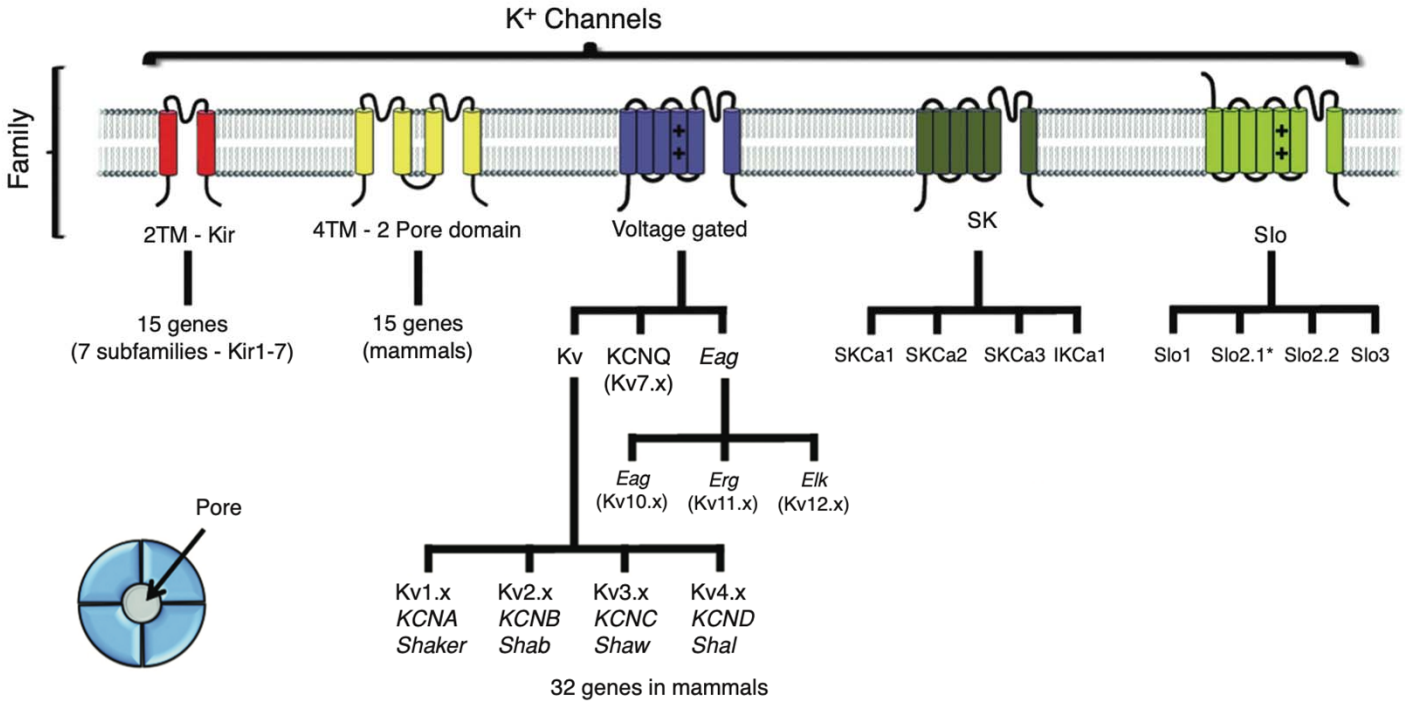
Potassium Channels

K+ Channels

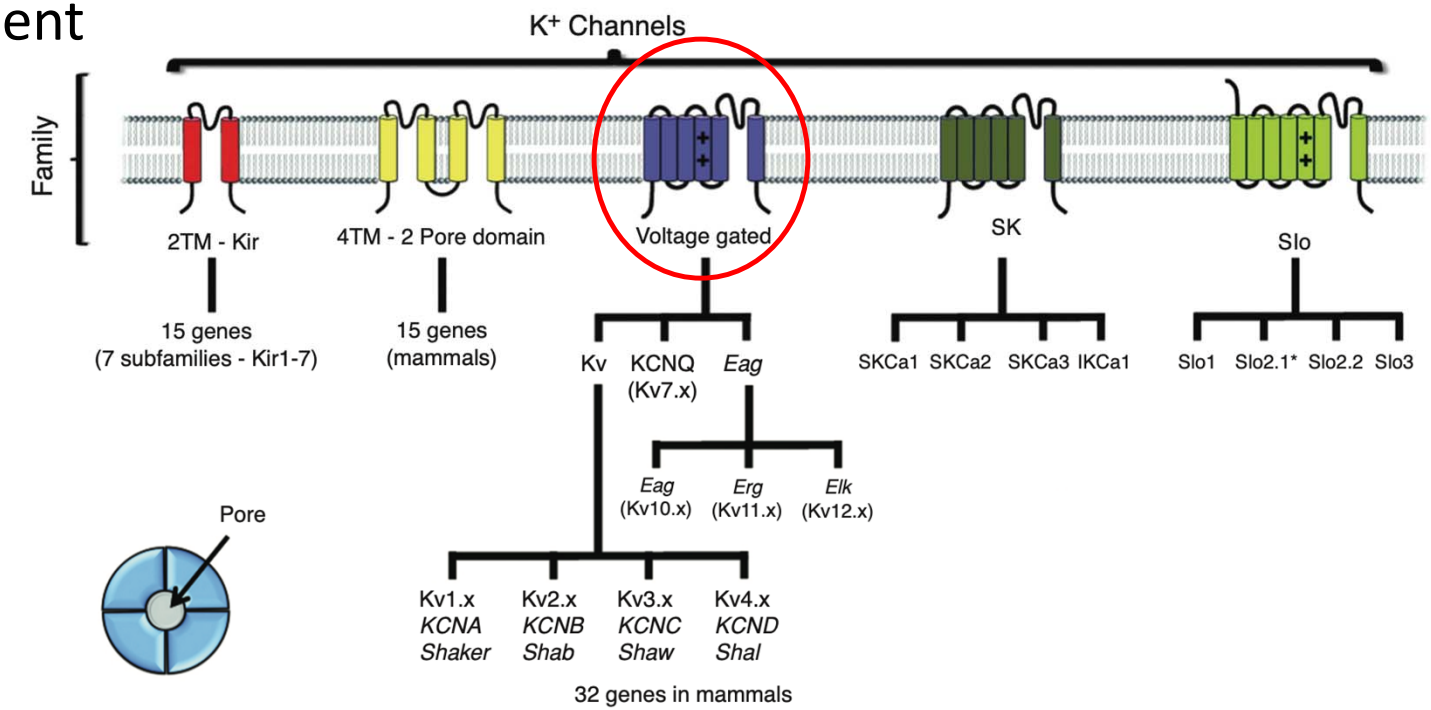
- Many different types of potassium channels



Voltage Gated K+ Channels

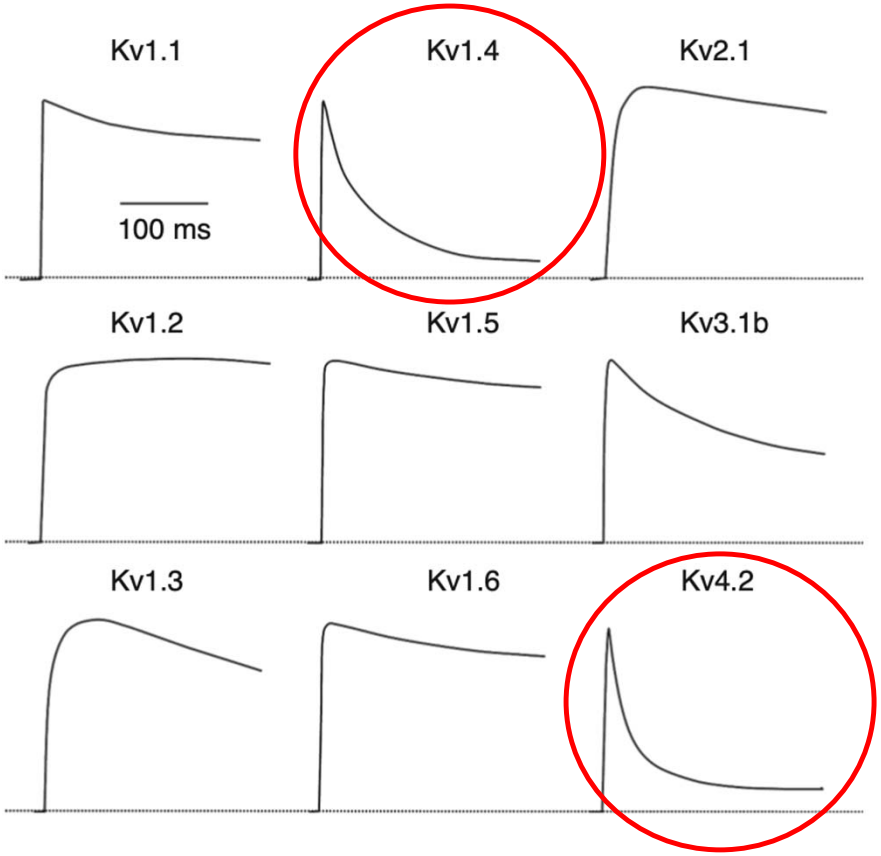
- A.K.A. Voltage-Dependent (Kv) Channels

- Two functional types:
  - A-type (inactivating)
  - Delayed rectifier (noninacitvating)



A-type Kv1 Channels

- N-type inactivating
- Include Kv1.4, Kv3.3, Kv3.4, Kv4.1, Kv4.2, and Kv4.3
- Recovers slowly from inactivation
- Primarily located in cell bodies and axons
- Relatively insensitive to TEA



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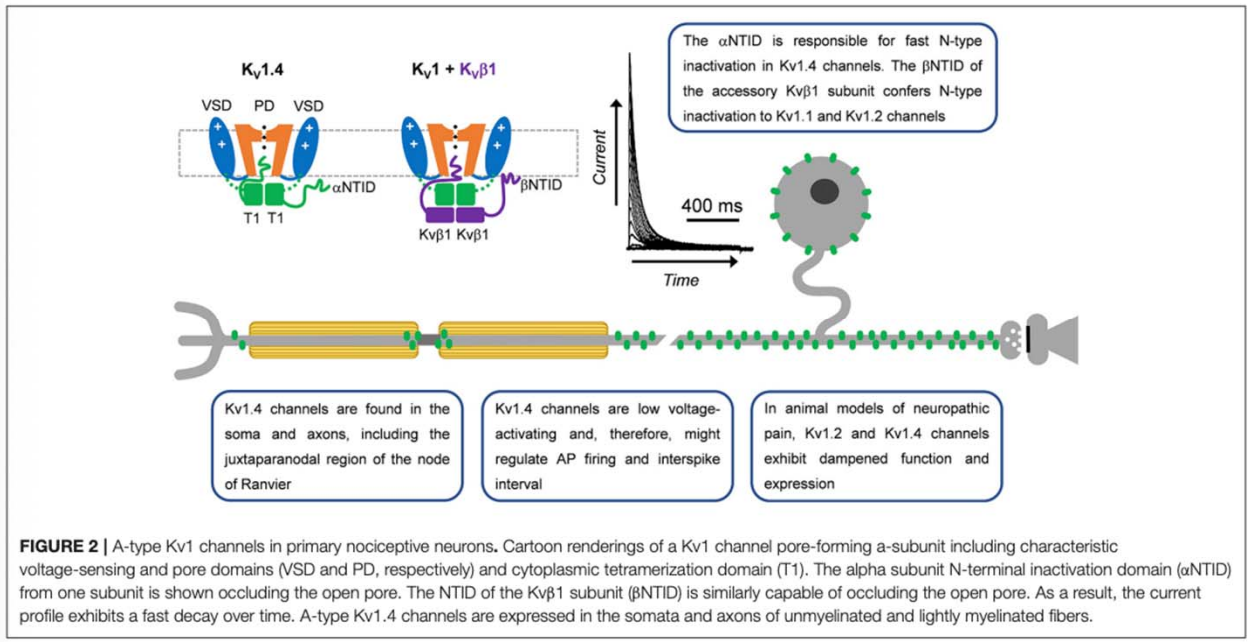
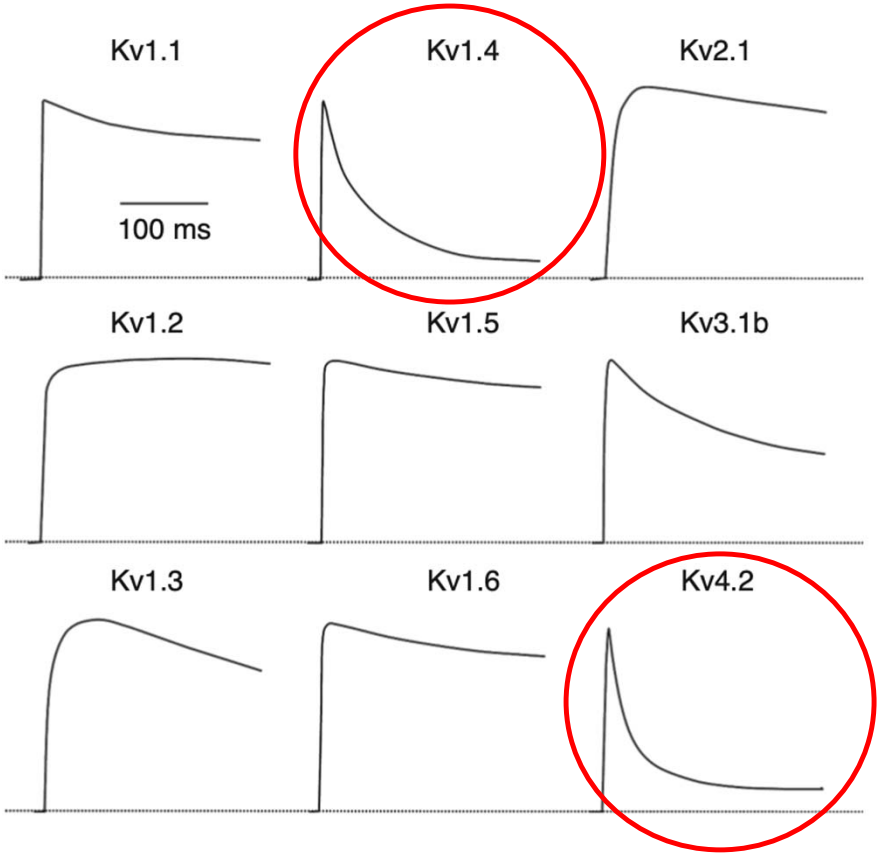


FIGURE 2 | A-type Kv1 channels in primary nociceptive neurons**.** Cartoon renderings of a Kv1 channel pore-forming a-subunit including characteristic voltage-sensing and pore domains (VSD and PD, respectively) and cytoplasmic tetramerization domain (T1). The alpha subunit N-terminal inactivation domain (αNTID) from one subunit is shown occluding the open pore. The NTID of the Kvβ1 subunit (βNTID) is similarly capable of occluding the open pore. As a result, the current profile exhibits a fast decay over time. A-type Kv1.4 channels are expressed in the somata and axons of unmyelinated and lightly myelinated fibers.

(Zemel et al., 2018)

A-type Kv Channels

- Include Kv1.4, Kv3.3, Kv3.4, Kv4.1, Kv4.2, and Kv4.3



A-type Kv4 Channels

- Non-N-Type Inactivating Channels
  - Instead bind K+ Channel Interacting Proteins (kCHIPs)
- Expressed in somatodendritic compartments
- Highly insensitive to TEA
- Recover quickly from inactivation

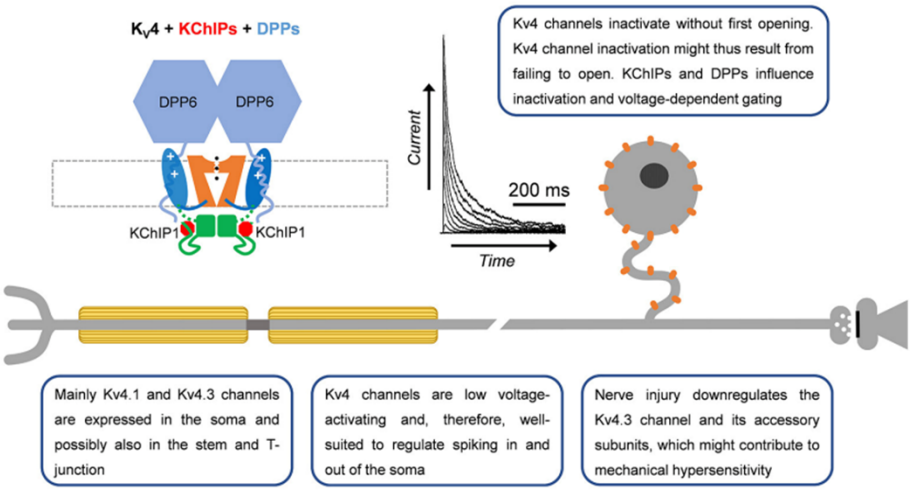
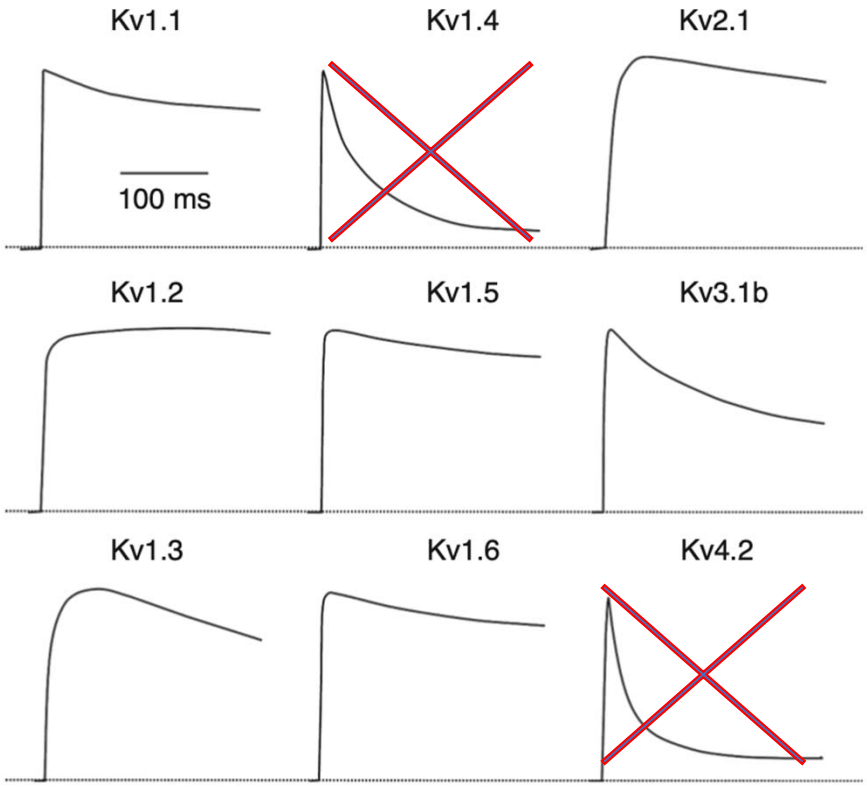


FIGURE 4 | A-type Kv4 channels in primary nociceptive neurons. Cartoon rendering of the Kv4 channel complex including the pore-forming a-subunit with its characteristic Kv channel functional domains (**Figure 2**; VSD, PD, and T1). Two distinct accessory subunits are also part of this complex: KChIPs and DPPs. Whereas KChIPs are cytoplasmic and bind to the vestigial αNTID to prevent N-type inactivation, DPPs are single-pass membrane spanning proteins that might interact with the VSD to determine the native voltage dependence of Kv4 channels. In addition, the cytoplasmic N-terminal region of DPP6 increases unitary conductance through long-range electrostatic interactions. Kv4.3 immunoreactivity has been detected mainly in somata of small-diameter DRG neurons. Based on the ability of Kv4 channels to regulate backpropagating APs in the CNS, and their particular subcellular localization in DRG neurons, they might act as 'shock absorbers' to actively regulate AP propagation into and out of the soma.

(Zemel et al., 2018)

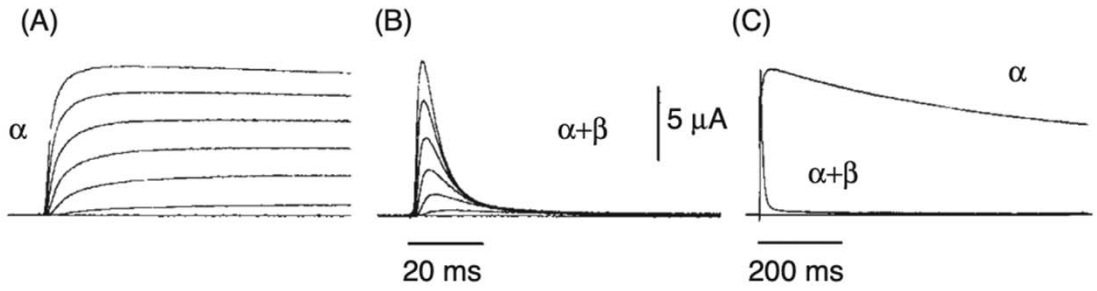
Delayed Rectifier Kv Channels

- Classic potassium channel
  - 1st ID'd by Hodgkin and Huxley in giant squid axon
  - Does not inactivate within msec
- Actions:
  - Shape action potential
  - Terminate action potential
  - Restore potassium permeability of resting membrane



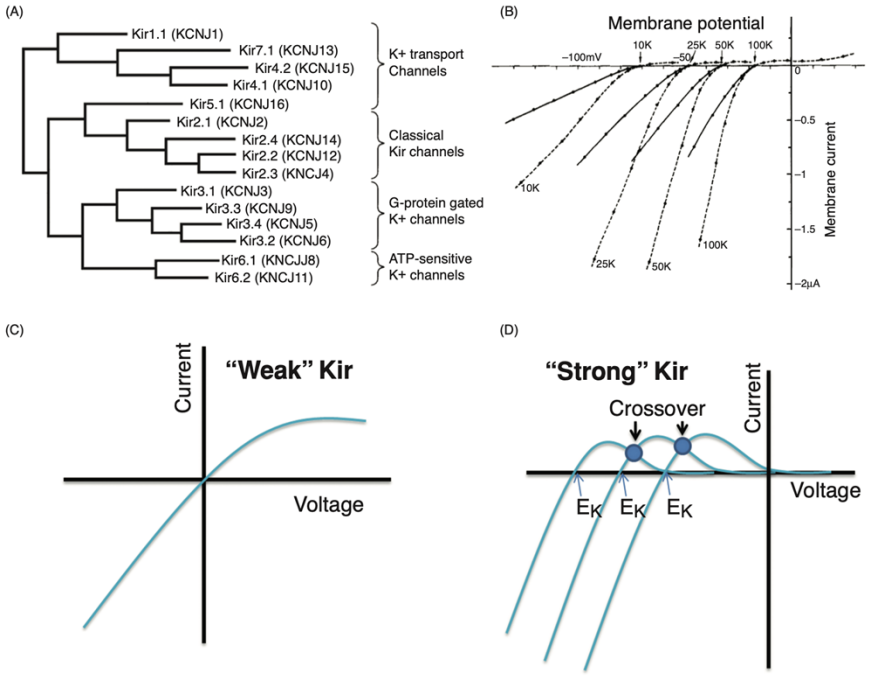
Delayed Rectifier Kv Channels

- Multimerization with β-subunits
  - can switch channel from delayed rectifying to inactivating
  - Kv1.1, Kv1.2, Kv1.3, and Kv1.5



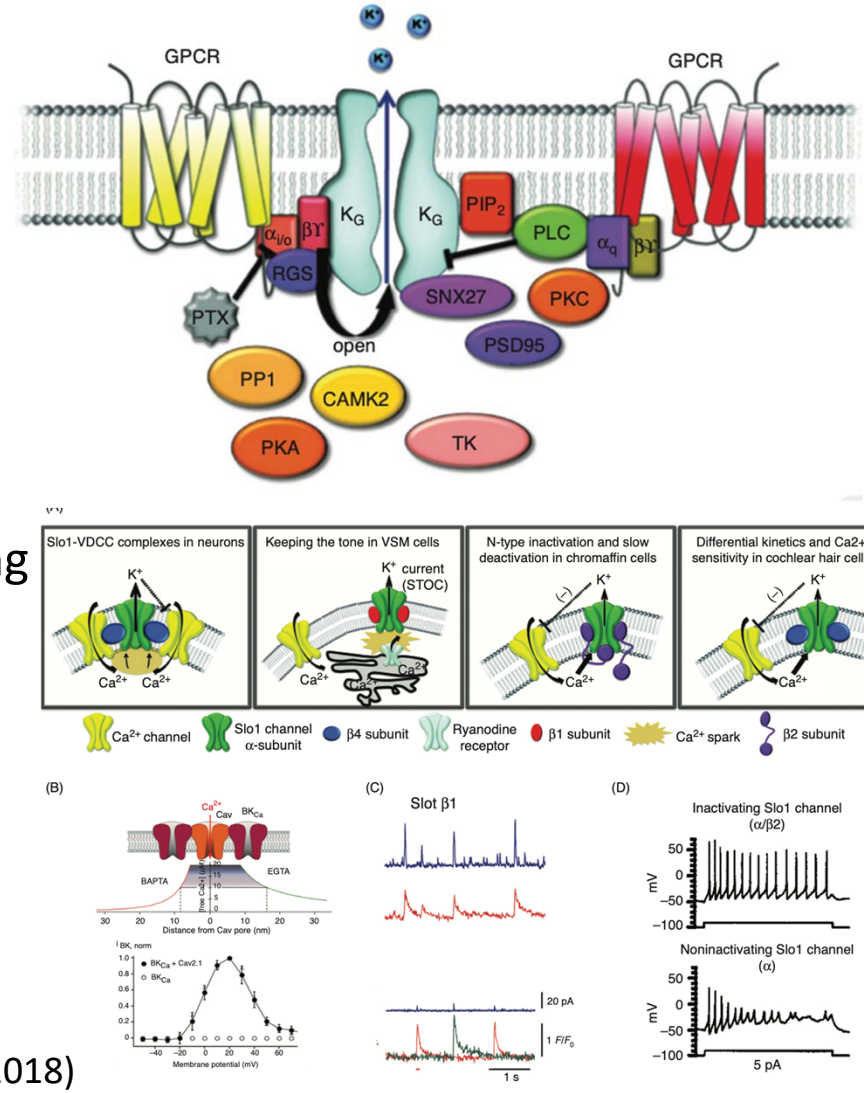
Inward Rectifier Kv Channels (Kir)

- First identified in skeletal muscle
- Greater flow of K+ into not out of the cell
  - Against the Nernst equation
  - Essentially voltage independent
- Cells with high proportion of Kir channels
  - RMP near E_K
  - Little to no spontaneous activity
- Regulates APs in electrically excitable cells
  - Such as cardiac muscle



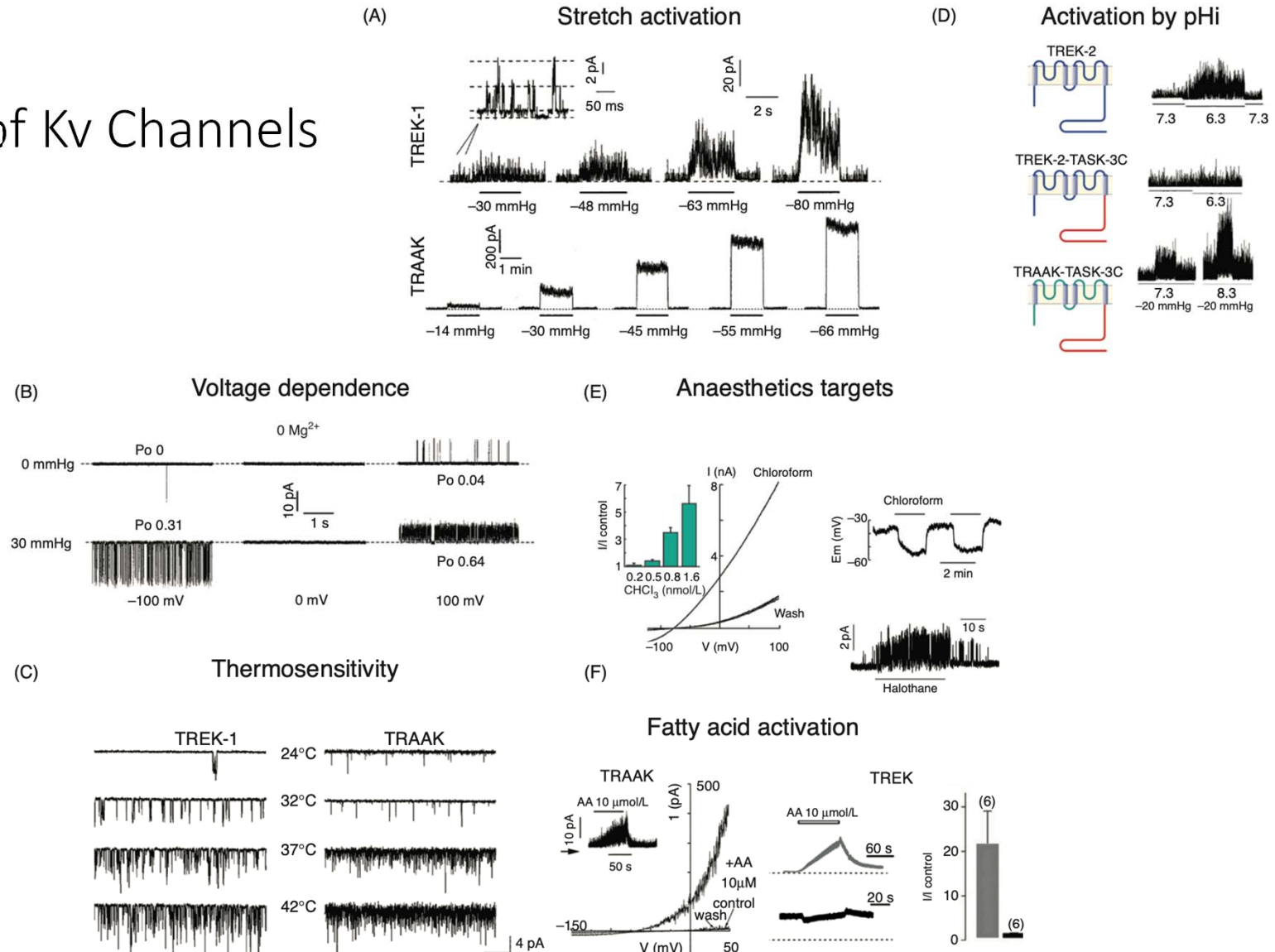
Other types of Kv Channels

- G-protein-gated channels (K_G or GIRK)
  - Activated by βγ-subunits
- ATP-sensitive channels (K_{ATP})
  - ATP inhibits opening, ADP stimulates opening
- Slo channels
  - Unusually large conductance
  - Activated by Ca2+ and voltage

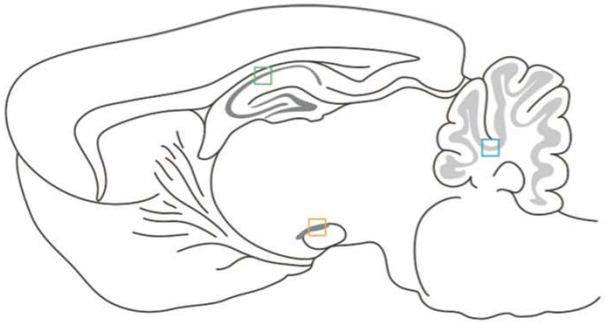


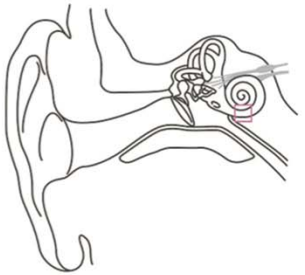
(Zemel et al., 2018)

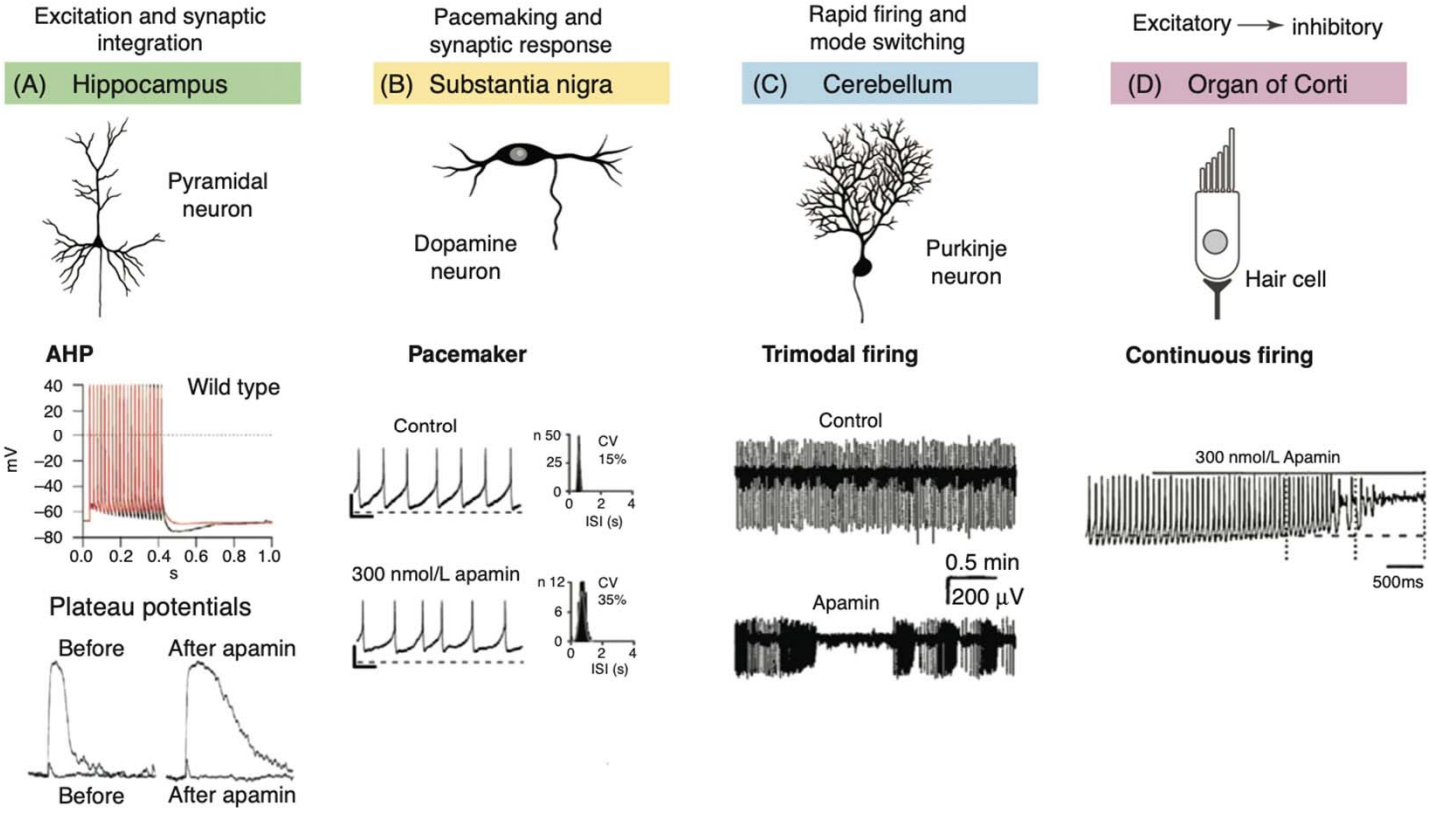
Other types of Kv Channels



Kv Channels & Physiology







Am J Physiol Heart Circ Physiol 313: H548–H557, 2017.
First published June 16, 2017; doi:10.1152/ajpheart.00216.2017.

RESEARCH ARTICLE | *Cardiovascular Neurohormonal Regulation*

A-type K^+ channels contribute to the prorenin increase of firing activity in hypothalamic vasopressin neurosecretory neurons

Soledad Pitra and Javier E. Stern

Department of Physiology, Medical College of Georgia, Augusta University, Augusta, Georgia

Submitted 18 April 2017; accepted in final form 12 June 2017

Background

- Prorenin (PR) stimulating its receptor
  a) Converts angiotensin I to II
  b) Initiates intracellular signaling, including MAPK and ERK1/2
- PR receptor is expressed in vasopressin neurons of the hypothalamus
- Recordings performed in vasopressin neurons in hypothalamus
  - Using eGFP-VP transgenic mice

PR inhibits A-type current (I_A) in a voltage-dependent way

A) Depolarizing steps pre and post application of PR

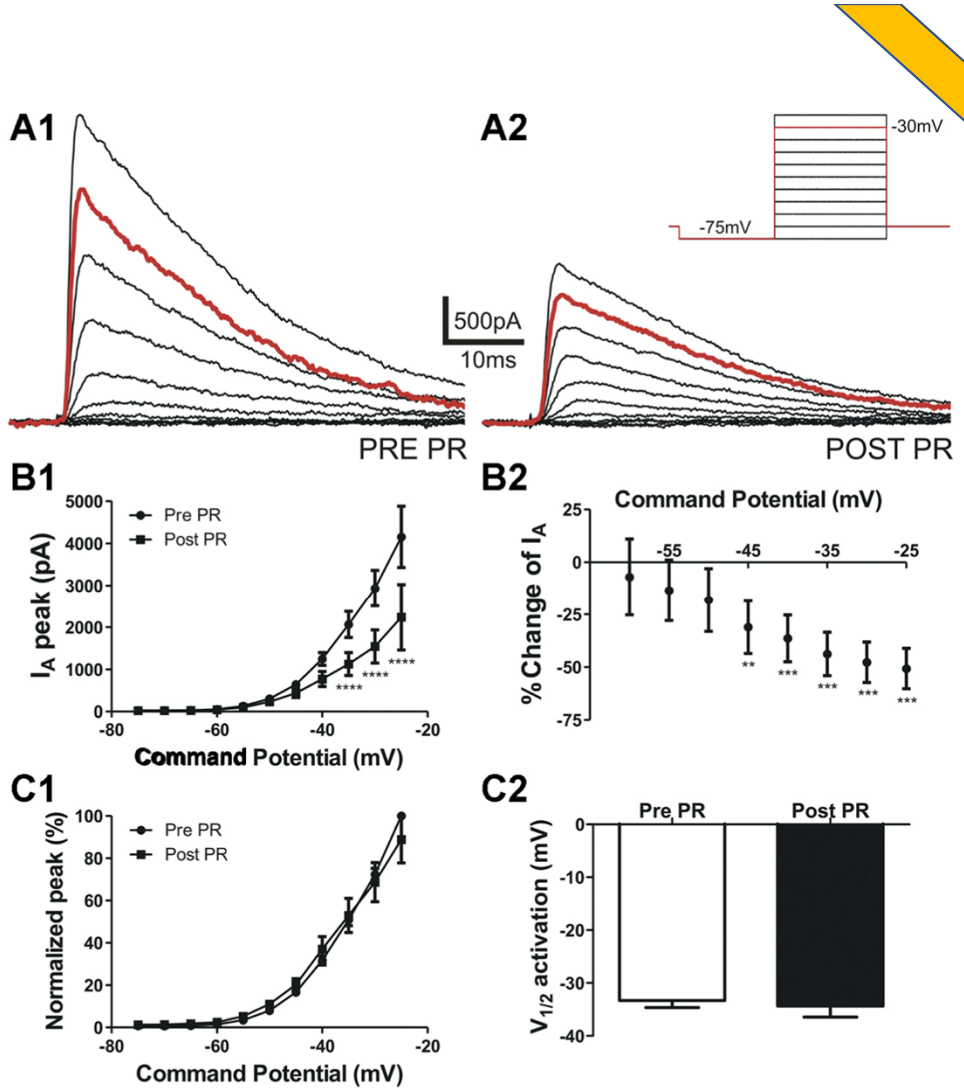
-- (5 mV steps between -75 and -25mV)

B) Mean plot of I_A amplitude and percent inhibition

-- post PR against command potential

C) Normalized I amplitude

-- plots fitted to Boltzmann to obtain V½



PR on activation and inactivation rates

A) Response to depolarization before and after PR

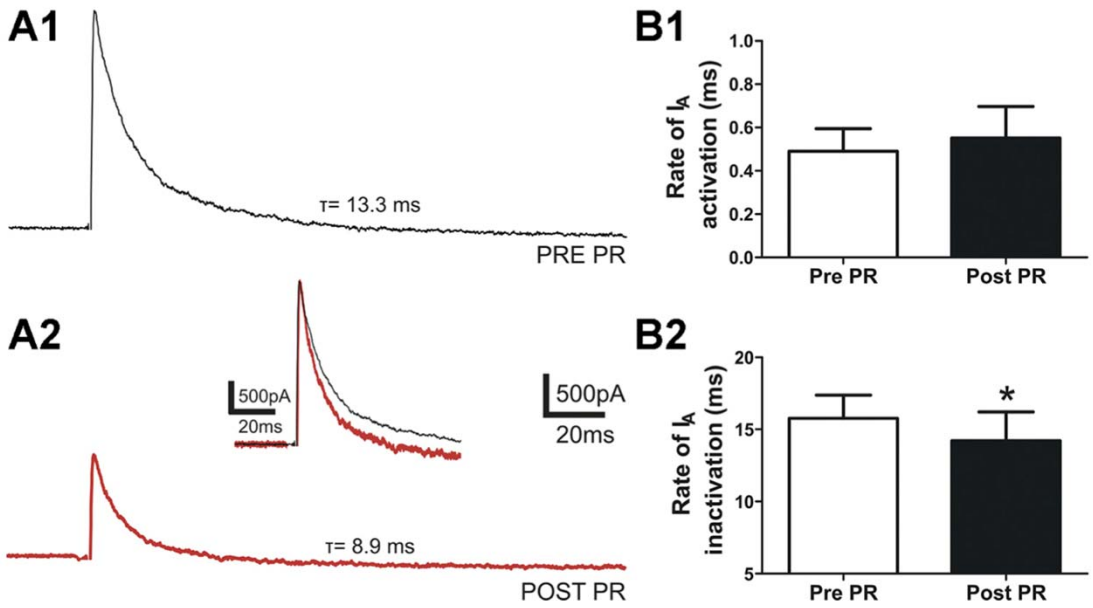
-- -75mV to -25mV

B) Mean rate of activation and inactivation pre and post PR

Post PR:

-- Activation rate: no change

-- Inactivation rate: faster



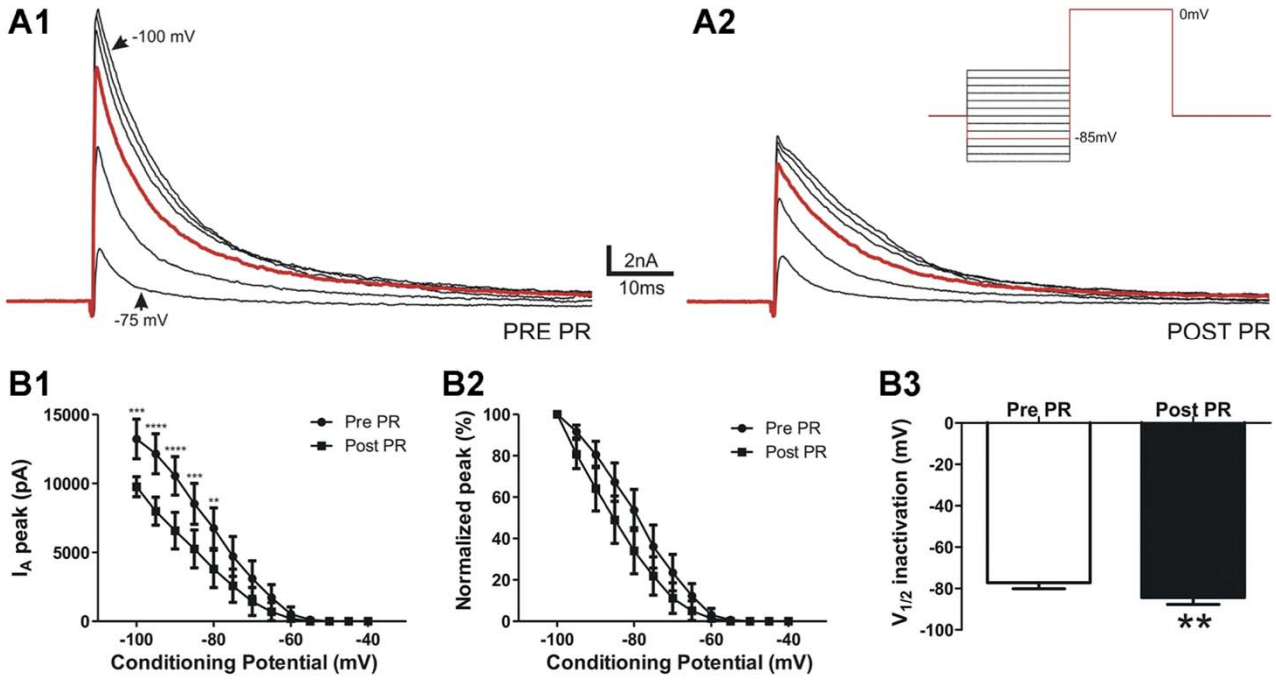
PR on steady-state inactivation

A) Prepulses of -100 to -40 mV prior to step to 0mV

B) Mean and normalized amplitude against conditioning potential

-- variable inactivating conditioning potentials

-- PR induced hyperpolarizing shift on steady-state inactivation curve



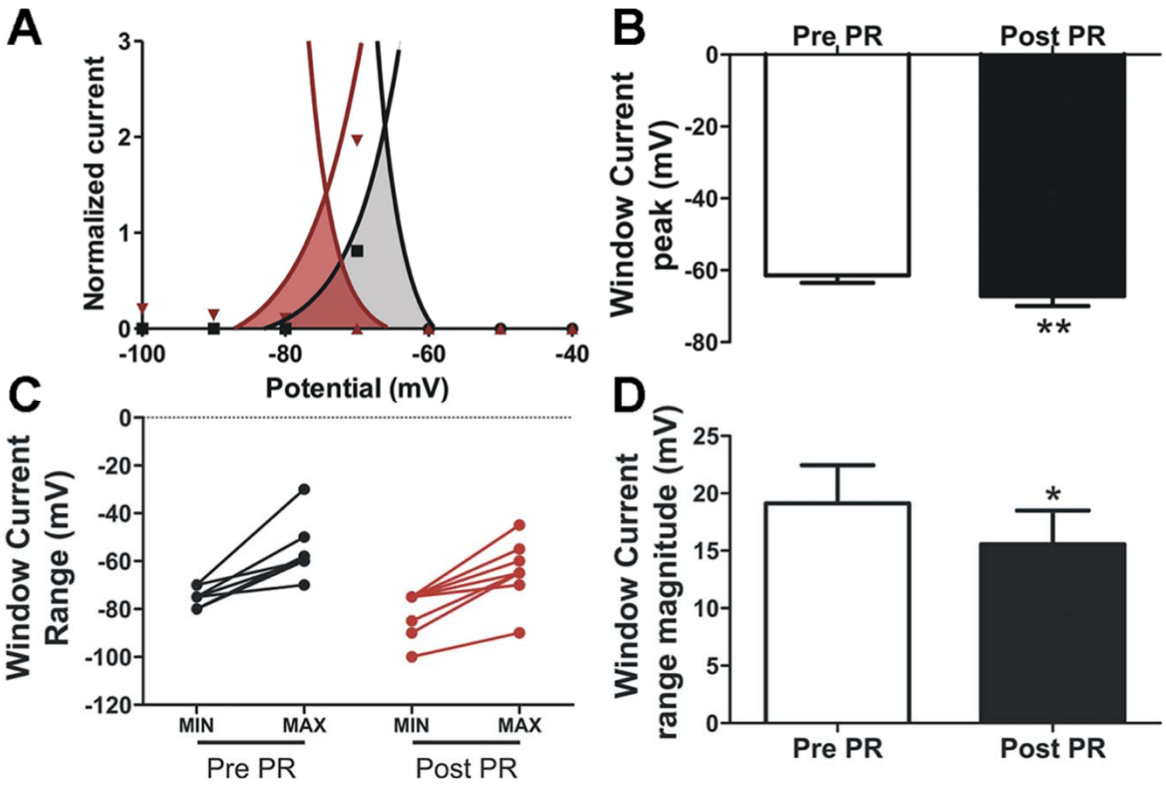
PR on Window current

A) Activation and inactivation plots of one neuron

-- Mean normalized steady-state plots

-- Area of overlap called window current

B) Voltage range of window current



PR on neuron firing

A) PR increases firing frequency of vasopressin neurons

B) PR excitation inhibited by 4-AP

-- 4-AP is a broad inhibitor of voltage-gated K+ channels

C) Action potential shape change between PR and PR + 4-AP

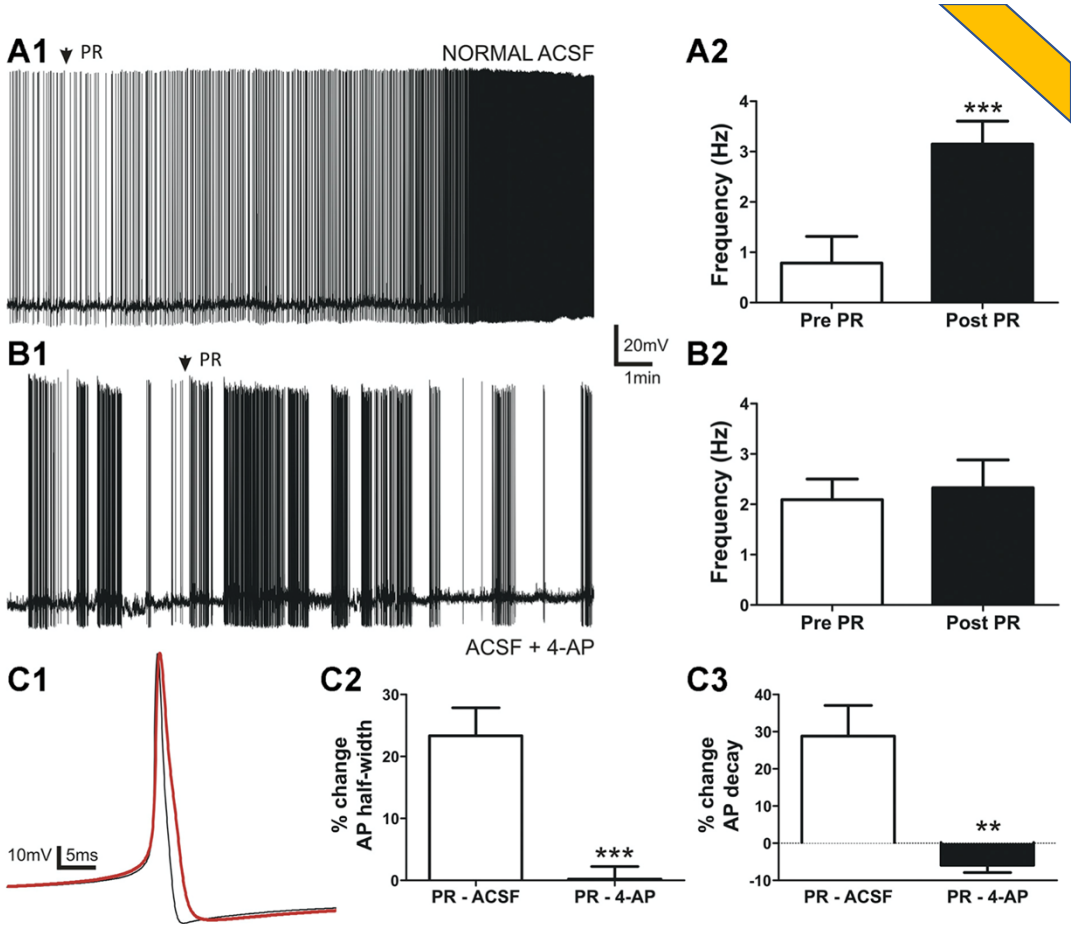


Table 1. *Properties of APs before and after PR*

Extracellular Solution	*n*	AP Half-Width Before PR, ms	AP Half-Width After PR, ms	AP Decay Before PR, ms	AP Decay After PR, ms	HAP Peak Before PR, mV	HAP Peak After PR, mV
aCSF	10	1.4 ± 0.1	1.8 ± 0.1‡	0.9 ± 0.1	1.2 ± 0.1†	−16.3 ± 0.7	−14.8 ± 0.5*
aCSF + 4-aminopyridine	9	3.8 ± 0.4	3.8 ± 0.4	3.3 ± 0.3	3.1 ± 0.3*	−16.3 ± 2.2	−16.2 ± 2.2

PR on oxidative stress

A) Reactive oxygen species indicator DHE in ACSF and ACSF+PR

-- imaged at 0min and 14 min

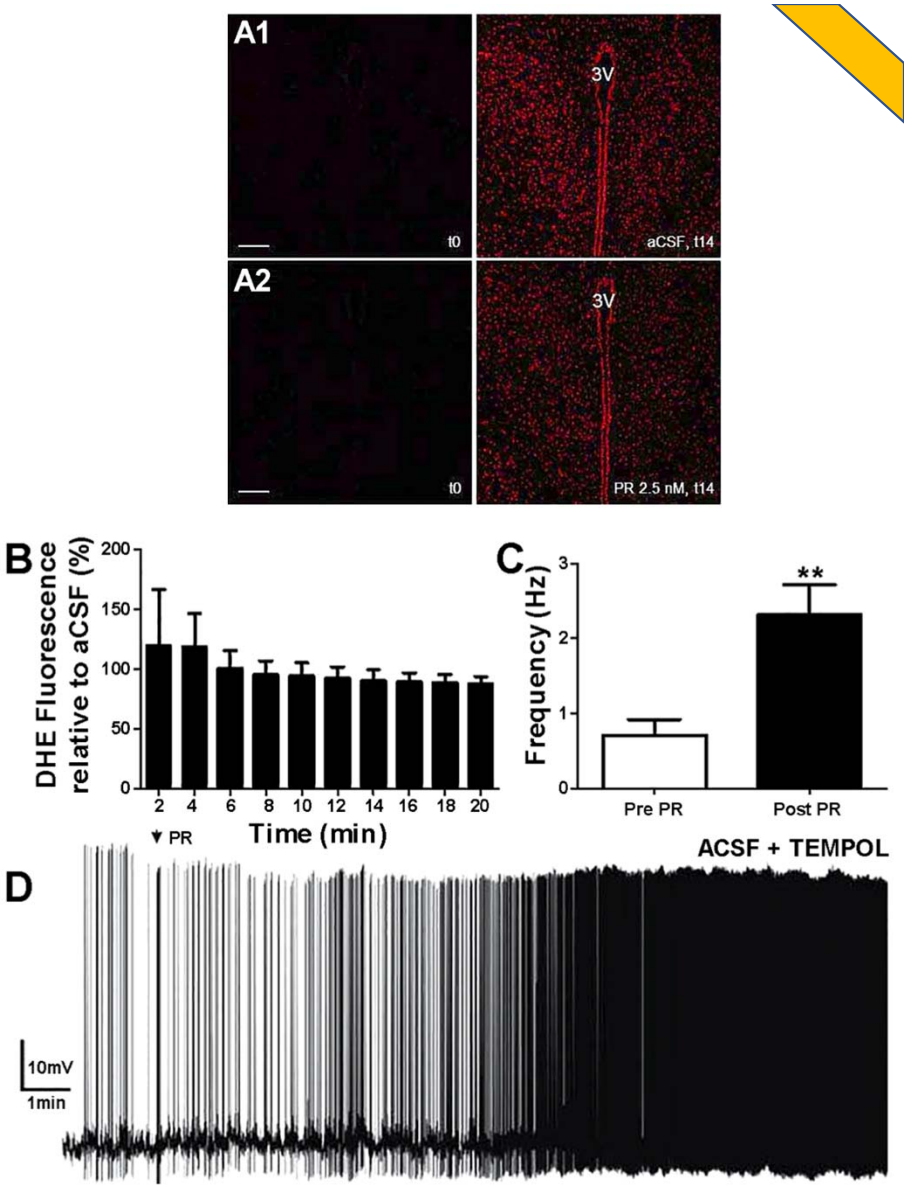
B) DHE fluorescence over time

-- in living cells, biomarker for aerobic respiration

C) Firing frequency of cultured cells in presence of tempol

-- tempol is a marker for superoxide and is used to measure oxidative stress

D) PR excitation still seen in ACSF + tempol



Brain Structure and Function (2020) 225:925–934
https://doi.org/10.1007/s00429-020-02043-4

ORIGINAL ARTICLE

A critical role for the inward rectifying potassium channel Kir7.1 in oligodendrocytes of the mouse optic nerve

Maria Papanikolaou[1] · Arthur M. Butt[1] · Anthony Lewis[1]

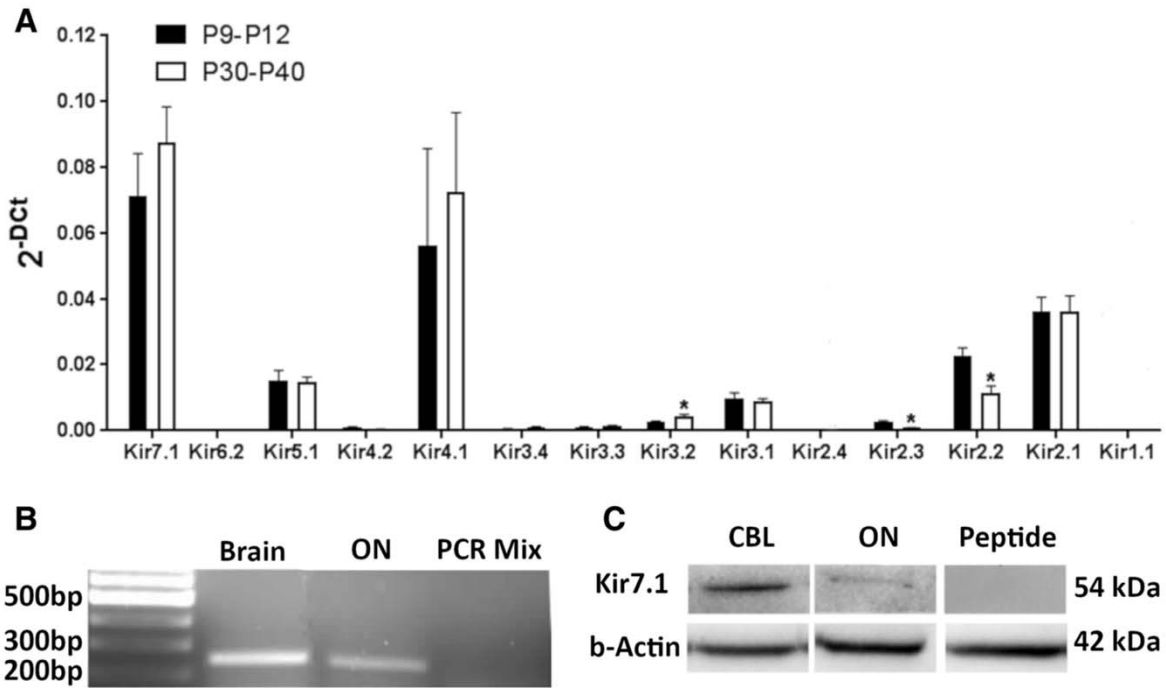
Background

- Oligodendrocytes: glia that myelinate axons in the CNS
  - Typically found in Purkinje and pyramidal neurons

- Kir7.1
  - Inward rectifying potassium channel
  - Low sensitivity to magnesium block
  - Low sensitivity to changes in extracellular K+

Kir expression in optic nerve and brain tissue

A) qRT-PCR of Kir channels in mouse optic nerve
B) RT-PCR of Kir7.1 in optic nerve and brain
C) Western blot of Kir7.1 in optic nerve and brain



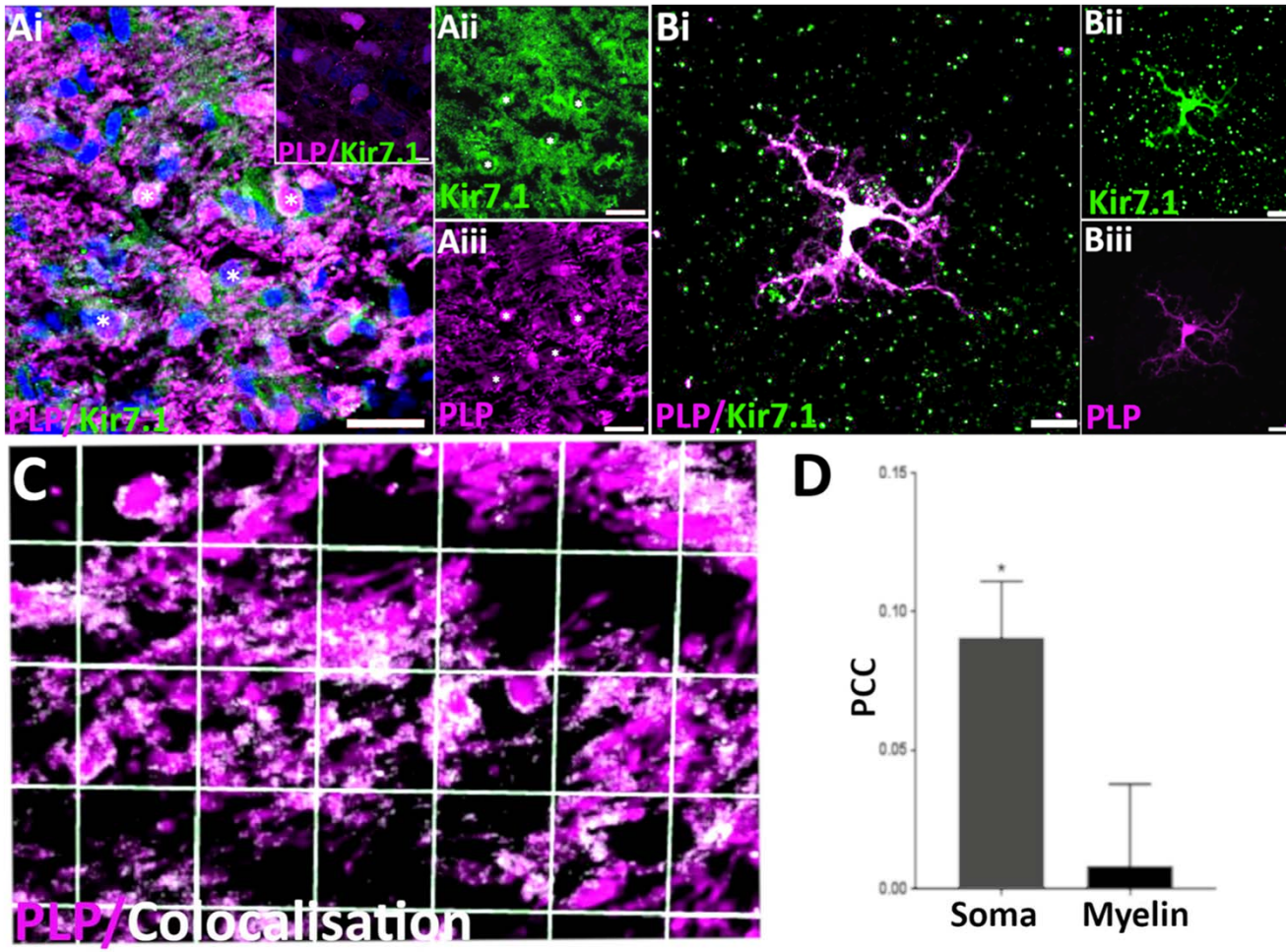
Oligodendrocytes and Kir7.1

A) Optic nerve stained for oligodendrocytes (purple) and Kir7.1 (green)

B) Cultured optic nerve oligodendrocytes stained for Kir7.1

C) Colocalization analysis

D) Pearson Correlation Coefficient revealing colocalization in soma vs myelin



Kir 7.1 and 4.1 currents

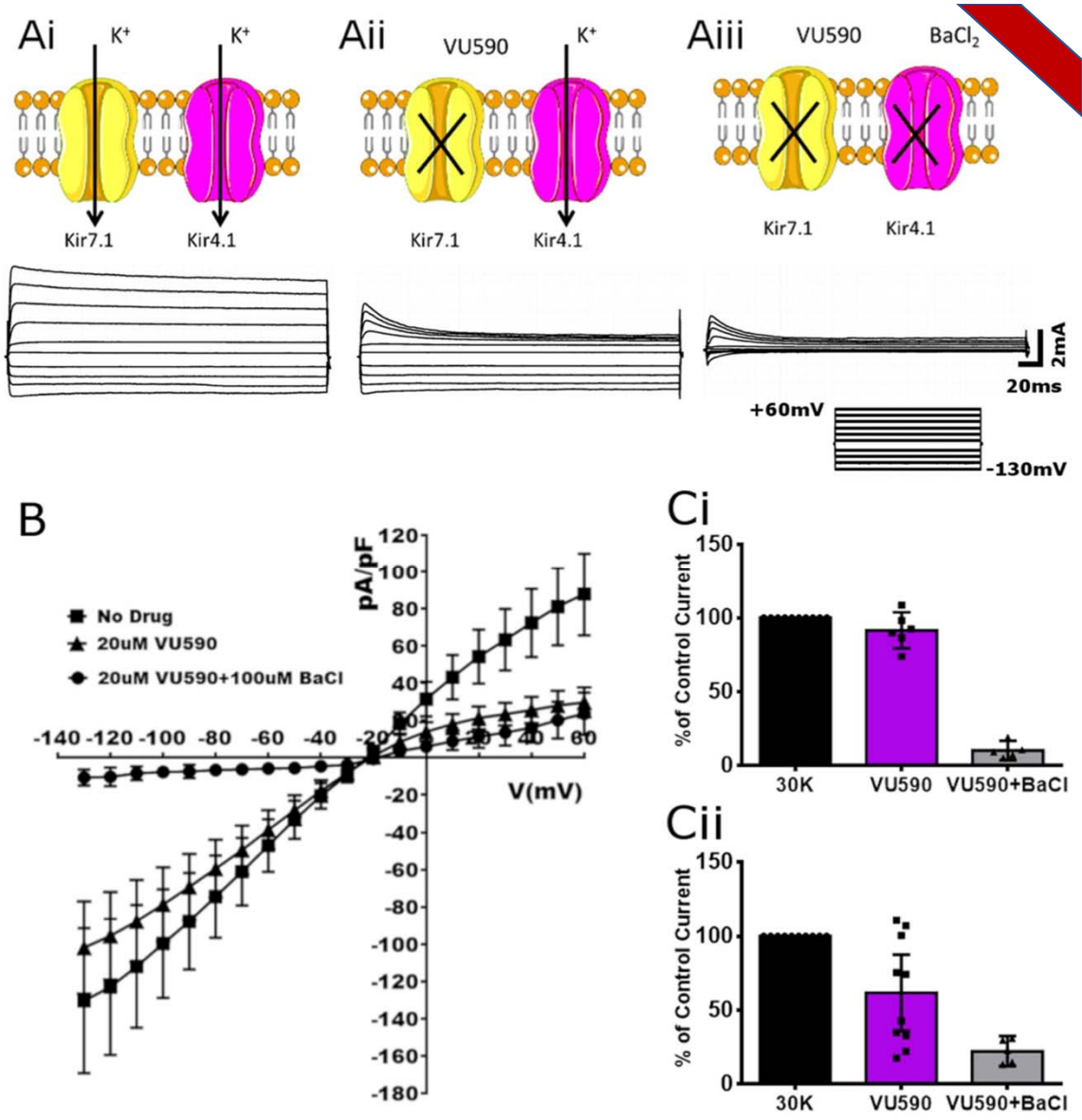
A) Kir7.1-like currents during -130 to +60mV steps

-- High extracellular K+

-- Aii in presence of Kir blocker VU590

-- Aiii in presence of a Kir4.1 blocker

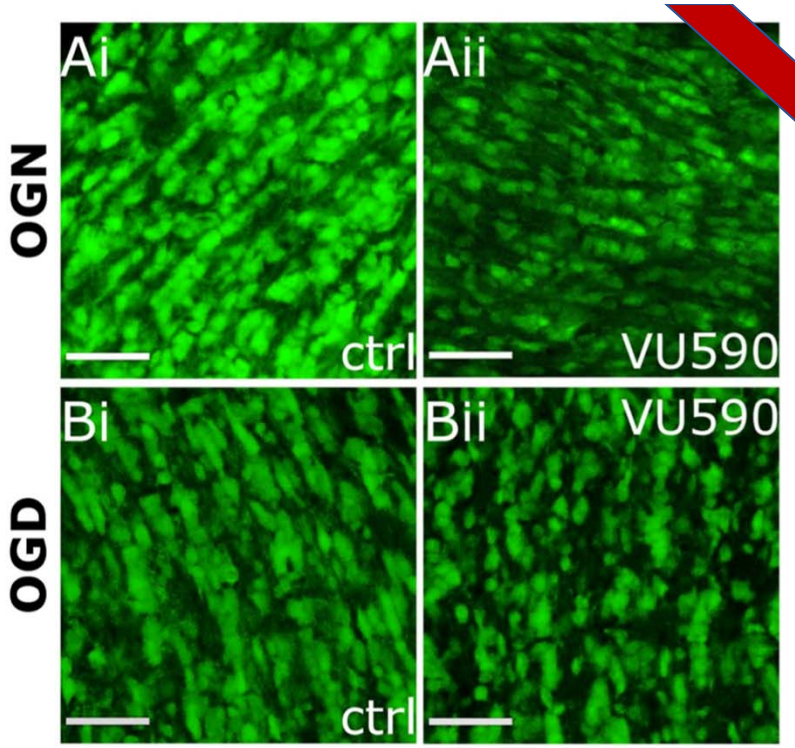
B) I-V relationship pre and post blockers

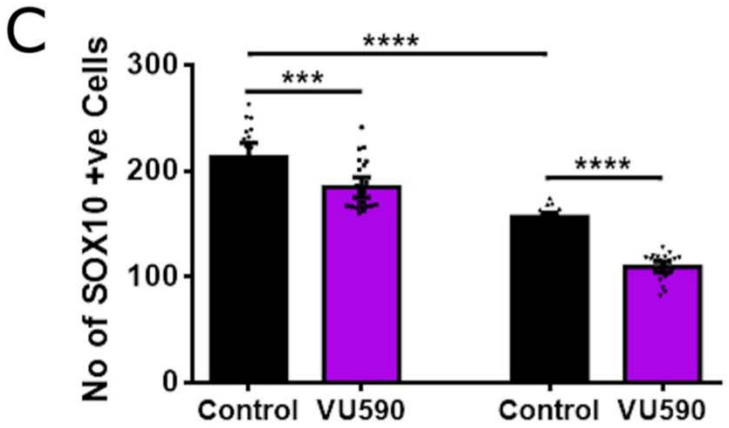


Oligodendrocyte survival with(out) Kir7.1

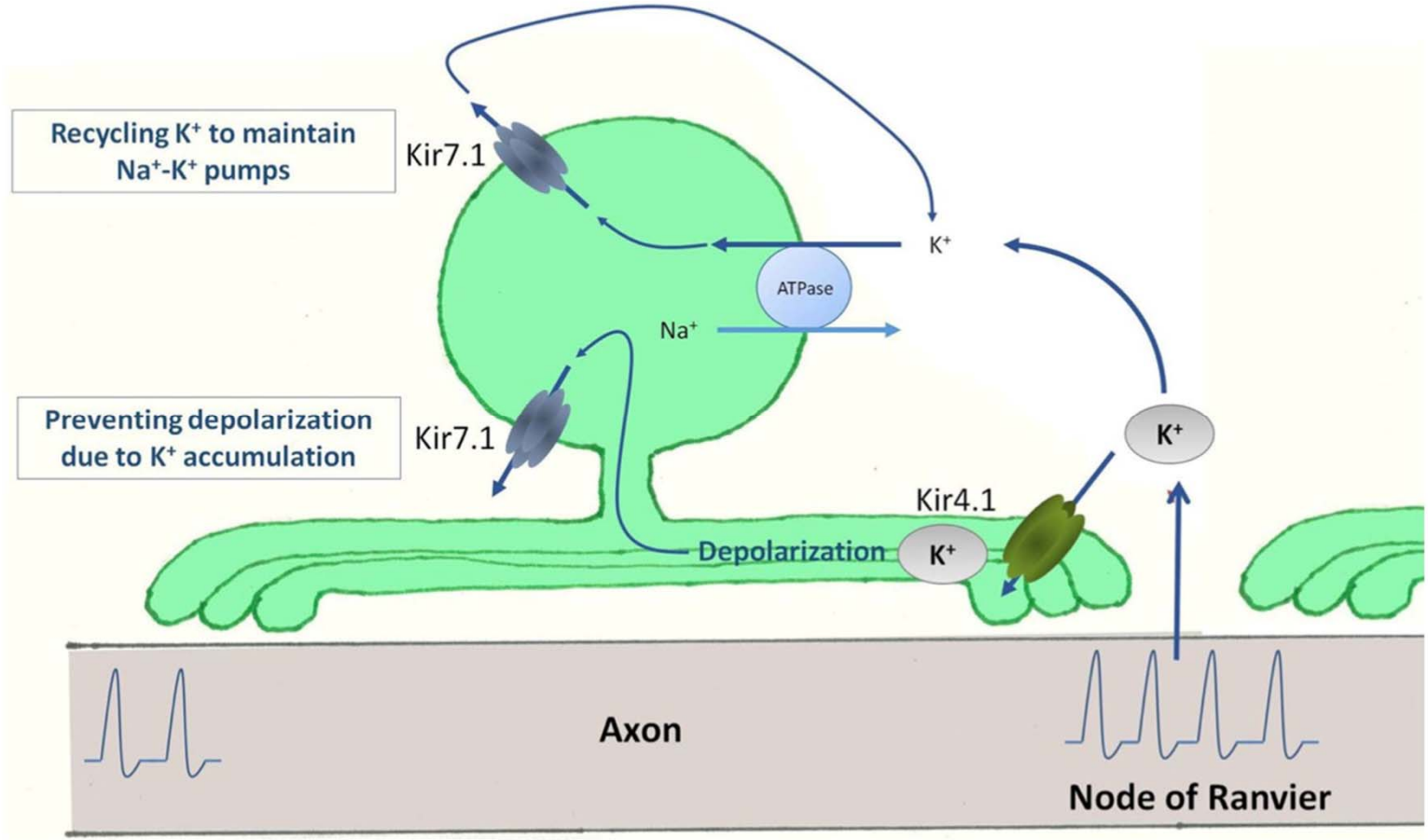
A) Oligodendrocyte somata in the optic nerve when exposed to normal oxygen and glucose
   -- Aii: plus Kir blocker VU590
B) Somata when deprived of oxygen and glucose
   -- Bii: plus Kir blocker VU590
C) Quantification of healthy cells
   -- ID'd as SOX10-eGFP cells

Test reveals survival rate in deprived conditions with and without Kir7.1 activity





Model of Kir7.1 function in oligodendrocytes



Journal of Physiology (2002), **544.2**, *pp.* 429–445

DOI: 10.1113/jphysiol.2002.022434
www.jphysiol.org

Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K^+ channels

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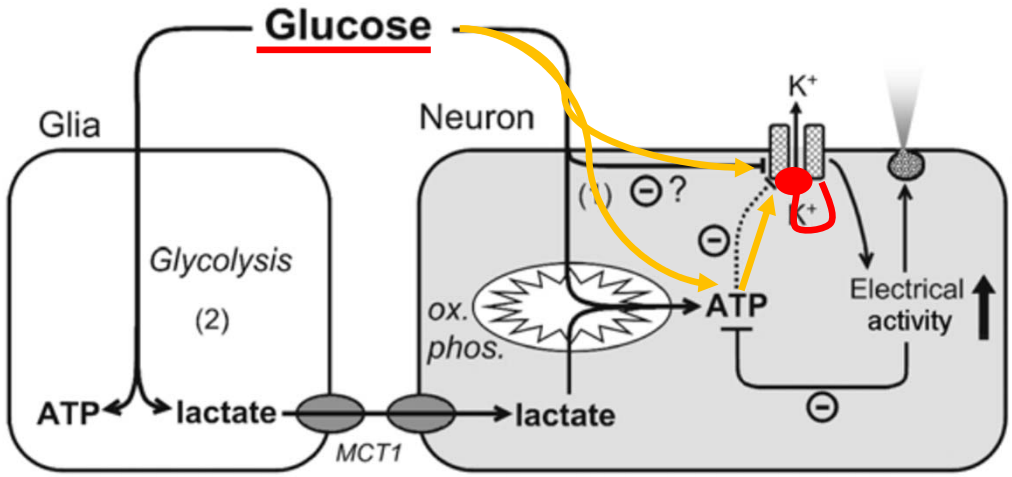
Proposed mechanisms

- Proposed glucose sensing in glucose-responsive hypothalamic neurons

- Direct inhibition of K channel
- Indirect inhibition via increased ATP



What they did…

- Confirmed fluorescent sensors worked in cultured cells
  - Recorded fluorescent changes in presence of 0, 3mM and 15mM Glucose
    - Also tolbutamide: glucose responsive neurons are expected to show increased internal Ca2+

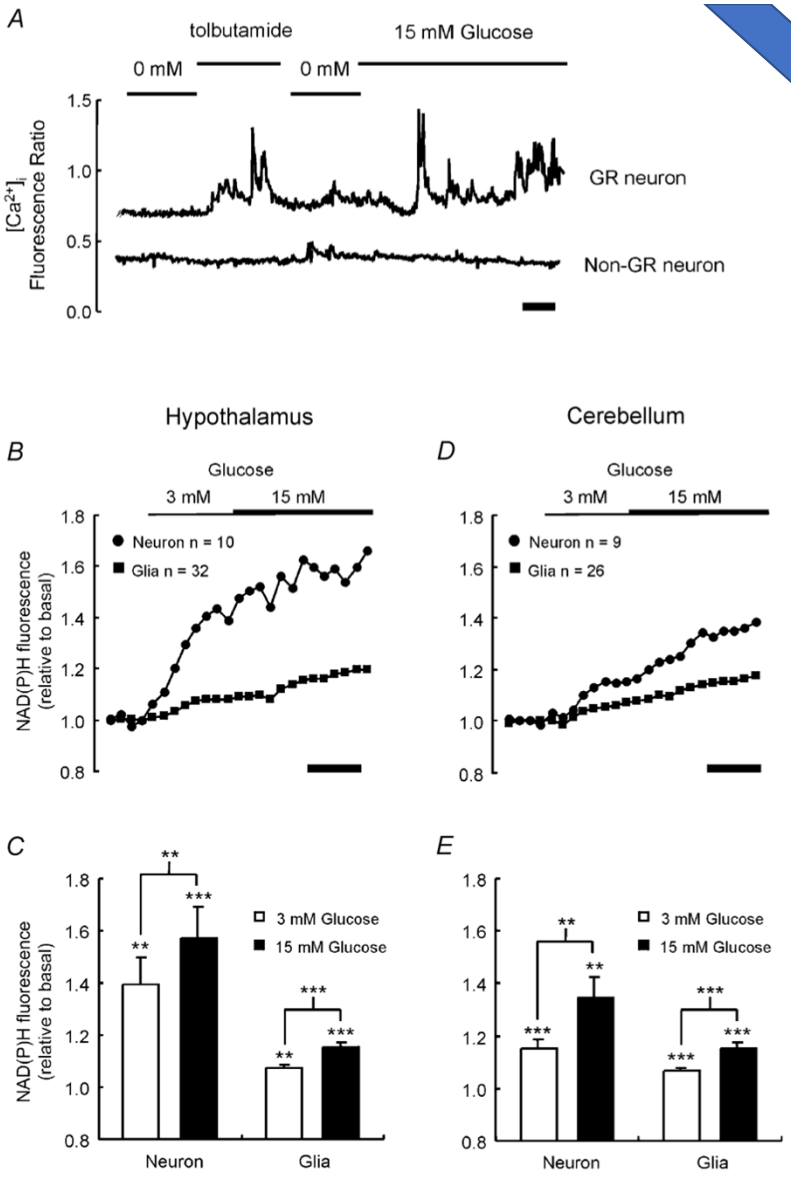
1) Confirmed Ca2+ sensor worked

a) Fura-2 – a commonly used Ca2+ sensor

2) Confirmed mitochondrial activity sensing worked

a) Changes in cellular autofluorescence indicate changes in NADH and NADPH concentration

– referred to as NAD(P)H fluorescence.

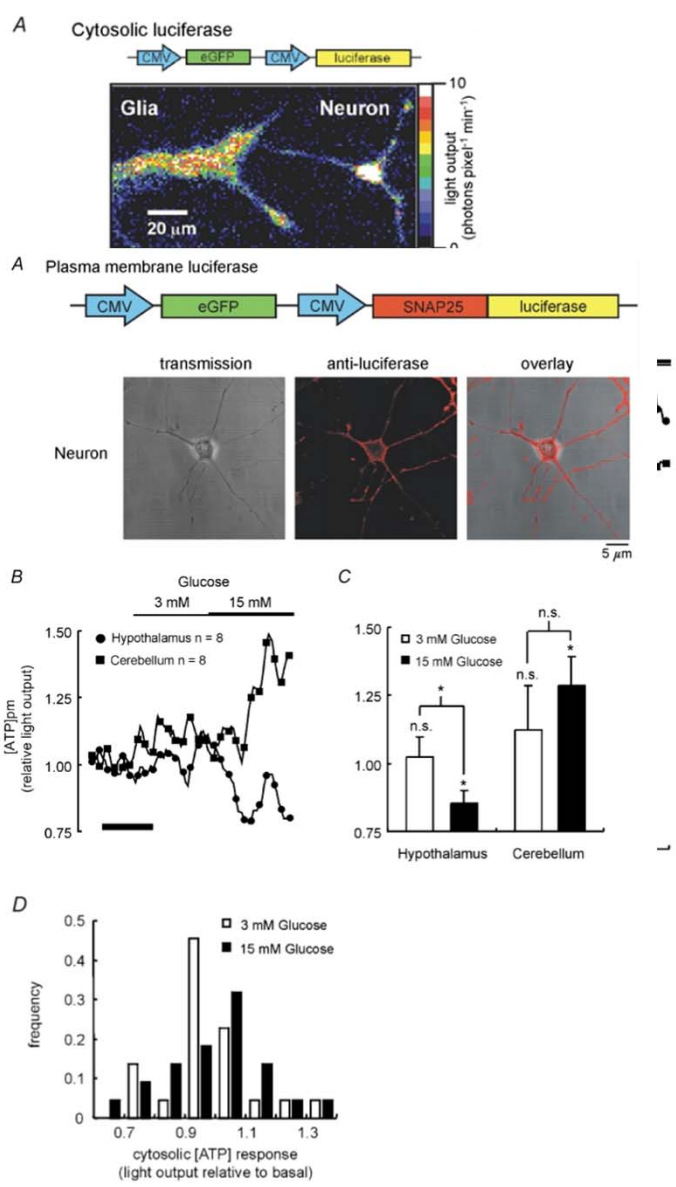


What they did…

- Confirmed fluorescent sensors worked in cultured cells
  - Recorded fluorescent changes in presence of 0, 3mM and 15mM Glucose

1) **Confirmed cytosolic ATP sensor worked**
   a) Cells infected with luciferase and eGFP
2) **Confirmed plasma membrane ATP sensor worked**

2)



What they did…

- Confirmed fluorescent sensors worked in cultured cells
  - Recorded fluorescent changes in presence of ouabain
    - An Na/K ATPase inhibitor
  - Recorded fluorescent changes in presence of oligomycin and 15mM glucose
    - Oligomycin inhibits ATP synthase

1) Confirmed cytosolic ATP sensor worked

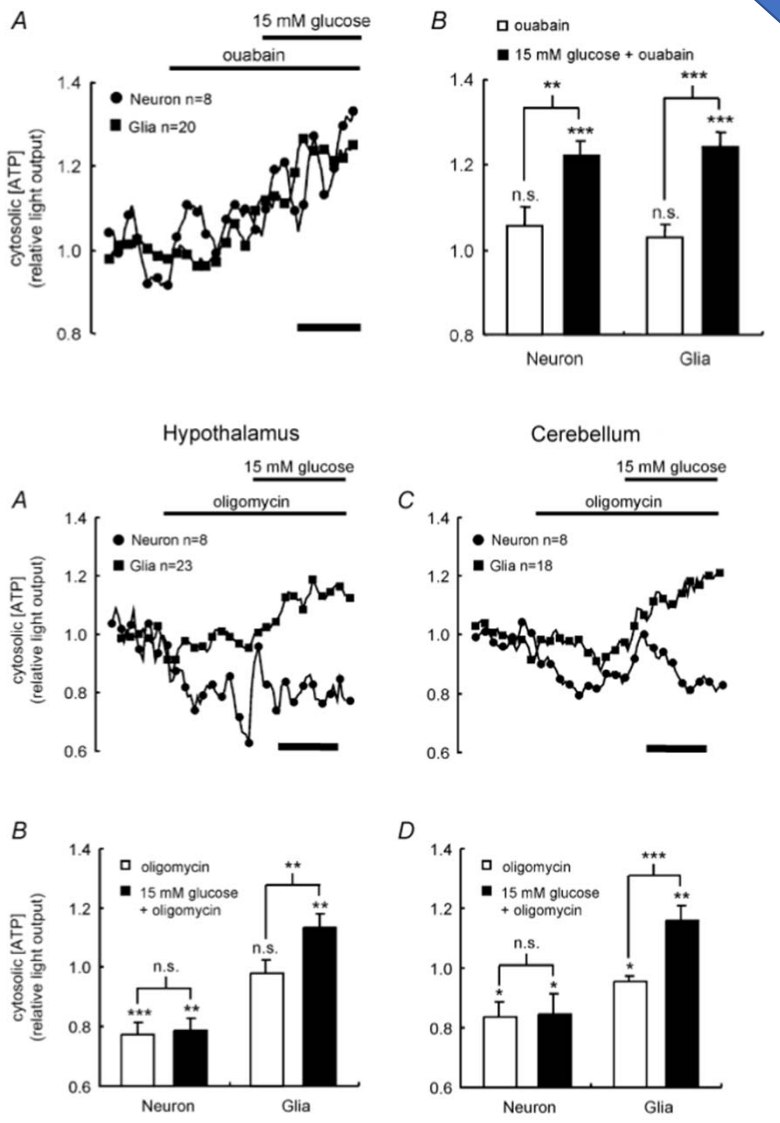
a) In the presence of ouabain and glucose

- Confirms glucose responsiveness independent of Na/K pump

2) Confirmed mitochondrial activity sensing worked

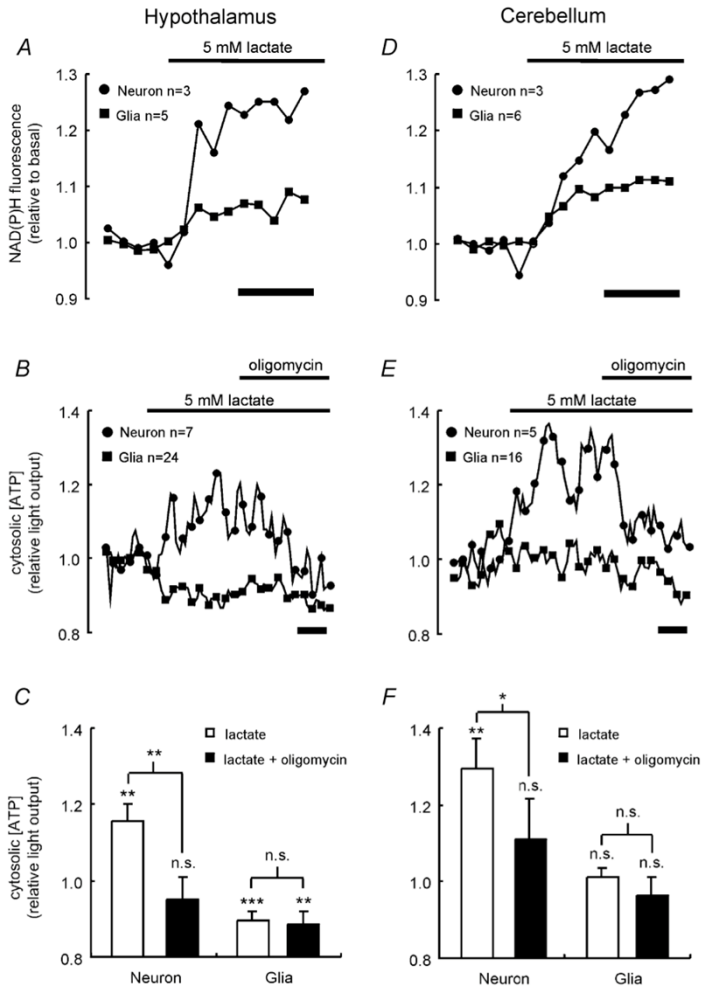
a) In the presence of oligomycin

- Neurons and glia metabolize glucose differently



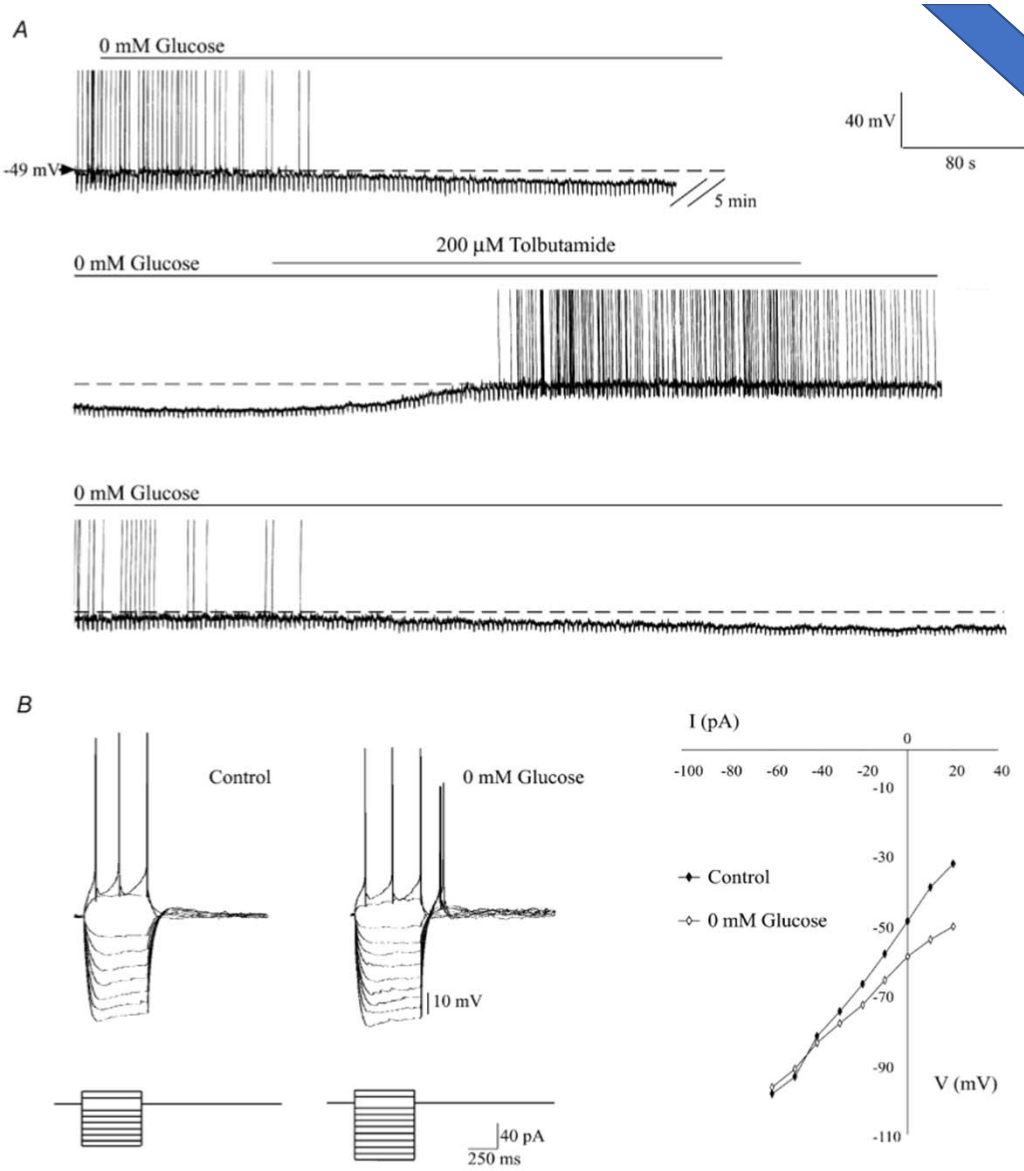
What they did…

- Showed neurons metabolize lactate, the biproduct of glial glycotic metabolism in both hypothalamic and cerebellar neurons



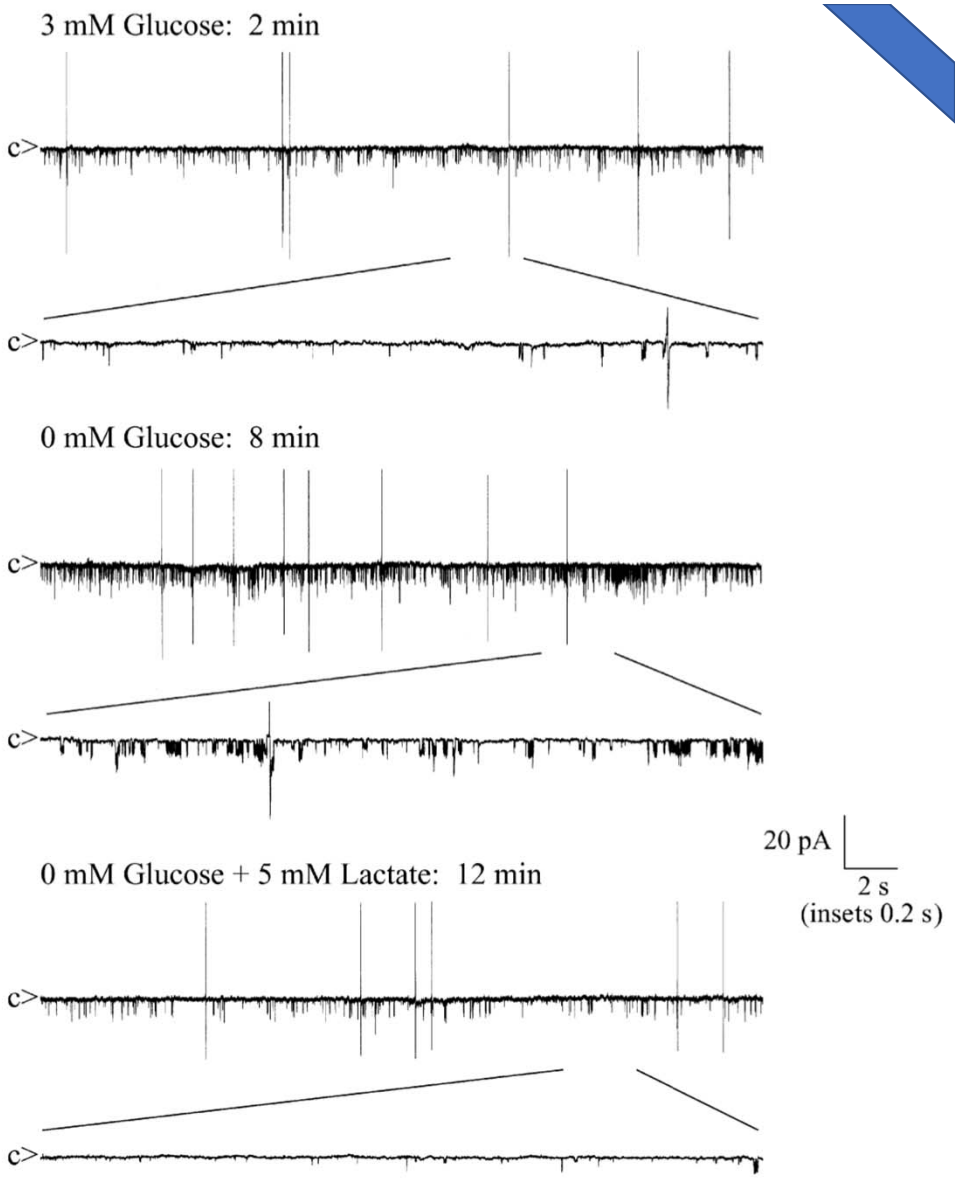
What they found…

- Removing extracellular glucose hyperpolarizes glucose-responsive cells of the hypothalamus.
  - Even with high intracellular [ATP]
- I-V relationship shows reversal potential near -90 mV
  - Indicating K+ conductance



What they found…

- Same cell recording:
  - Removal of glucose increased K-ATP activity
  - Lactate reduce this activity



What they found…

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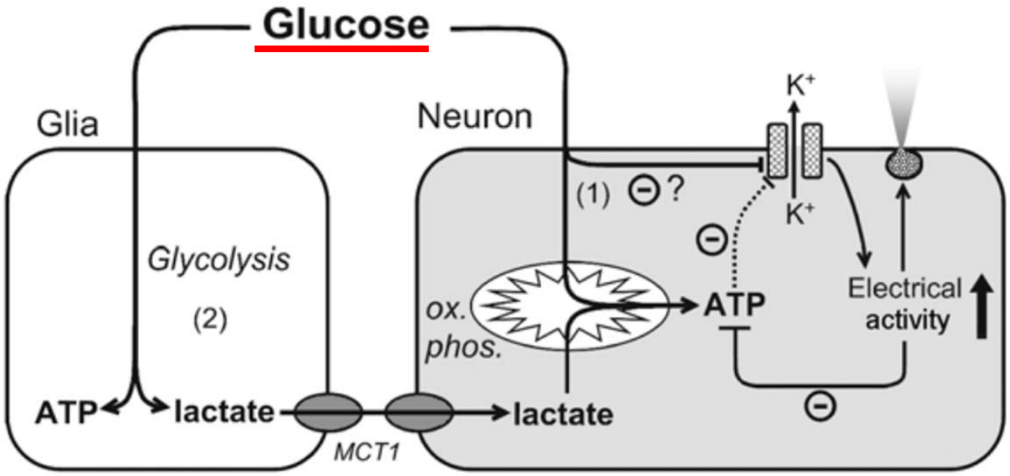
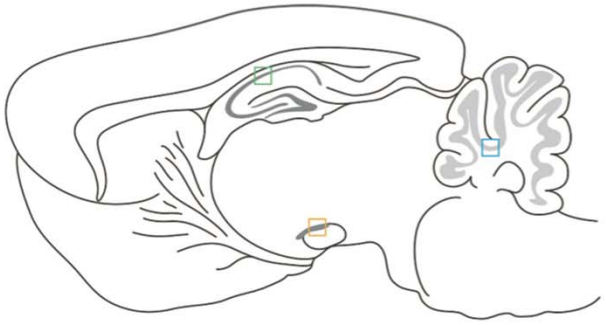
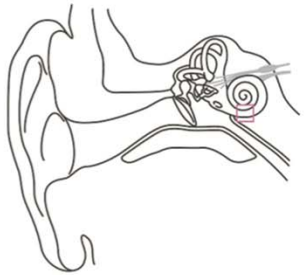


Kv Channels & Physiology

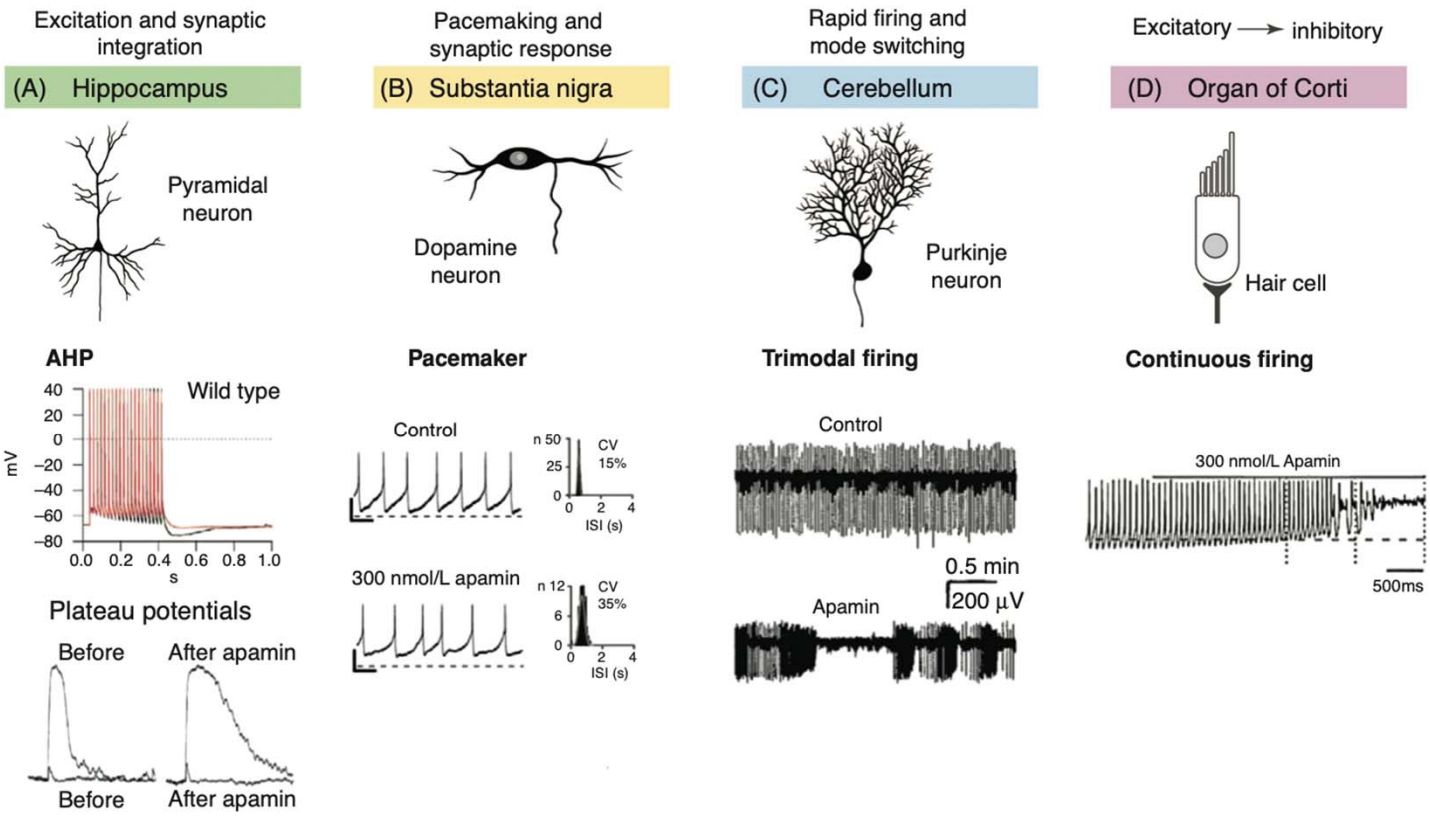




As you can see,

Potassium channels are highly diverse

With a variety of channel types essential for cell function



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