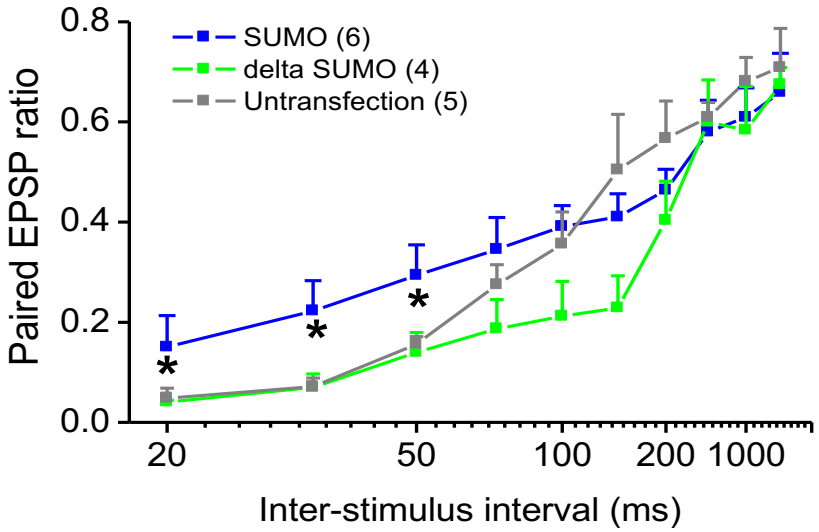


Confocal images of SCG neurons 48 h after injection with A) SUMOΔGG-YFP or B) SUMO-YFP constructs

SUMO increases paired EPSP ratio in SCG neurons



representative EPSP recording

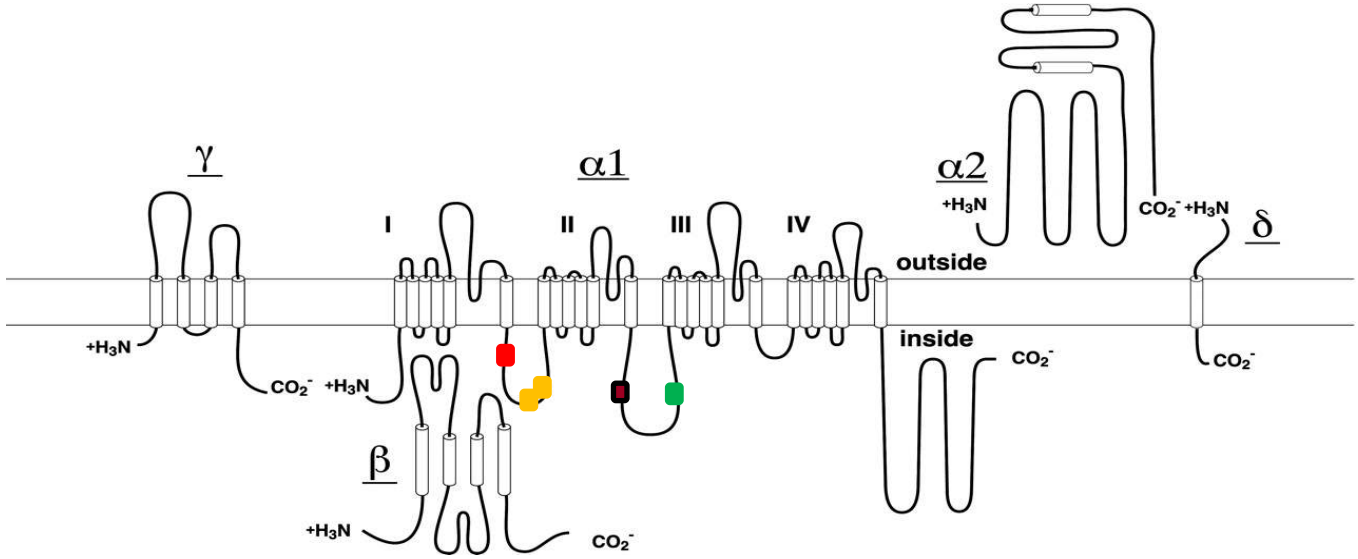


* p＜0.05 (one-way ANOVA)

Identification of potential $Ca_V2.2$ SUMOylation sites using SUMOplot™ and SUMOsp2.0™

Five high probability (> 65%) SUMO interaction motifs with the sequence Ψ-K-x-E, where ψ is a large hydrophobic residue, K is a lysine residue, x can be any residue and E is a (acidic) glutamic acid residue identified

Identification of potential $Ca_V2.2$ SUMOylation sites using SUMOplot™ and SUMOsp2.0™



= K394 – FKAE – I-II loop Gβγ interaction region

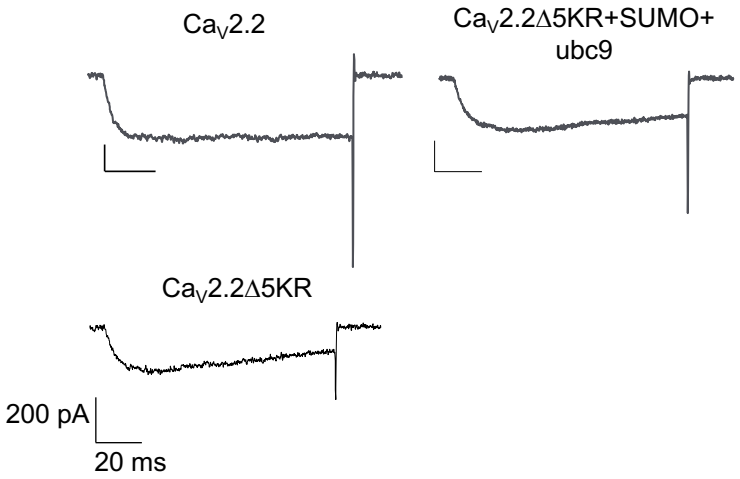
= K454/457 – LKSG/ GKTE - I-II loop

= K951 – AKGE – II/III loop

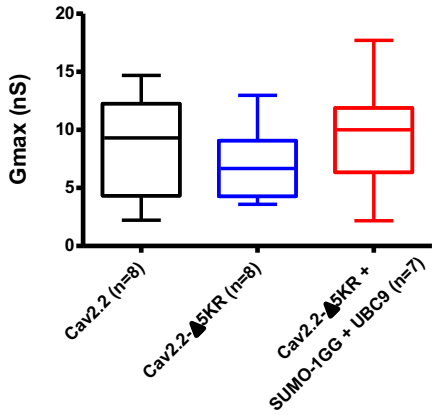
= K1108 – GKKE - II/III loop

SUMO has no effect on $Ca_V2.2\Delta5KR$ Ca^{2+} current density

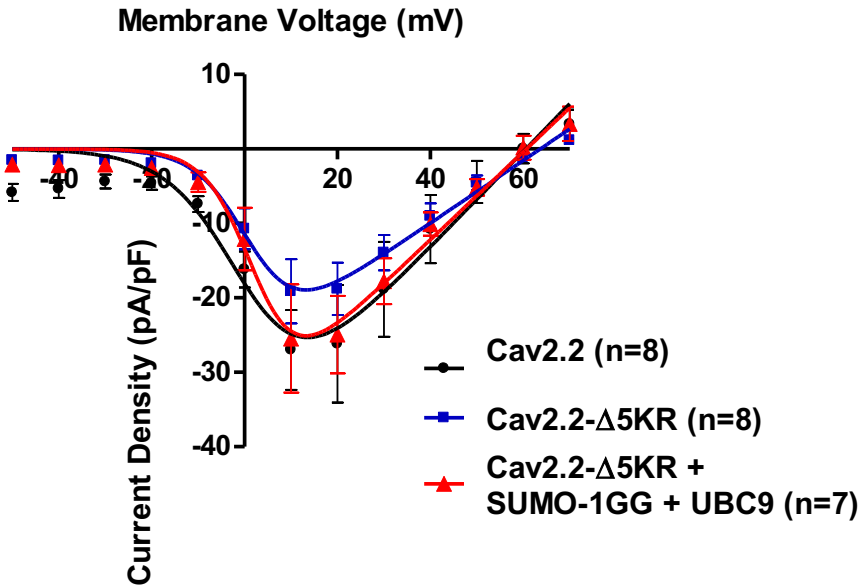
A



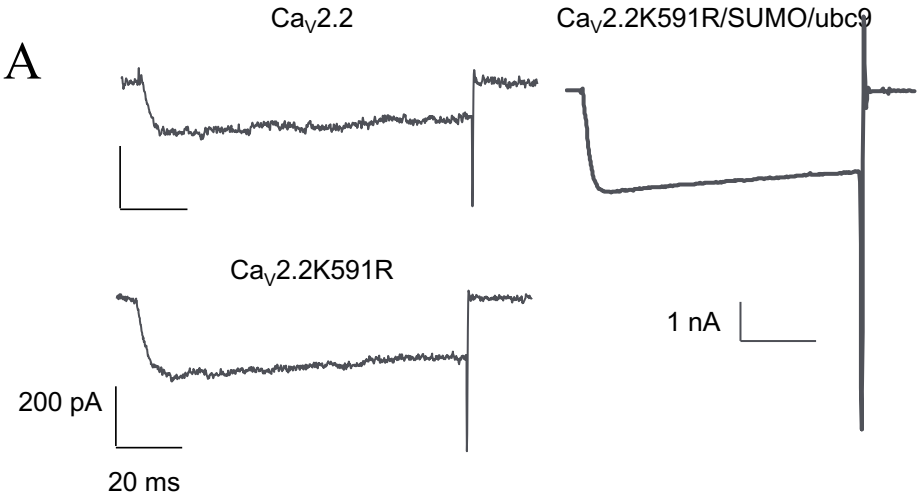
B

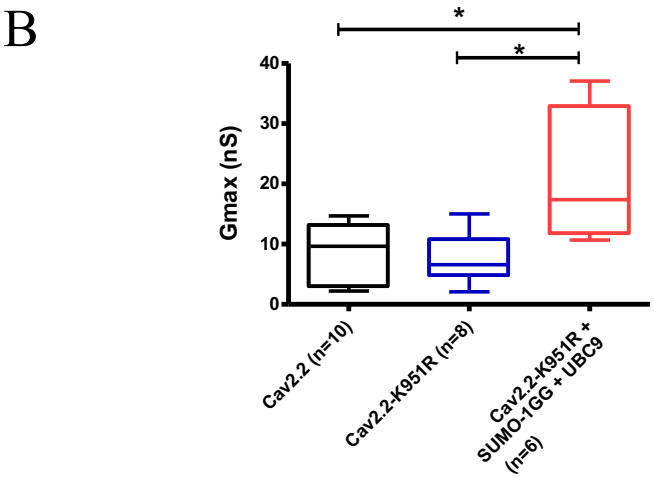


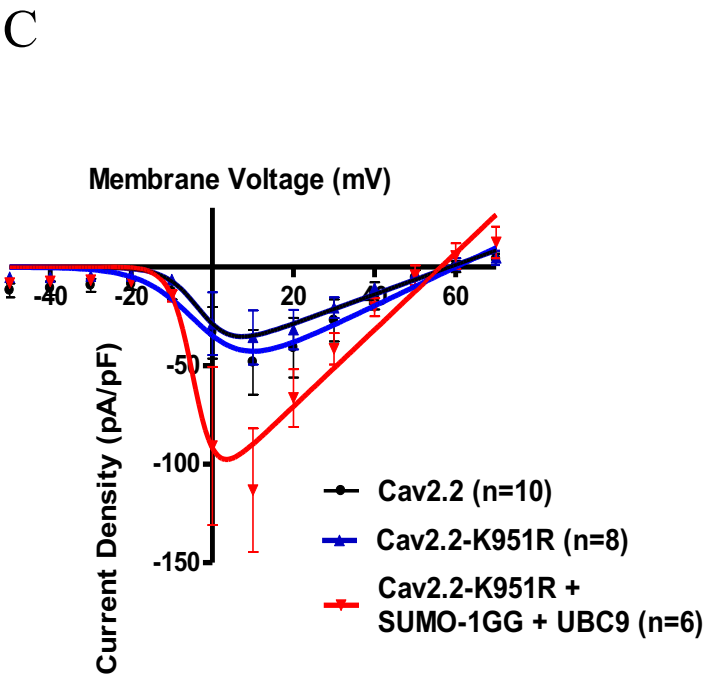
C



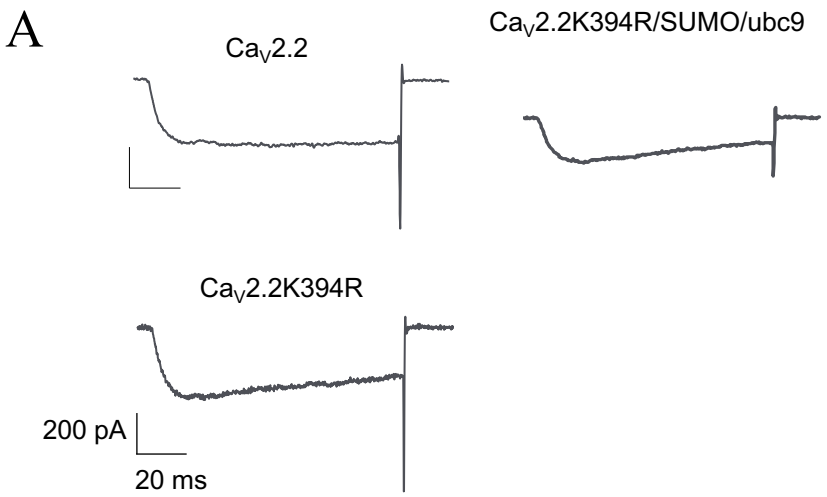
SUMO increases $Ca_V2.2K951R$ Ca^{2+} current density

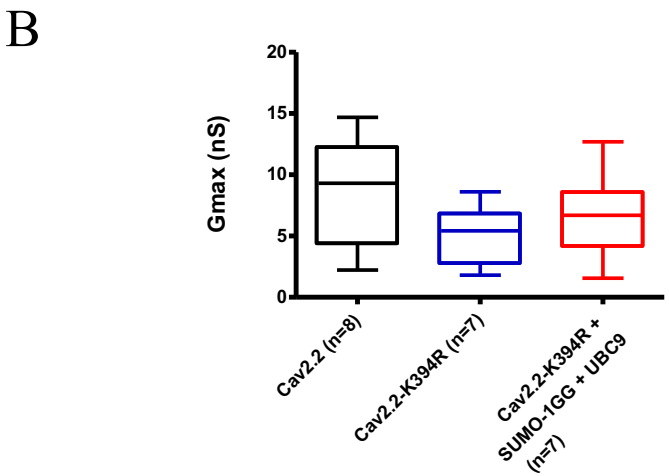


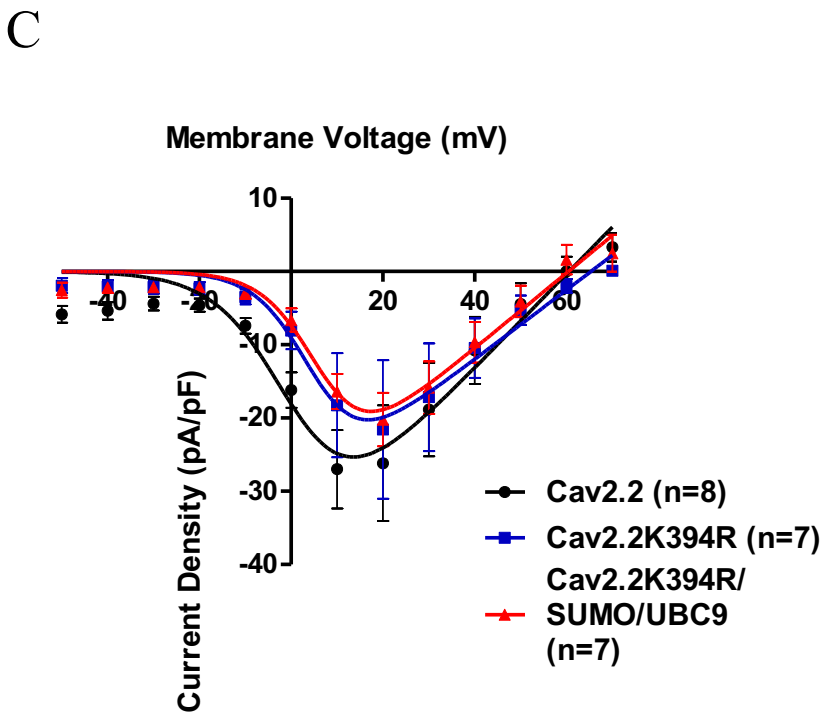




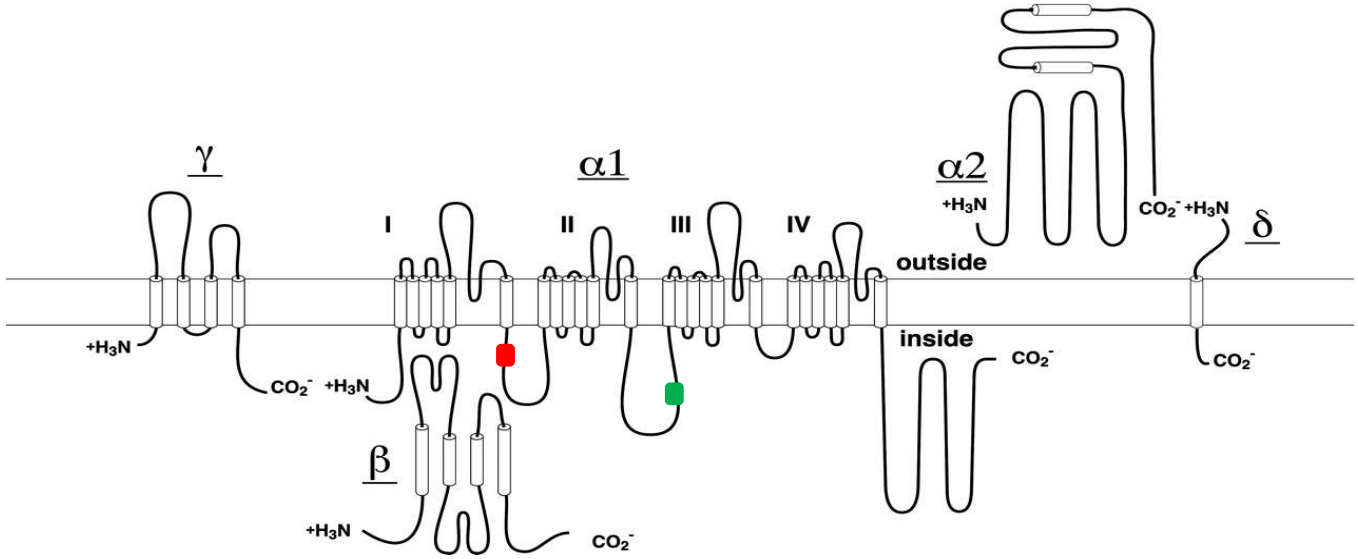
SUMO has no effect on $Ca_V2.2K394R$ Ca^{2+} current density







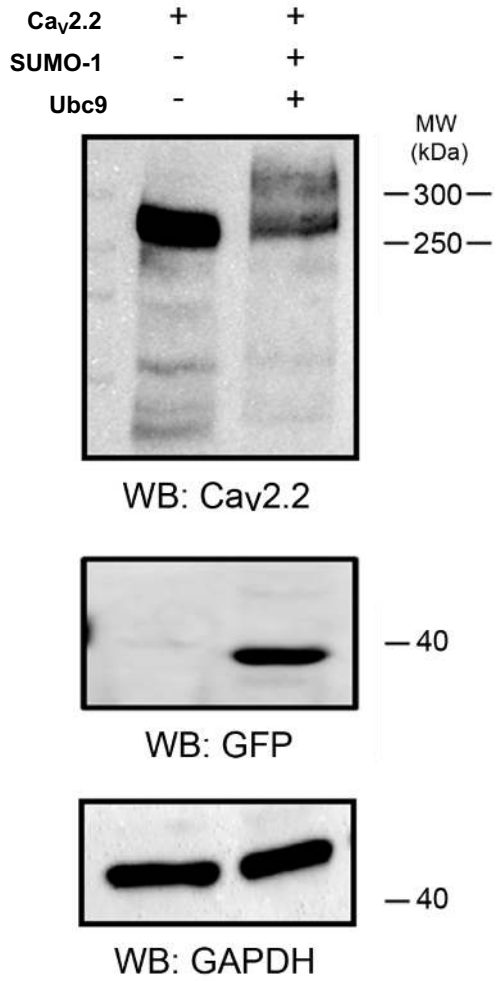
$Ca_V2.2$K394, but not $Ca_V2.2$K1108 is a molecular determinant for SUMOylation



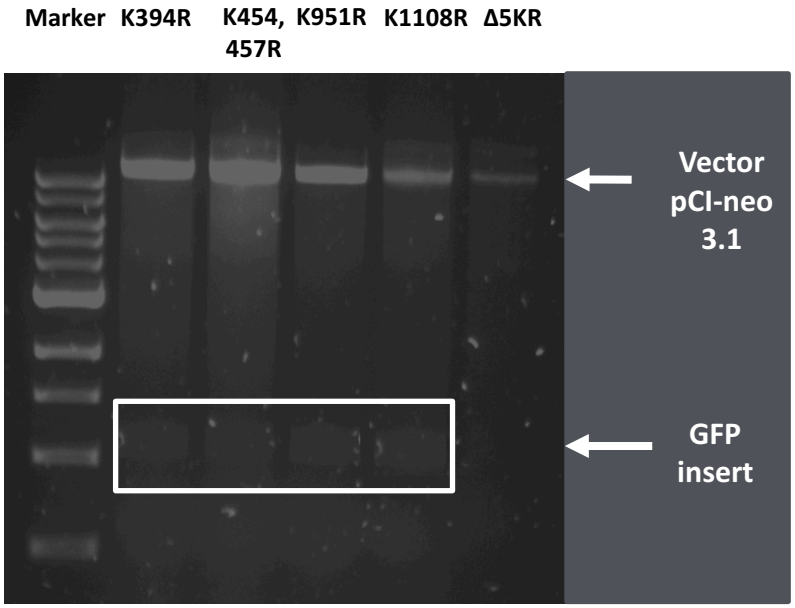
= K394 – FKAE – I-II loop Gβγ interaction region

= K1108 – GKKE - II/III loop

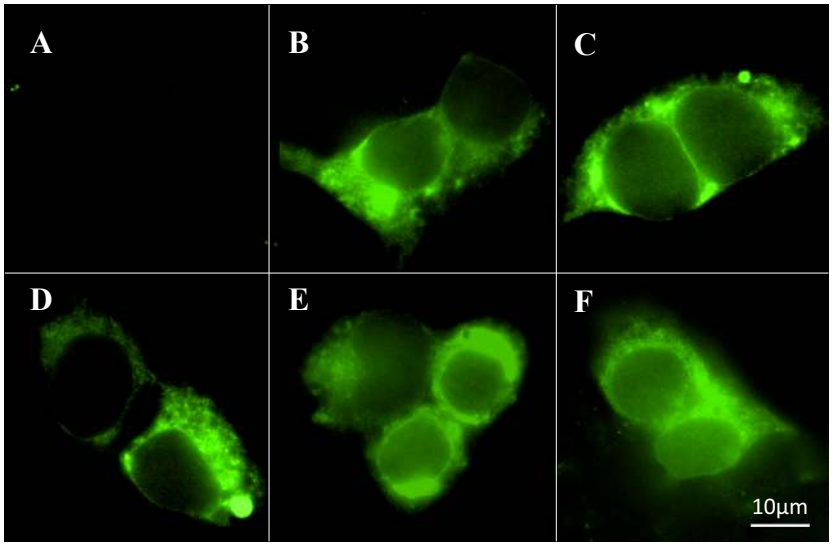
SUMOylation is associated with biochemical changes



Tools to investigate $Ca_V2.2$/SUMO function:



DNA electrophoresis of mutant maxipreps



Microscopy of the **GFP-tagged mutants.**
A) vector control, B) WT $Ca_V2.2$, C) $Ca_V2.2$ K394R, D) $Ca_V2.2$ K454,7R E) $Ca_V2.2$ K951R, F) $Ca_V2.2$ K1108R

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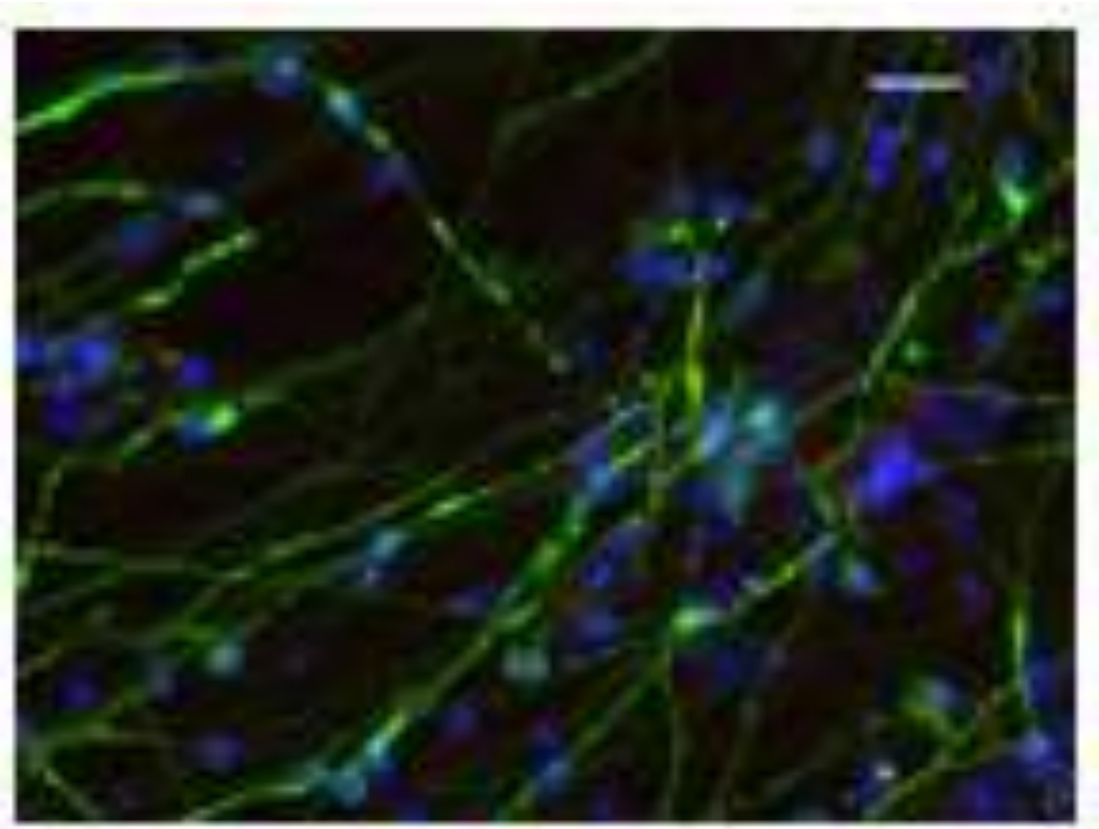
It's not just electrophysiology…….



Neuronal subtype specification of differentiating hippocampal progenitor (HPC03A/07) stem cells after 50 days in vitro

Blue: DAPI (DNA stain)

Green: β tubulin

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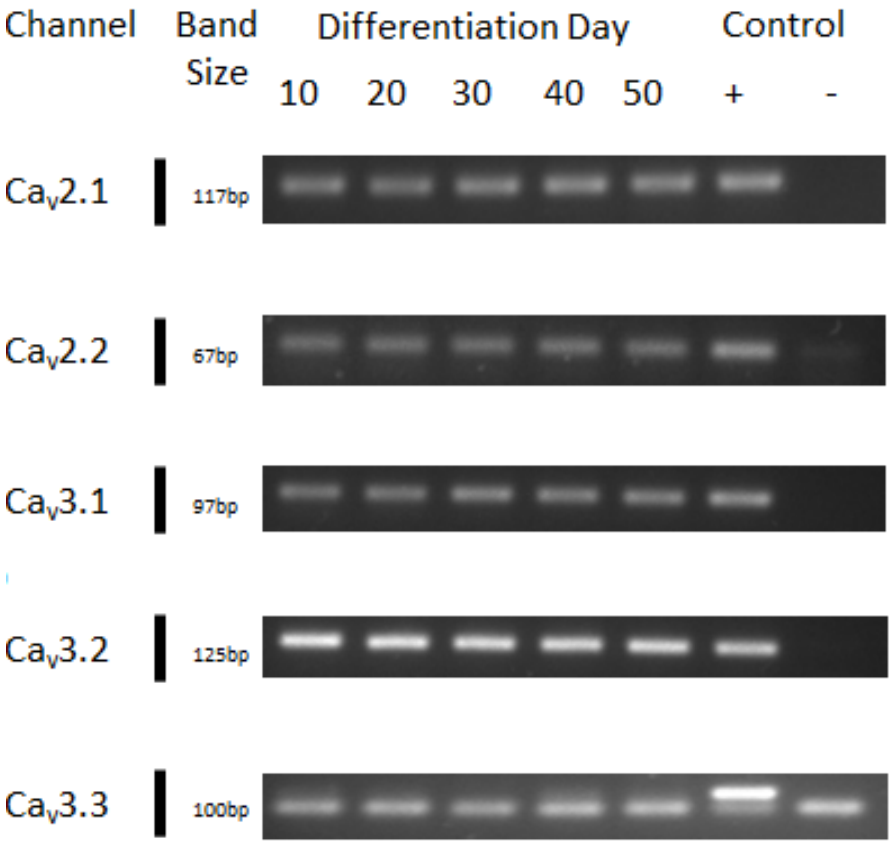
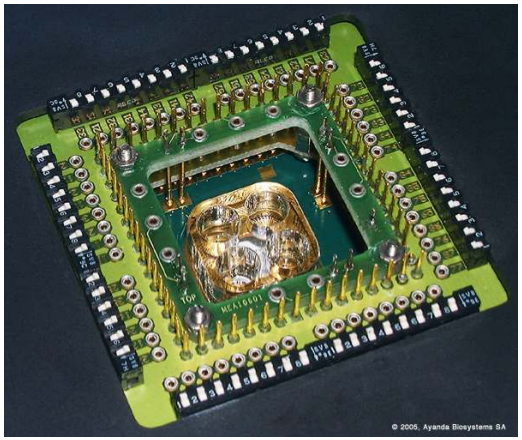
It's not just electrophysiology…….

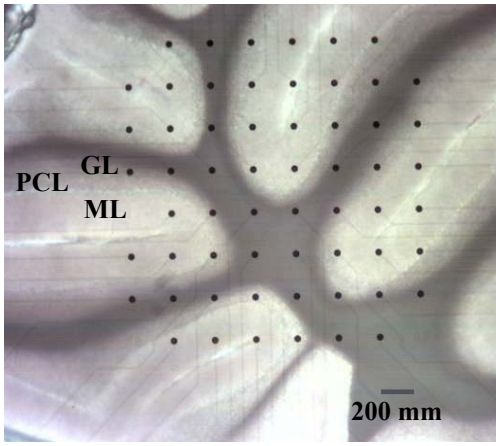


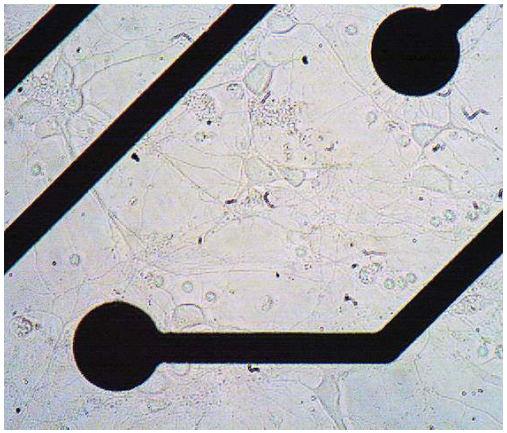
Expression of Cav channel mRNA in differentiating HPC03A/07 stem cells

Multi-electrode array recording in the CNS (Ben Whalley group)

- The information provided by single electrode extracellular recording can be improved by increasing electrode number using planar multi-electrode arrays (**MEA**)







- Simultaneous acquisition across ~60 channels allows description of spatio-temporal information.

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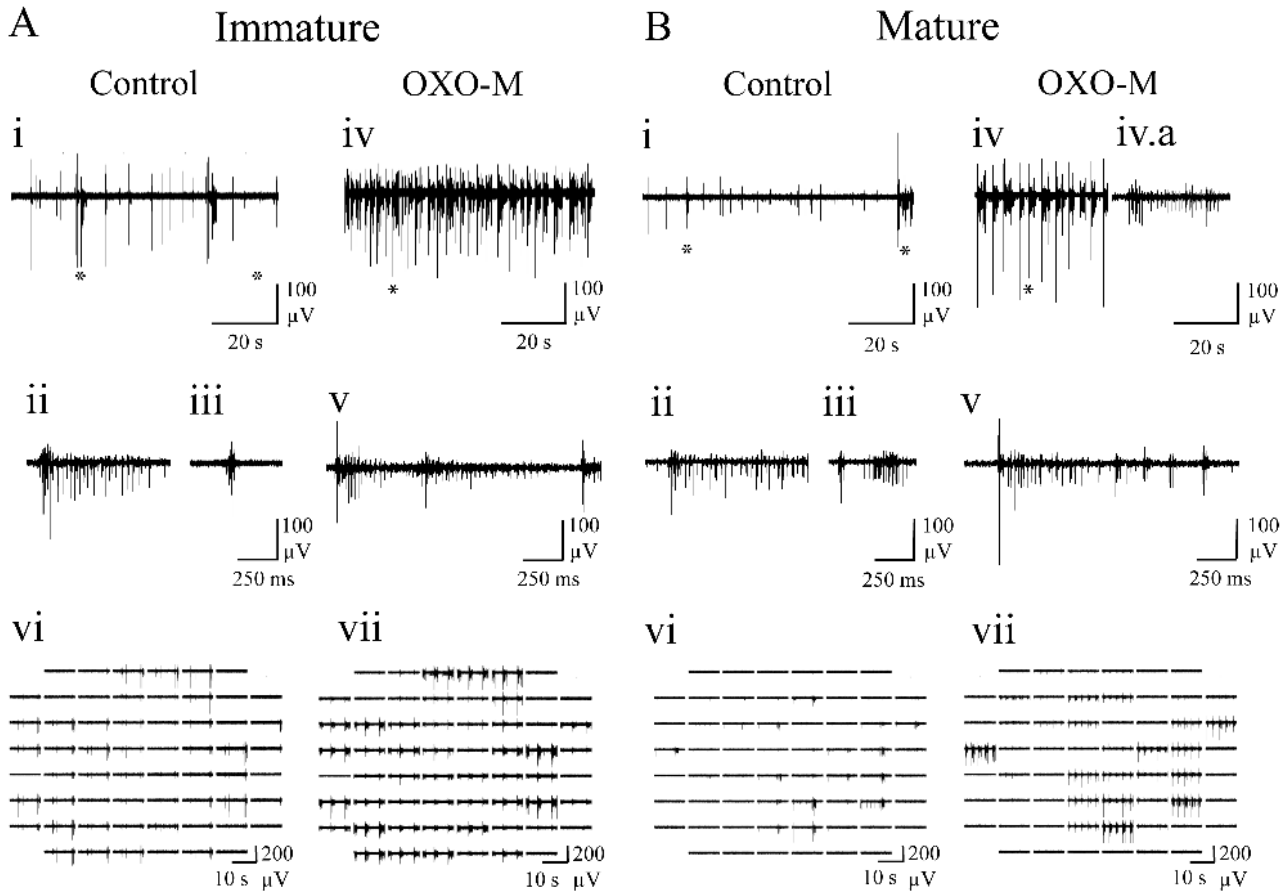
Multi-electrode array recording in the CNS



Hammond et al. BMC Neurosci 2013 Mar 26;14:38

Pharmacology Group, Hopkins Building