Potassium channels:
important drug targets of the future?

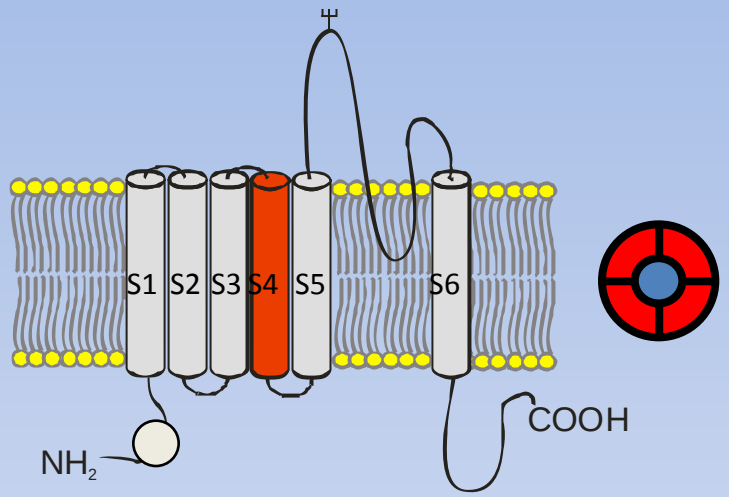
- ✓Physiology
- ✓Molecular biology
- ✓Pharmacology

Neuroprotection and Preconditioning

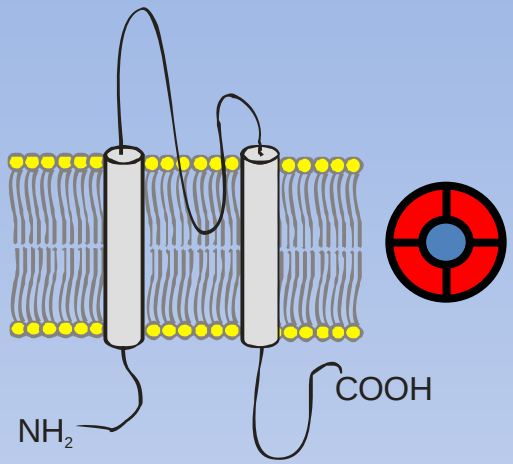
K^+-channel function in excitable tissue

- Dampen excitation
- Stabilize membrane potential
  - Set resting potential
  - Keep action potential short
  - Terminate periods of intense activity
  - Time the inter-spike interval
  - Lower the effectiveness of excitatory inputs

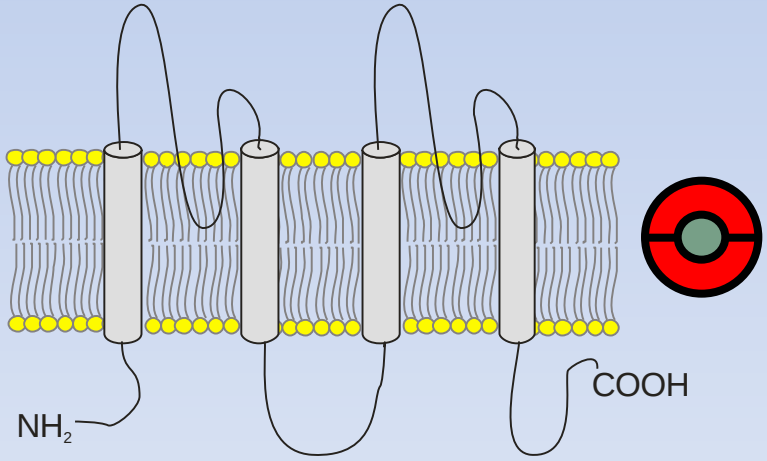
K^+-channels are the most diverse ion channel family



Voltage-gated K channels (K_v)
(Delayed rectifiers)
KCNQ channels
(slow delayed rectifiers)
Ca-activated K channels (BK, SK)
(KCNM, KCNN)

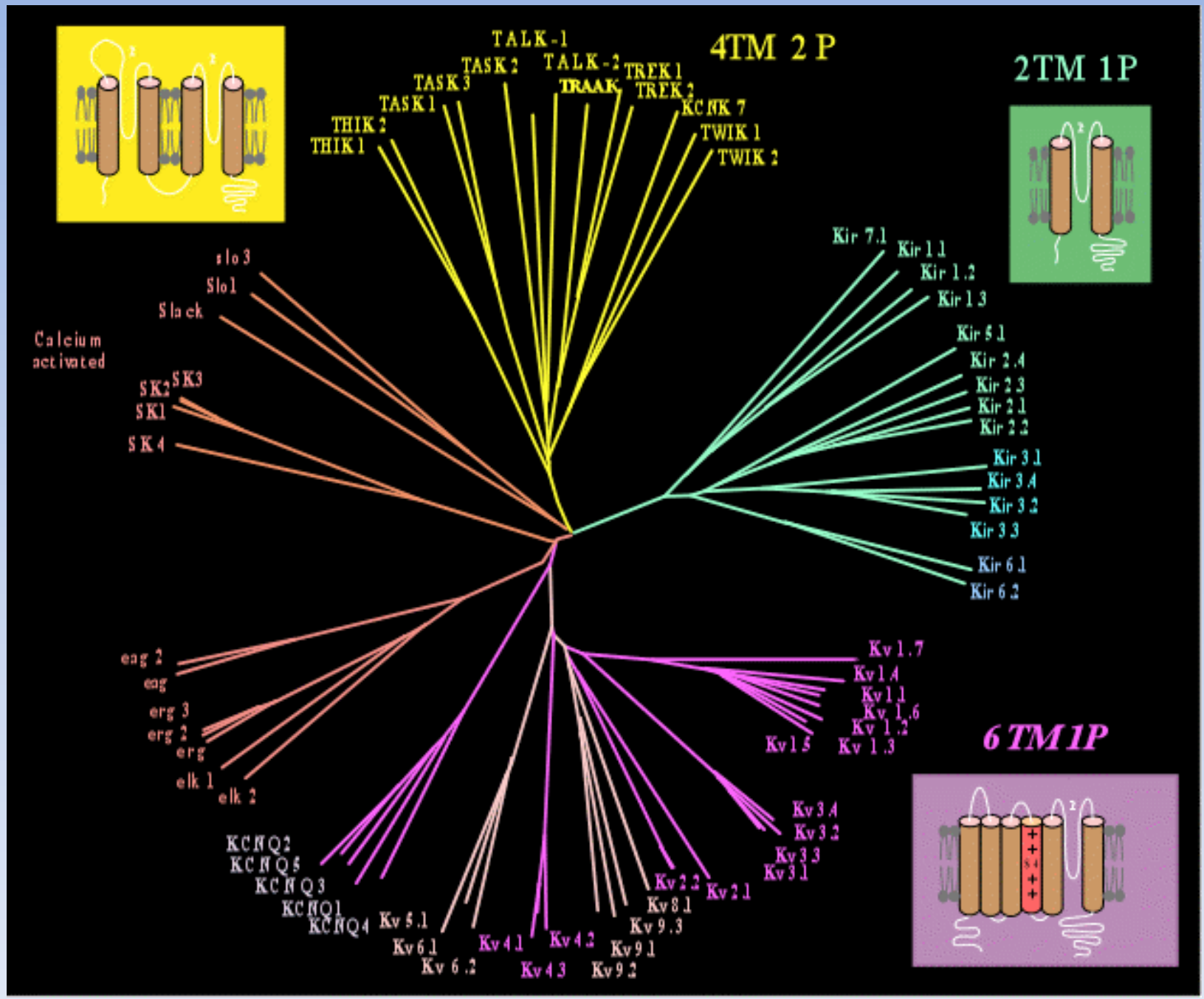


Inward rectifiers (K_{ir})
KCNH



2 pore domain K channels
KCNK

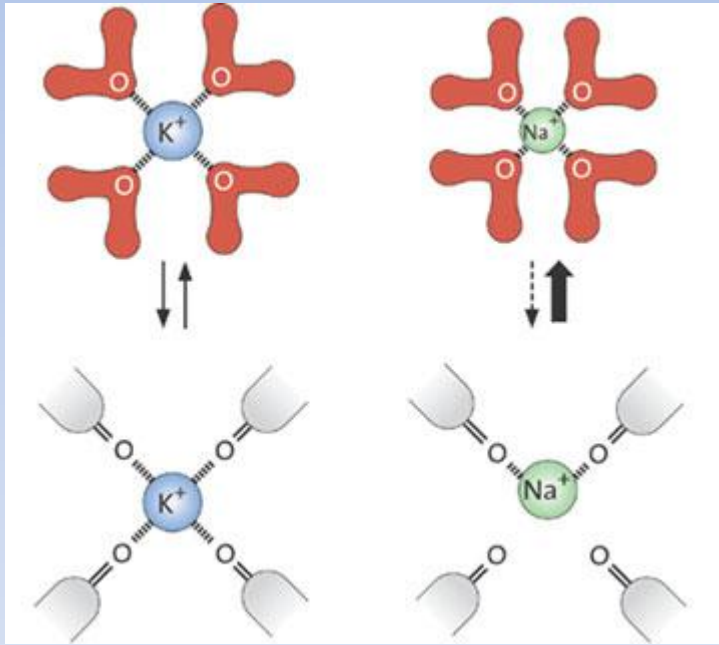
Modular design allows heteromultimers!


4TM 2 P
2TM 1P
6 TM 1P
TALK-1
TASK 2
TALK-2
TASK 3
TRAAK
TREK 1
TREK 2
TASK 1
KCNK 7
THIK 2
TWIK 1
THIK 1
TWIK 2
Kir 7.1
Kir 1.1
Kir 1.2
Kir 1.3
slo 3
Slo1
Slack
Calcium activated
Kir 5.1
Kir 2.4
Kir 2.3
Kir 2.1
Kir 2.2
SK2
SK3
SK1
SK 4
Kir 3.1
Kir 3.4
Kir 3.2
Kir 3.3
Kir 6.1
Kir 6.2
eag 2
eag
erg 3
erg 2
erg
elk 1
elk 2
Kv 1.7
Kv 1.4
Kv 1.1
Kv 1.6
Kv 1.2
Kv 1.5
Kv 1.3
Kv 3.4
Kv 3.2
Kv 3.3
Kv 3.1
KCNQ2
KCNQ5
KCNQ3
KCNQ1
KCNQ4
Kv 5.1
Kv 6.1
Kv 6.2
Kv 4.1
Kv 4.2
Kv 4.3
Kv 2.2
Kv 2.1
Kv 8.1
Kv 9.3
Kv 9.1
Kv 9.2

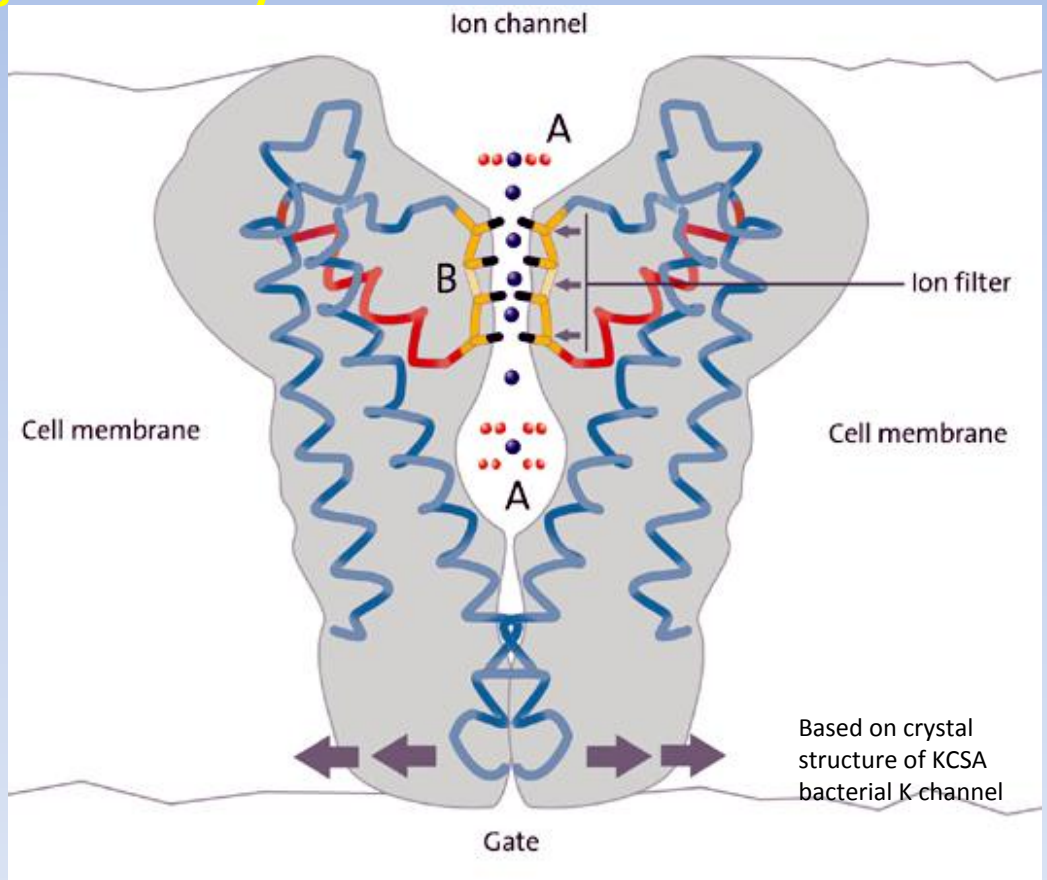
K channel structural features

2 essential functional features

- High throughput
- **High selectivity**



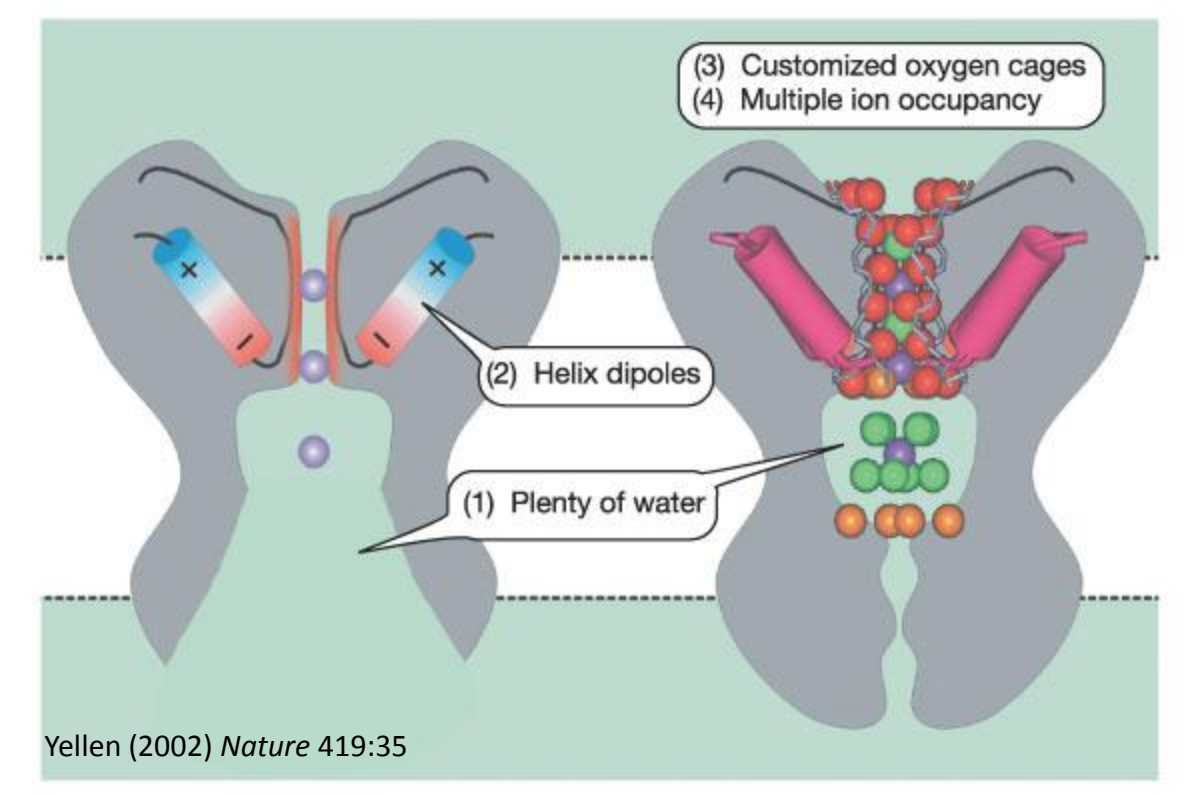
Selectivity filter



Consensus sequence in pore loop:TXXTXGYGD

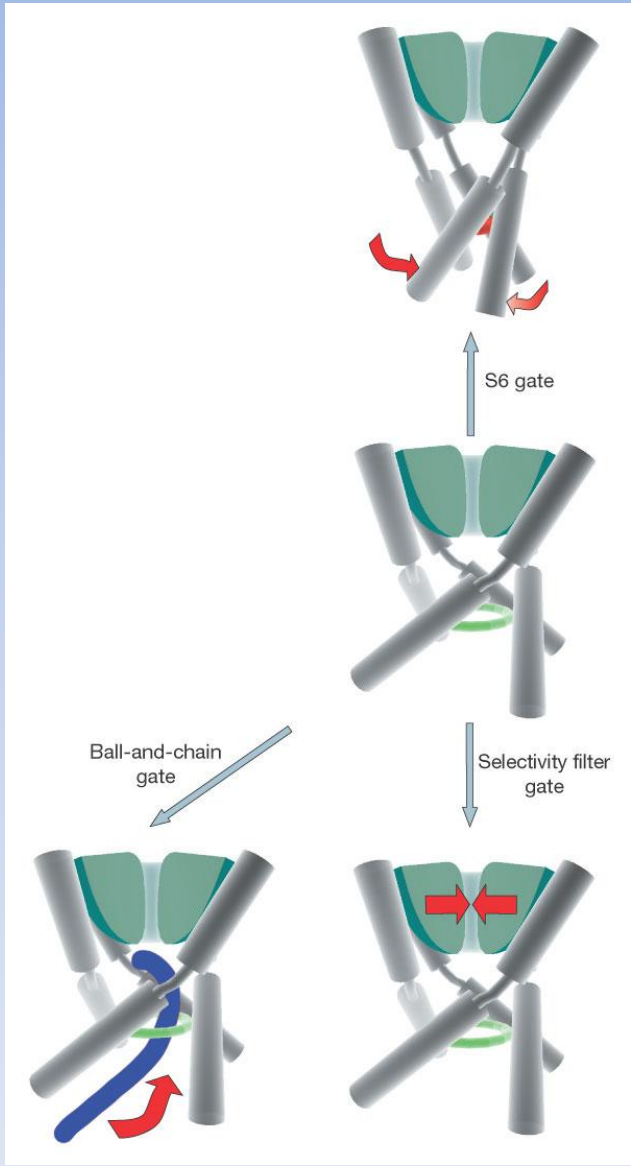
K channel signature sequence

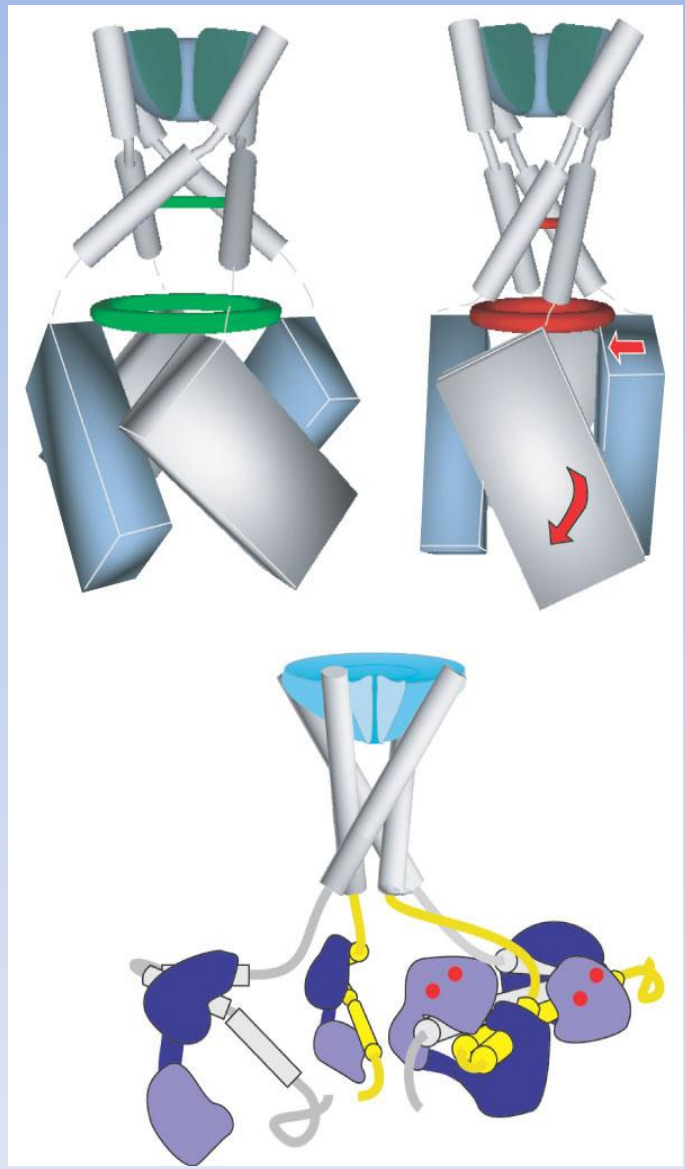
High throughput



No ionic charges involved!

K channel gating



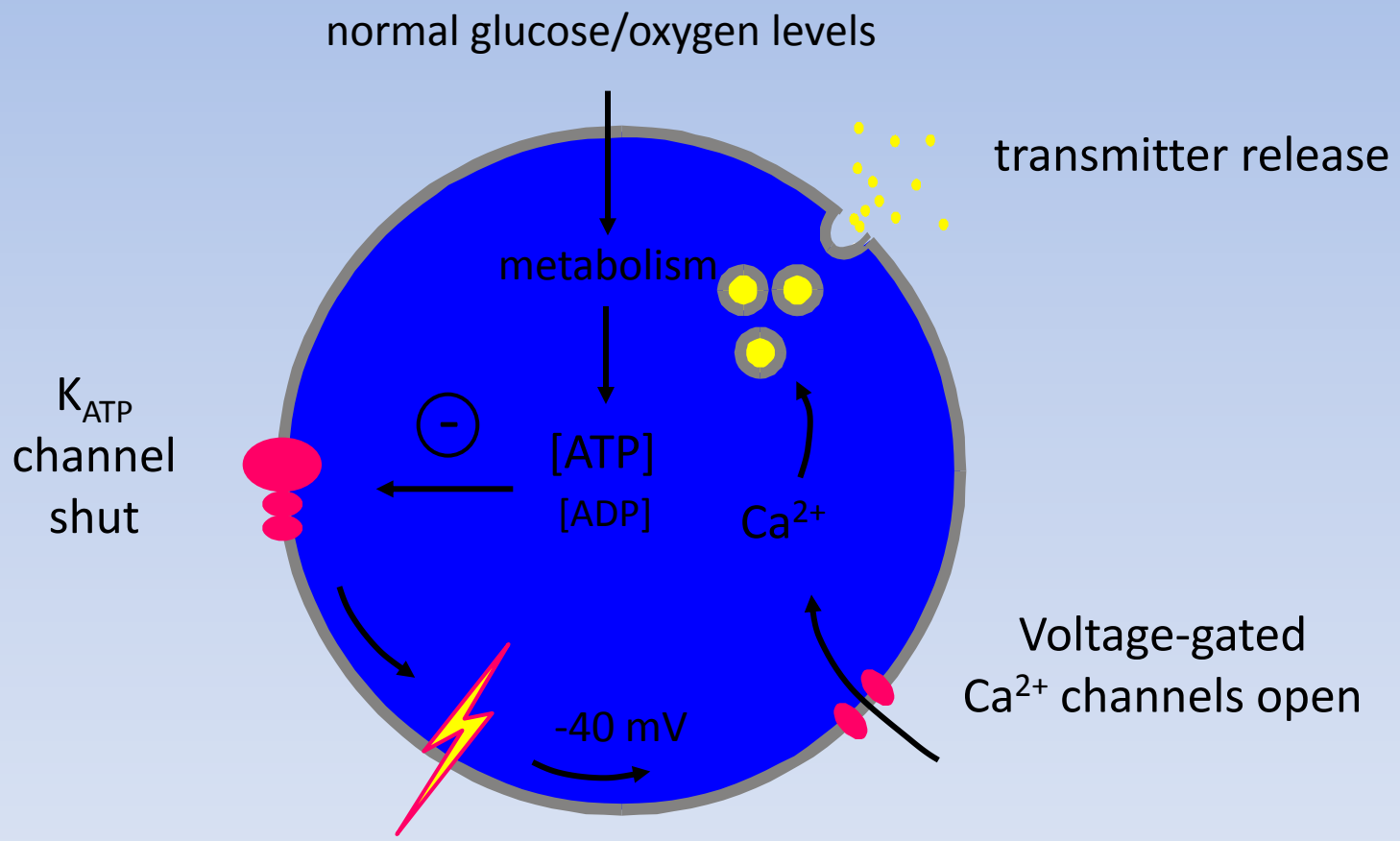


Proposed Ca^{2+} sensor mechanism for SK channels

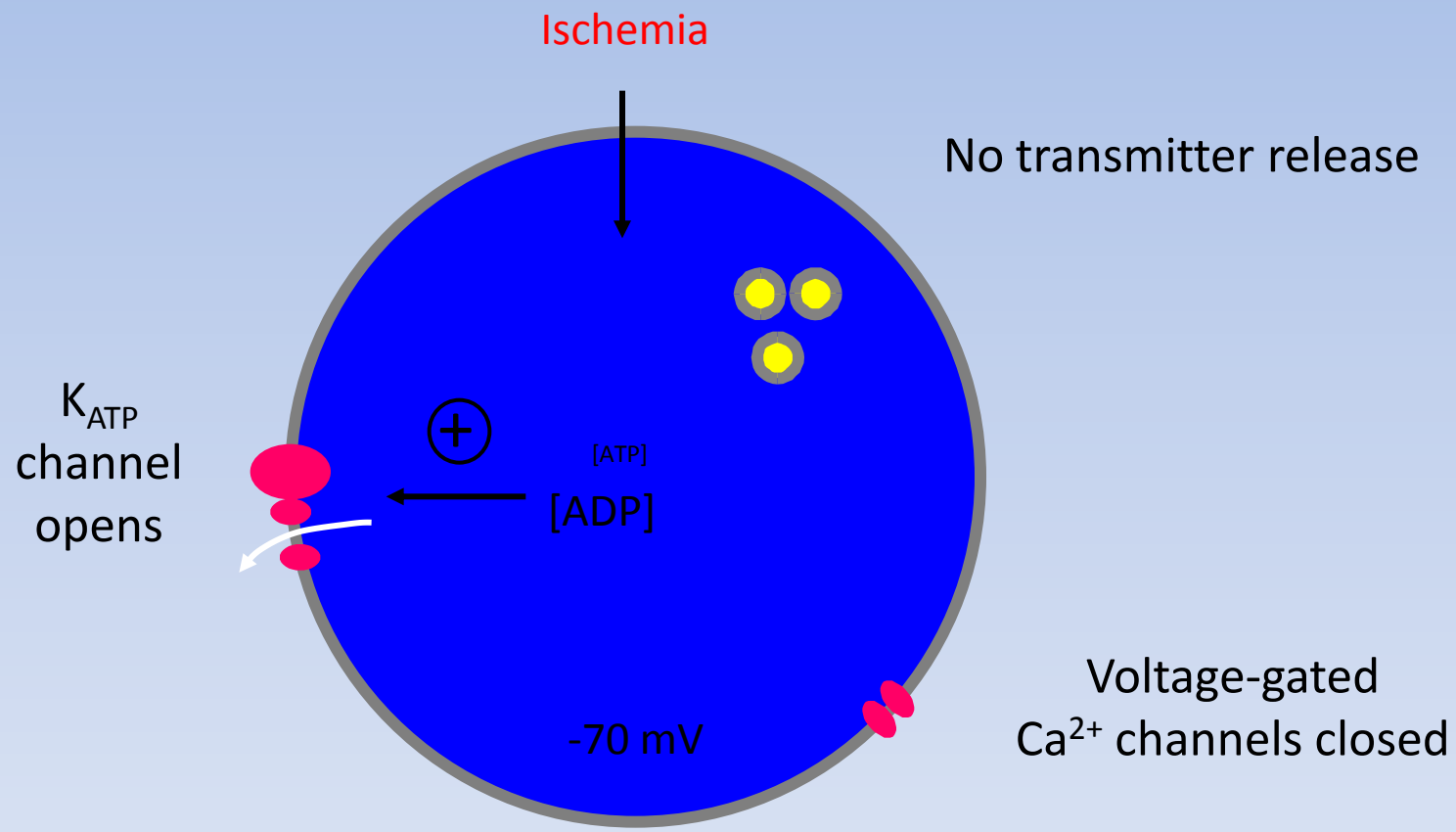
Neuroprotection & Preconditioning

- What is preconditioning?
  - What is the difference between acute neuroprotection and preconditioning?
- What are the potential cellular pathways?
- Preconditioning by Xenon and Sevoflurane

Acute Neuroprotection by K^+ channel: Metabolism-linked activity

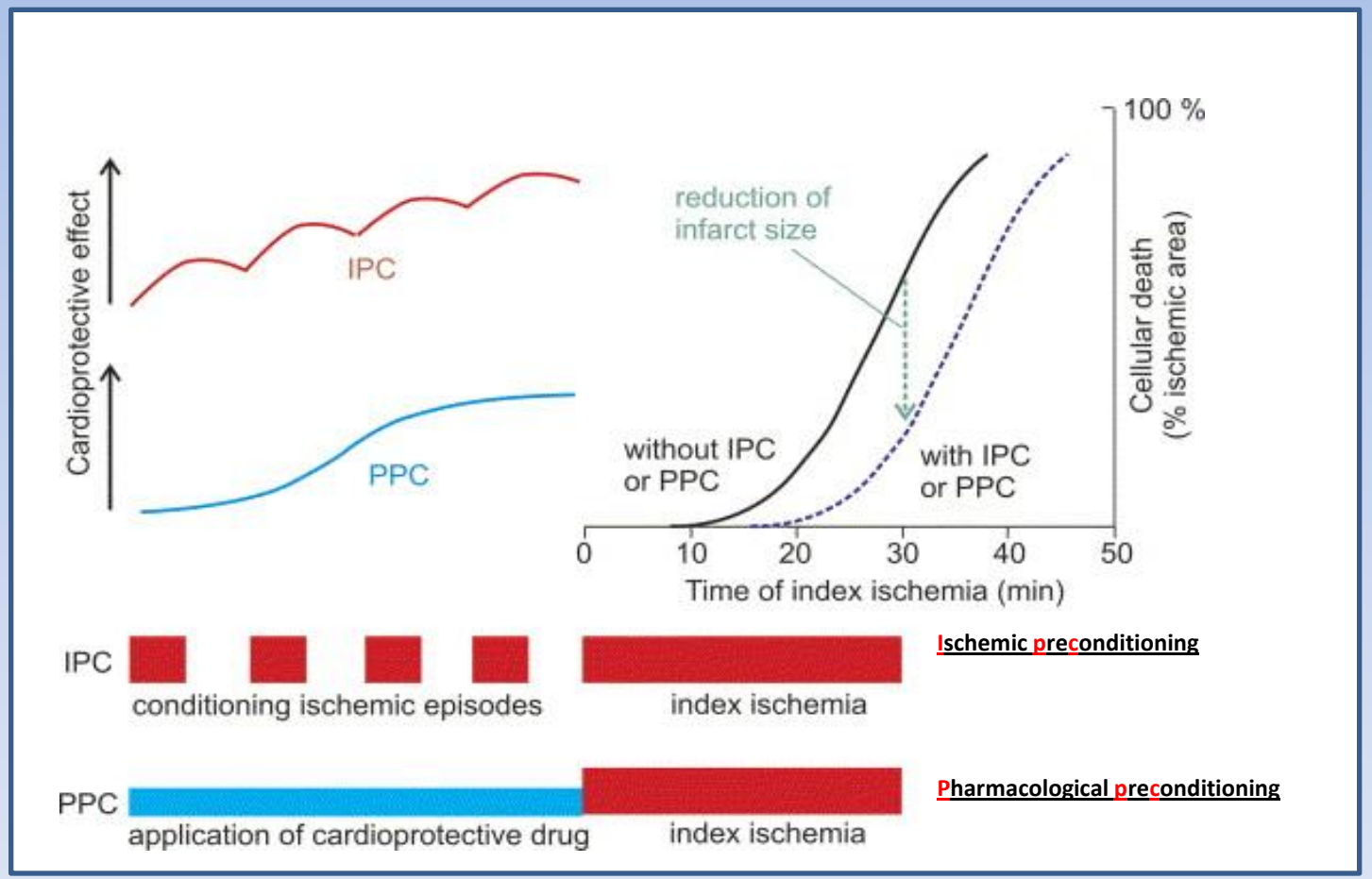


Metabolism-linked activity



Cardiac Preconditioning

"Short periods of ischemia protect tissue against injury by a prolonged period of ischemia"



Hanley & Daut 2005; Journal of Molecular and Cellular Cardiology 39:17-50

Preconditioning is induced by:

- K_{ATP}channel openers
- Agonists of G-protein coupled receptors
- …

Preconditioning is prevented by:

- K_{ATP} channel blockers
- …

Inhalational Anaesthetics have preconditioning effects *in vitro* and *in vivo* (review: Wang et al 2008; Curr opin Pharmacol 8:104)

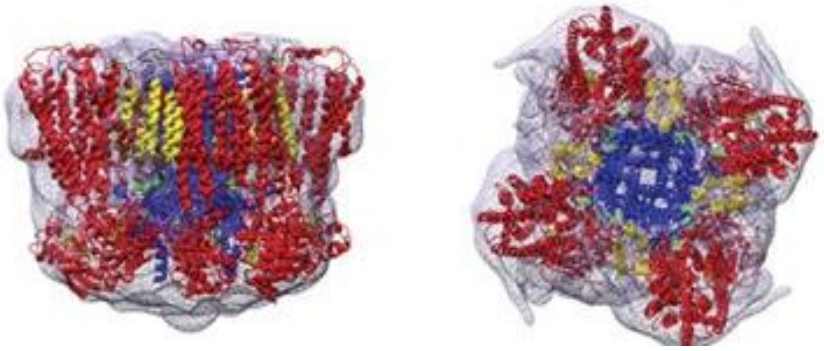
- Mechanisms/primary target: ???

K_{ATP} Channels:

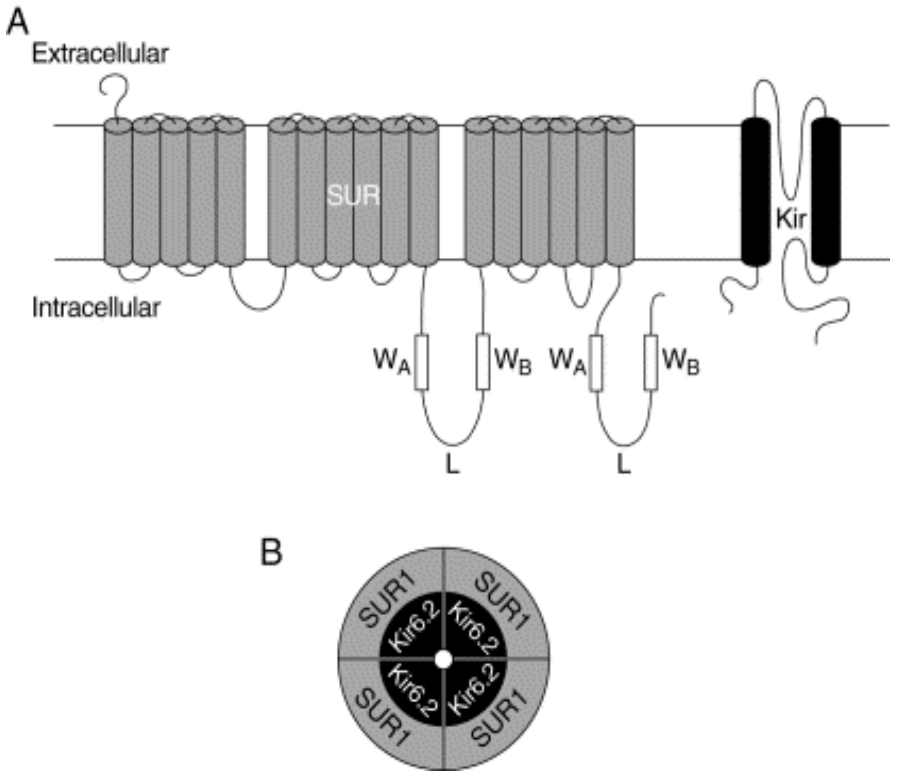
- Two Types:
  - mK_{ATP}:
    - Mitochondrial (unknown)
  - sK_{ATP}:
    - Plasmalemmal (defined)

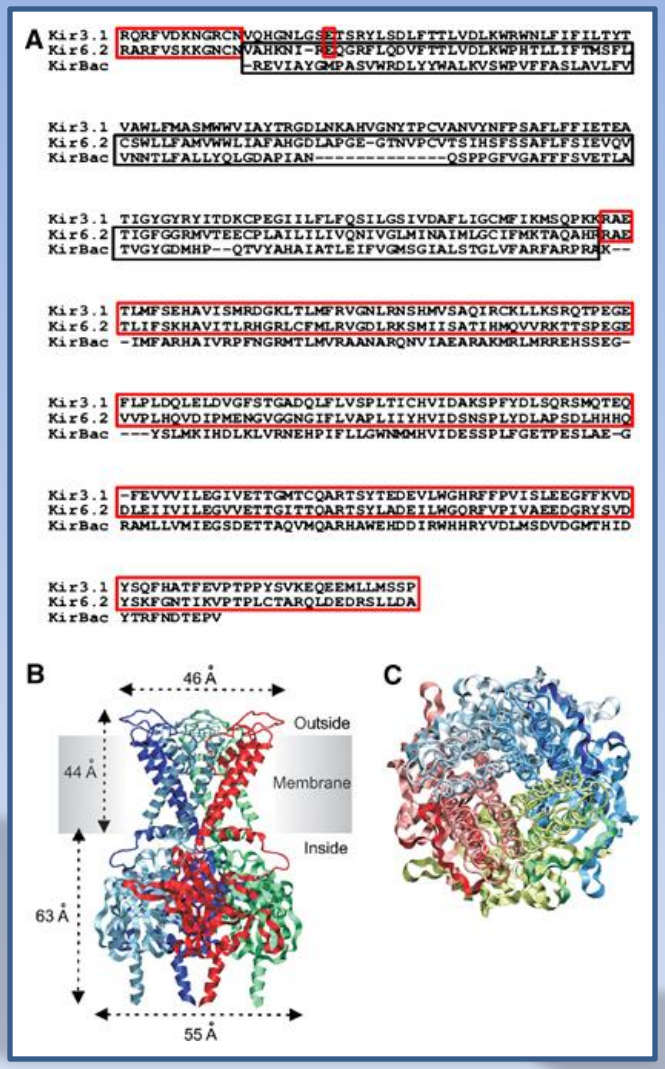
- sK_{ATP}:
  - Inwardly rectifying K^+ channels (K_{ir})
    - $K_{ir}6$ family: ATP-sensitive (ATP inhibition)
    - β-subunit: Sulphonylurea receptor

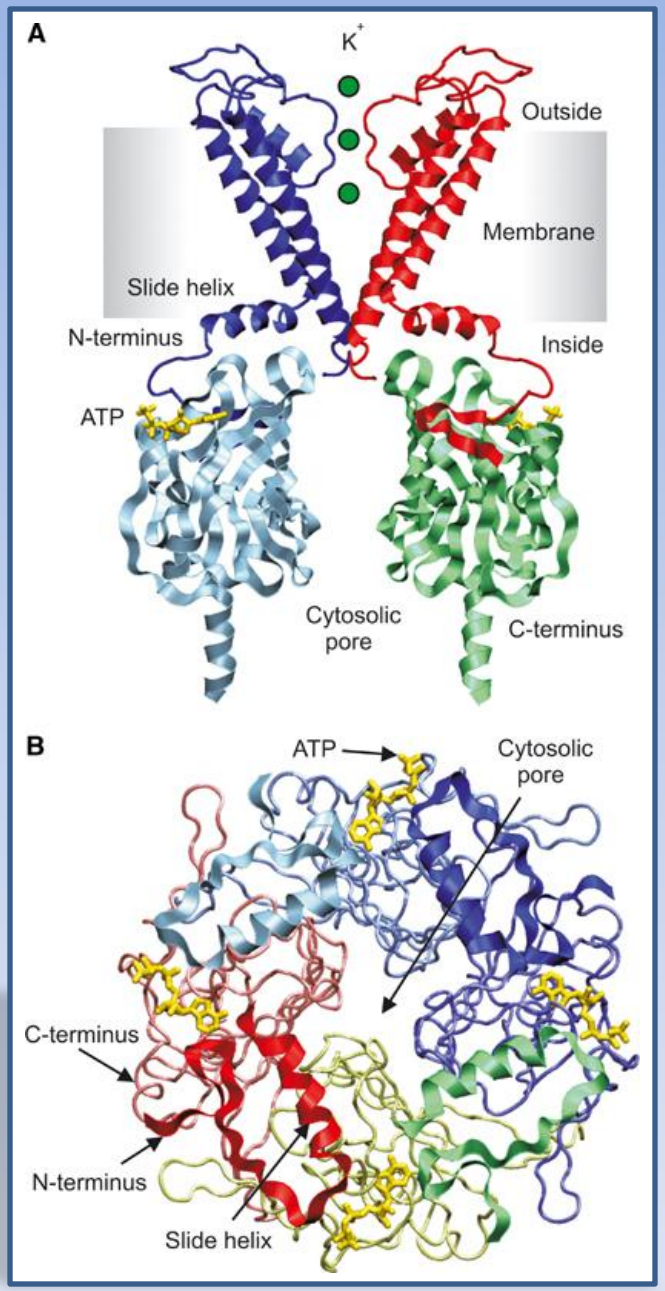
  – Heart muscle: $K_{ir}6.2$ and SUR2A
  – Smooth muscle: $K_{ir}6.1$ and SUR2B
  – Pancreatic beta cells: $K_{ir}6.2$ and SUR1
  – Neurons: primarily $K_{ir}6.2$ and SUR1



Mikhailov et al., (2005) *EMBO J* **24,** 4166

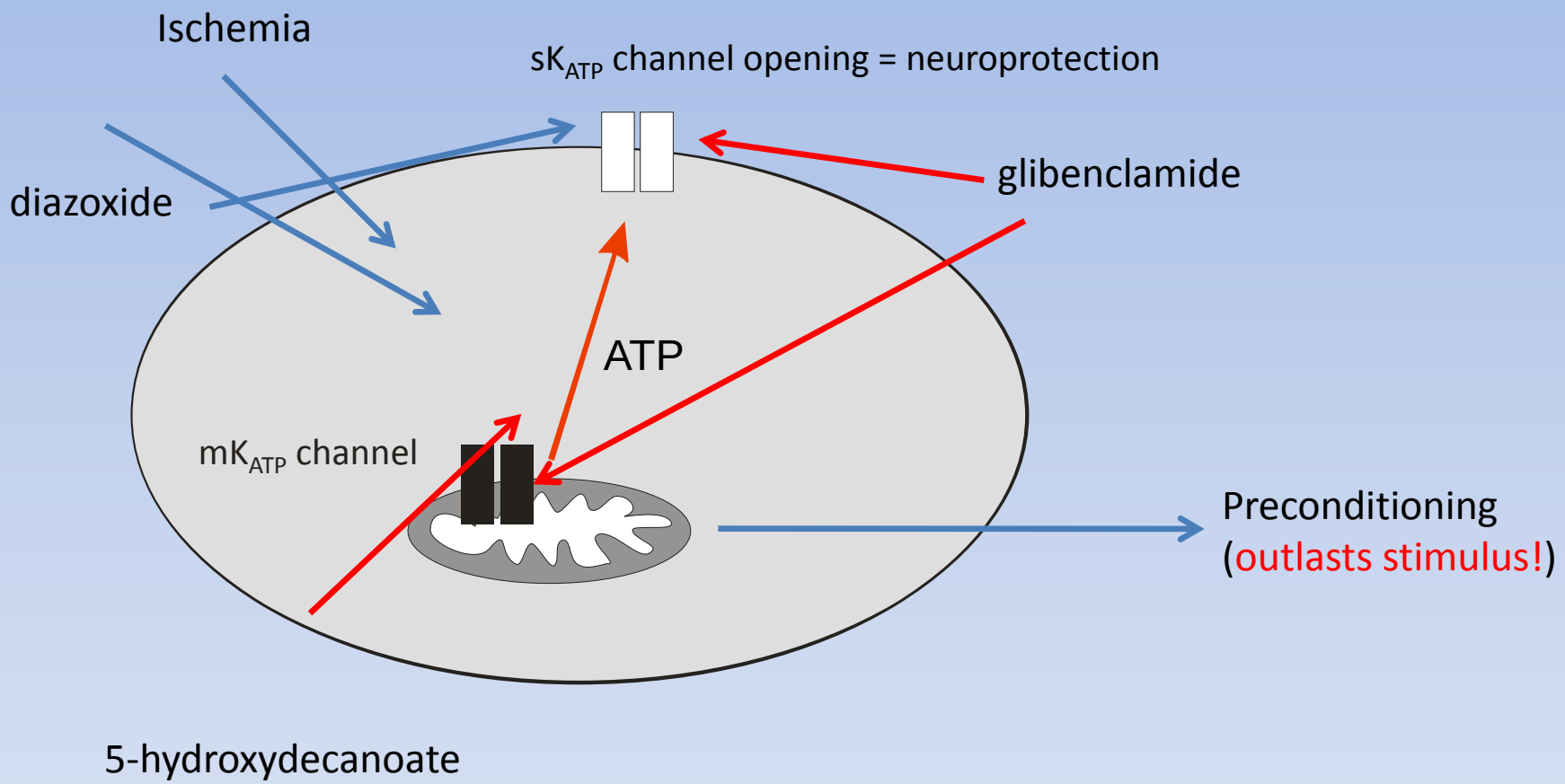






Homology model of Kir6.2 (GenBank D50581) based on the X-ray crystal structures of KirBac1.1 (Kuo *et al*, 2003) and the intracellular (IC) domains of Kir3.1 (Nishida and MacKinnon, 2002)

Antcliff et al., (2005) *EMBO J* **24**:229-239.


Ischemia
sK$_{ATP}$ channel opening = neuroprotection
diazoxide
glibenclamide
ATP
mK$_{ATP}$ channel
Preconditioning
(outlasts stimulus!)
5-hydroxydecanoate

Are sK_{ATP}, but not mK_{ATP}, channels essential for neuronal anaesthetic preconditioning?

If yes:

- Block of these channels must prevent preconditioning
- Drugs that act on mK_{ATP} channels must be without effects
- The anaesthetic must activate sK_{ATP} channels

K_{ATP} channel pharmacology

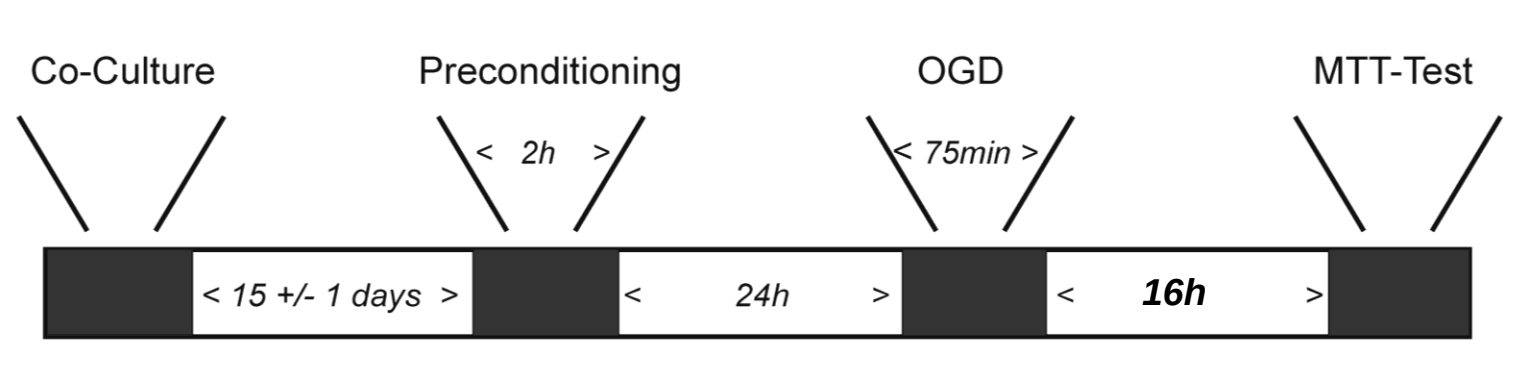
- **Diazoxide**
  - Opens ***brain*** sK_{ATP} & mK_{ATP}
- **Pinacidil**
  - Opens ***heart*** sK_{ATP} & mK_{ATP}

- **5-Hydroxydecanoate (5-HD)**
  - Blocks mK_{ATP}
- **Tolbutamide**
  - Blocks sK_{ATP}
- **Glibenclamide**
  - Blocks sK_{ATP} & mK_{ATP}

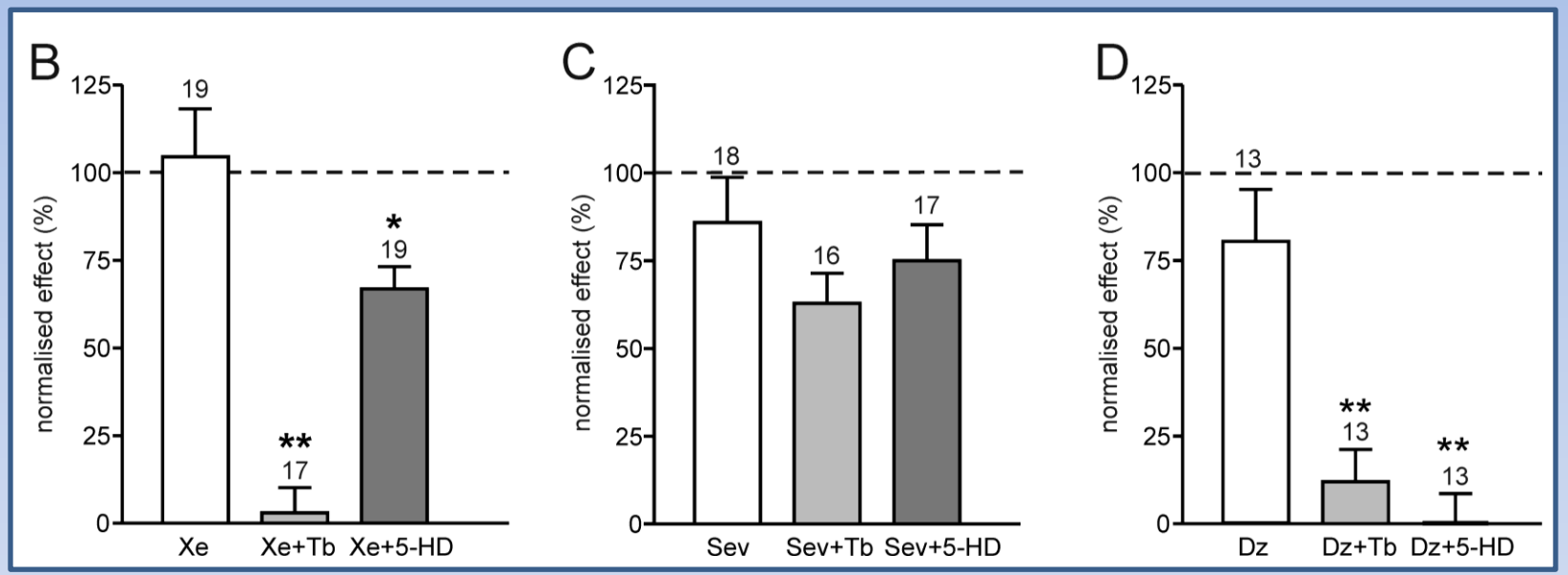
Methods: Preconditioning

1. Neuronal Glial Co-Culture generated from cortices of foetal and neonatal Balb/c mice
2. Preconditioning for 2 hours
3. 24h later exposure to oxygen – glucose deprivation (OGD) for 75 minutes
4. 16h later cell viability tested with MTT reduction test

   (requires active mitochondria to reduce MTT > blue colour > quantified in spectrophotometer)



Does the preconditioning effect of inhalational anaesthetics involve K_{ATP} channels?



- Xenon, sevoflurane and diazoxide elicit preconditioning in neuronal glial co-cultures
- Xenon's effect is dependent on opening of sK_{ATP} channels (only minor involvement of mK_{ATP} channels)
- Sevoflurane's effect is K_{ATP} channel independent

Electrophysiology

- **Heterologous expression of Kir6.2/SUR1 in HEK293 cells**
  - **Whole-cell voltage-clamp recordings**
  - **Inside-out macropatches**

Do inhalational anaesthetics modulate the activity of K_{ATP} channels?

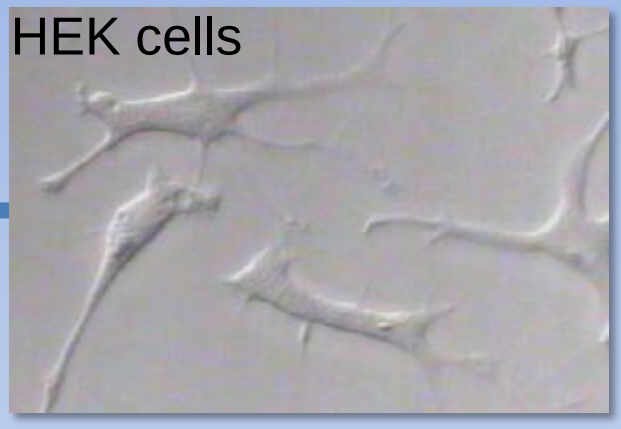
A drug that preconditions via K_{ATP} channels should increase K_{ATP} currents

HEK293 cell expression system

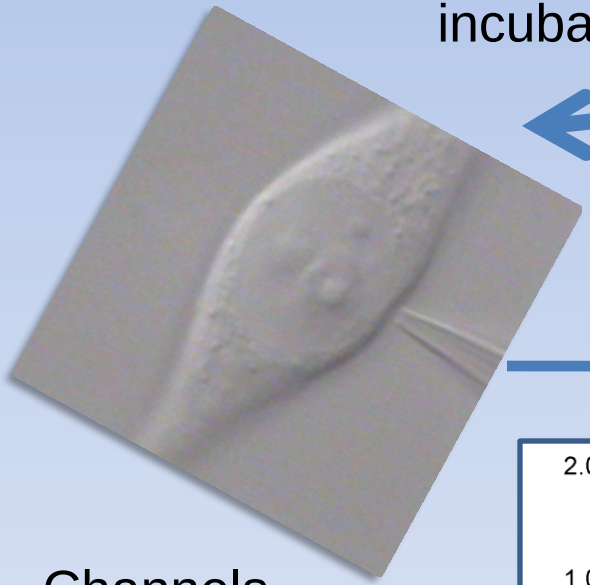
cDNA plasmids

Transfection

HEK cells

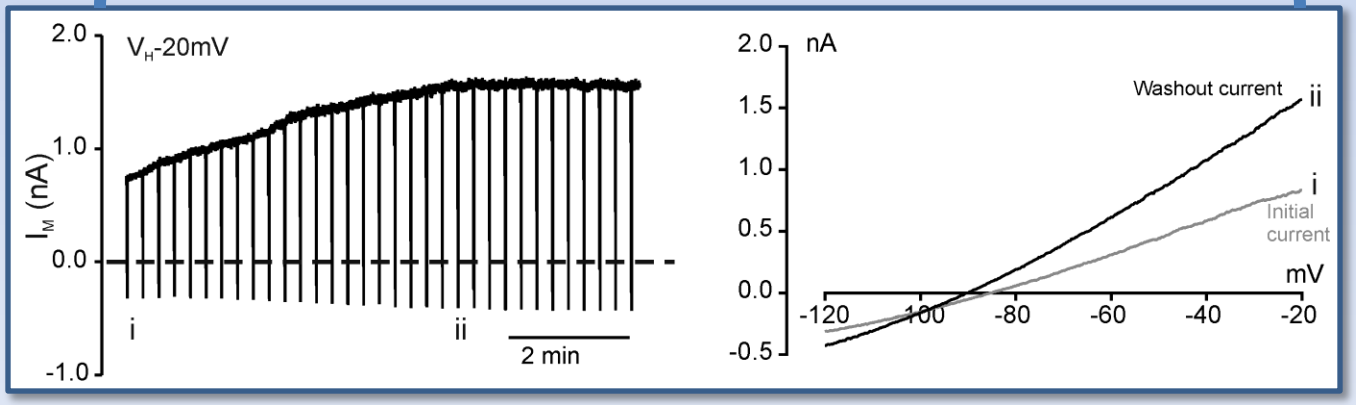


1-4 days
incubation

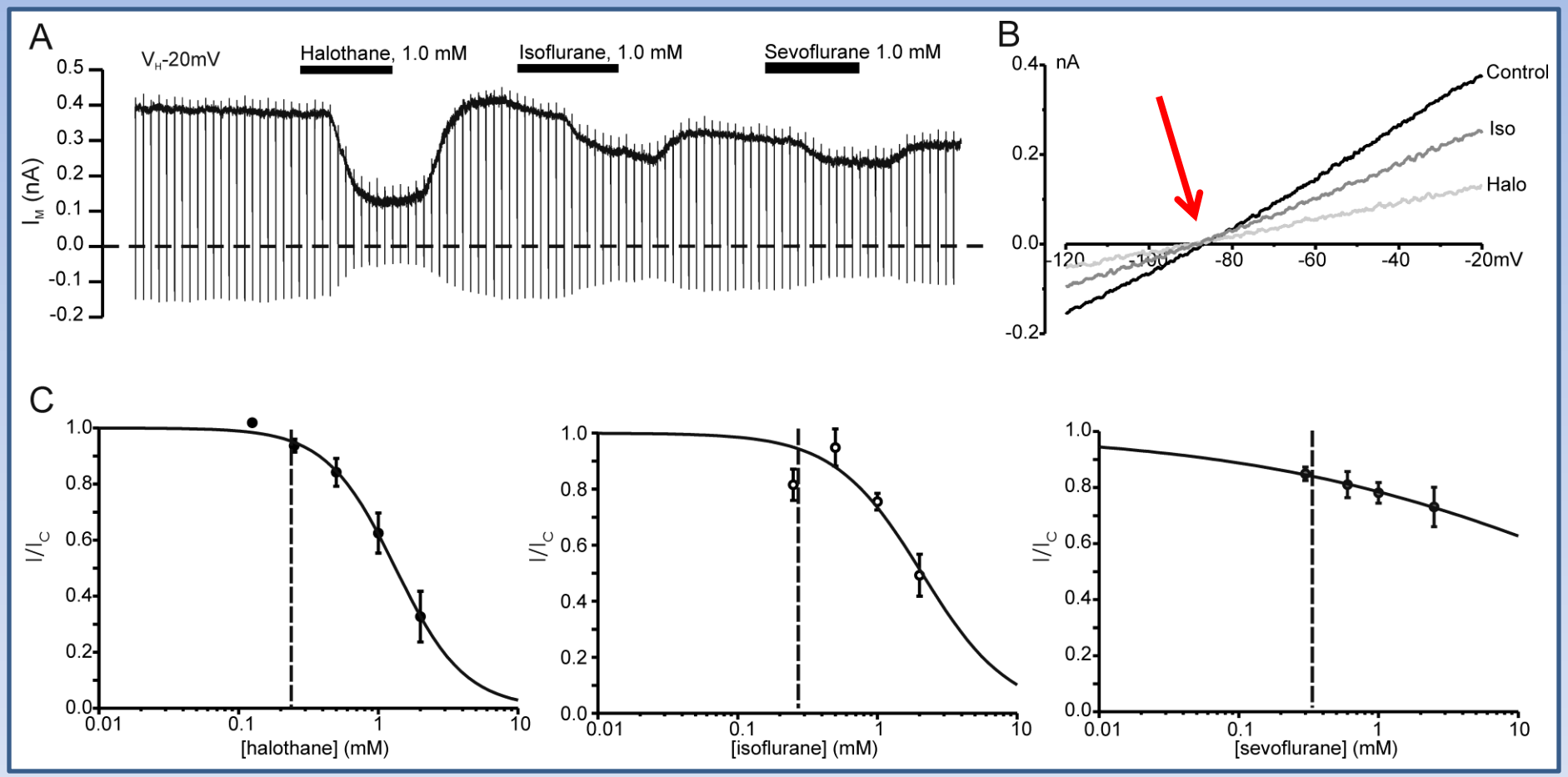


Channels
expressed

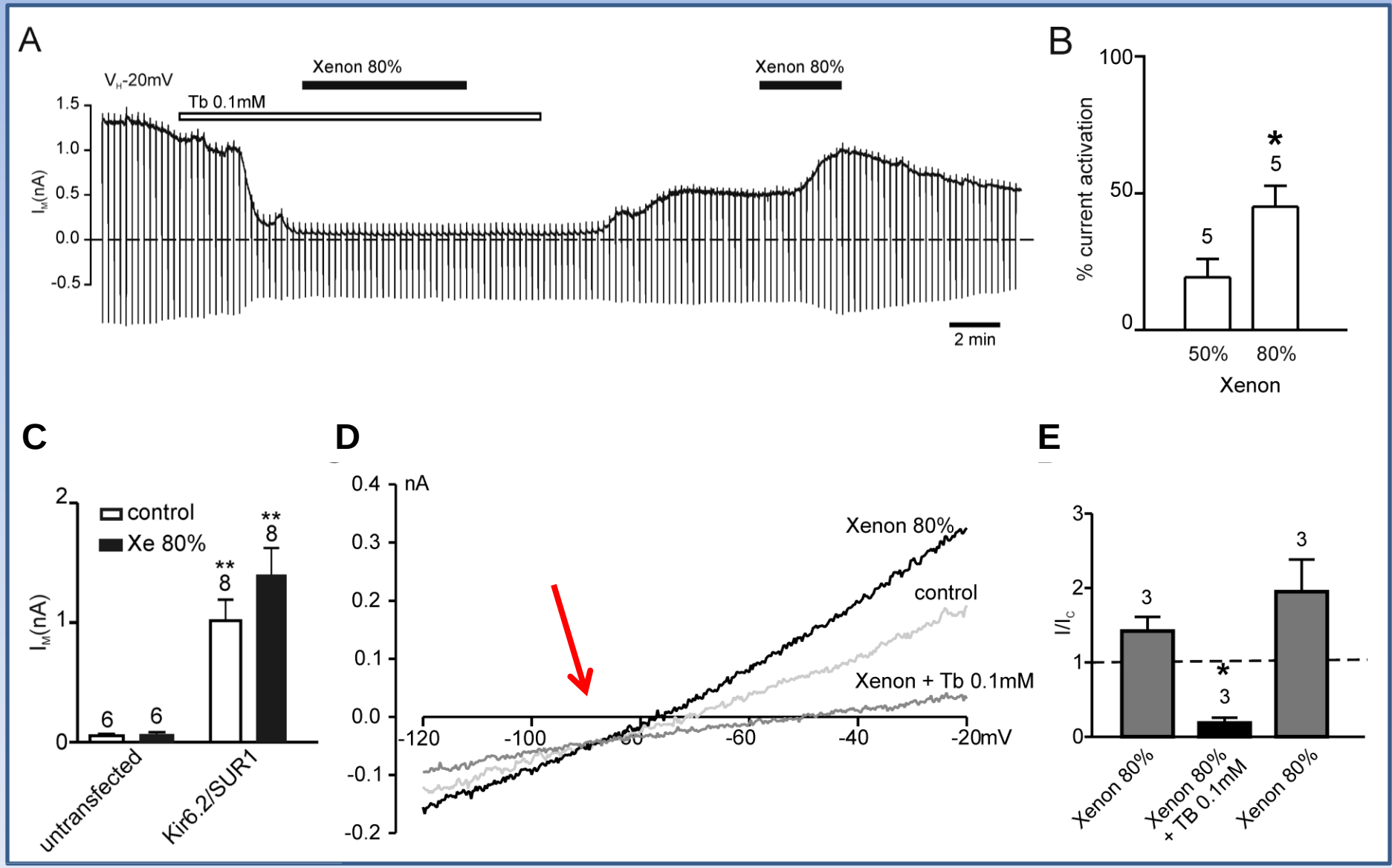
K_{ATP} currents



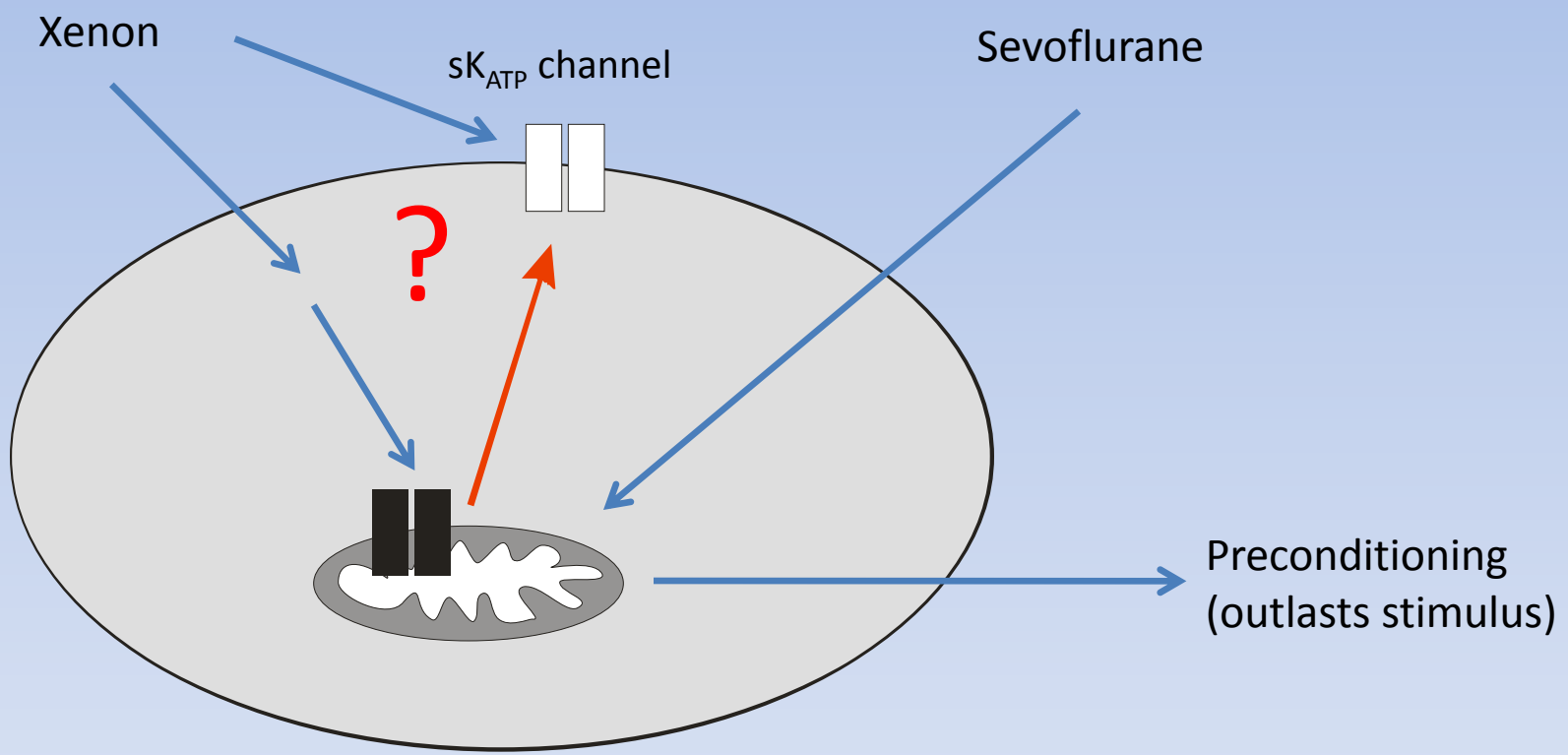
Sevoflurane & other volatiles



Xenon

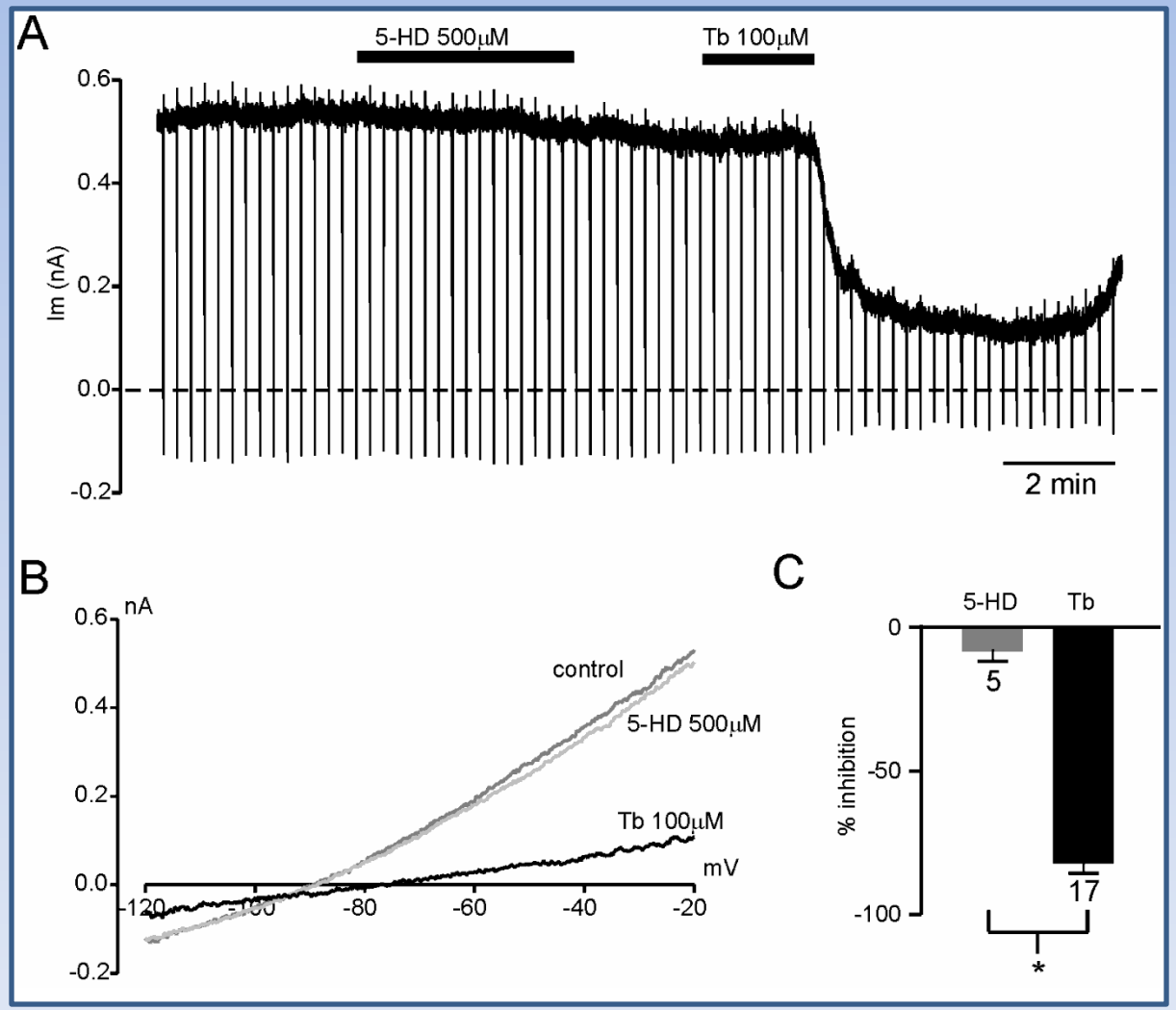

A
V_H-20mV
Tb 0.1mM
Xenon 80%
Xenon 80%
I_M(nA)
2 min
B
% current activation
Xenon
50%
80%
C
control
Xe 80%
I_M(nA)
untransfected
Kir6.2/SUR1
D
nA
Xenon 80%
control
Xenon + Tb 0.1mM
-20mV
E
I/I_C
Xenon 80%
Xenon 80% + TB 0.1mM
Xenon 80%

Direct or Indirect????



Do drugs that interact specifically with mK_{ATP} influence the activity of sK_{ATP}?

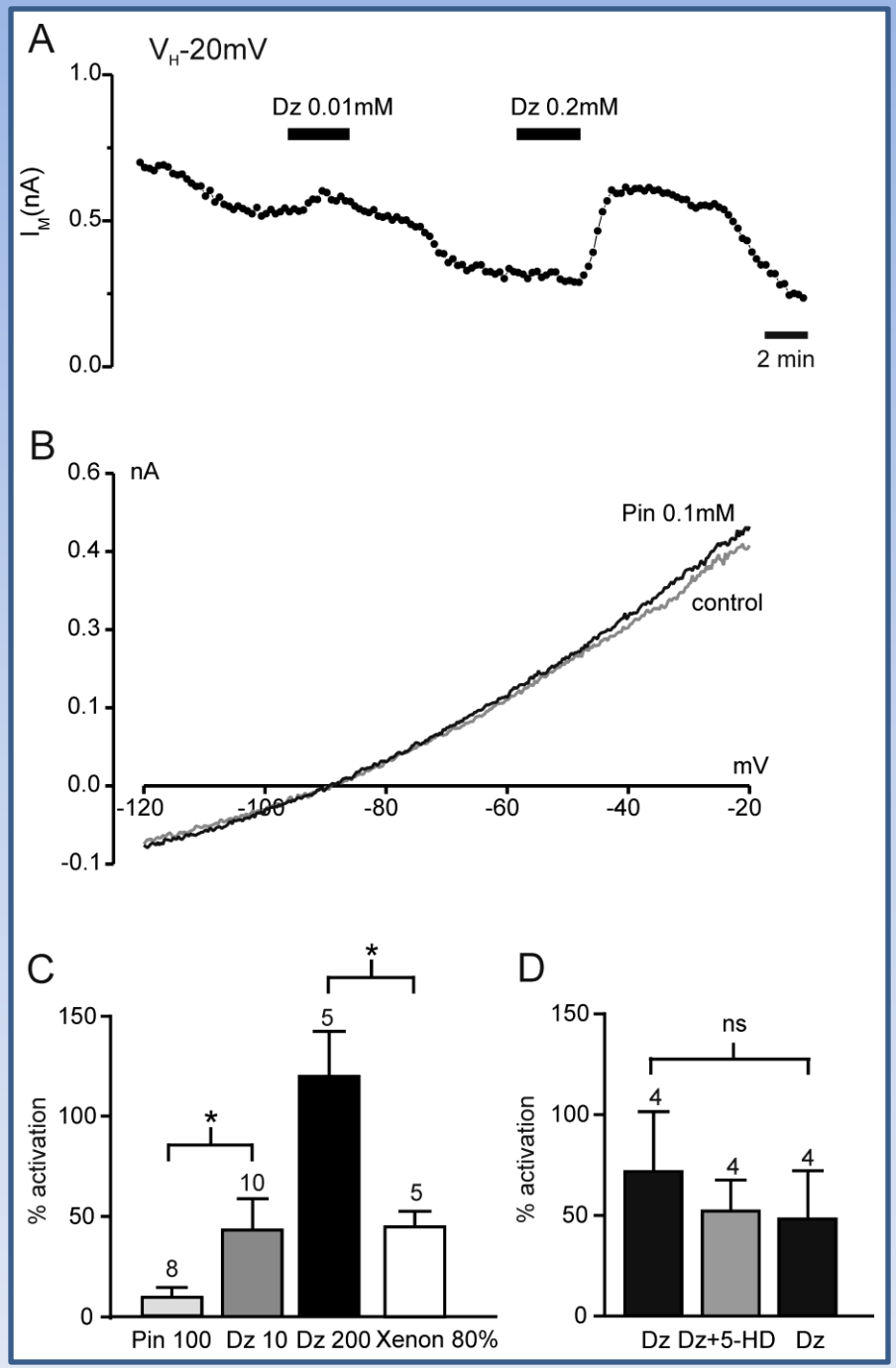
5-HD has no effect on sK_{ATP} currents in whole-cell recordings



Pinacidil has no effect on sK_{ATP} currents

5-HD does not prevent diazoxide activation of sK_{ATP} currents

→ Diazoxide activates sK_{ATP} independently of mK_{ATP} channels

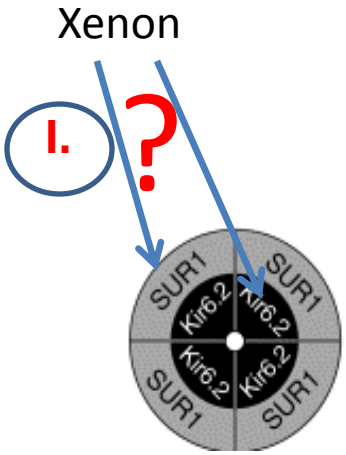


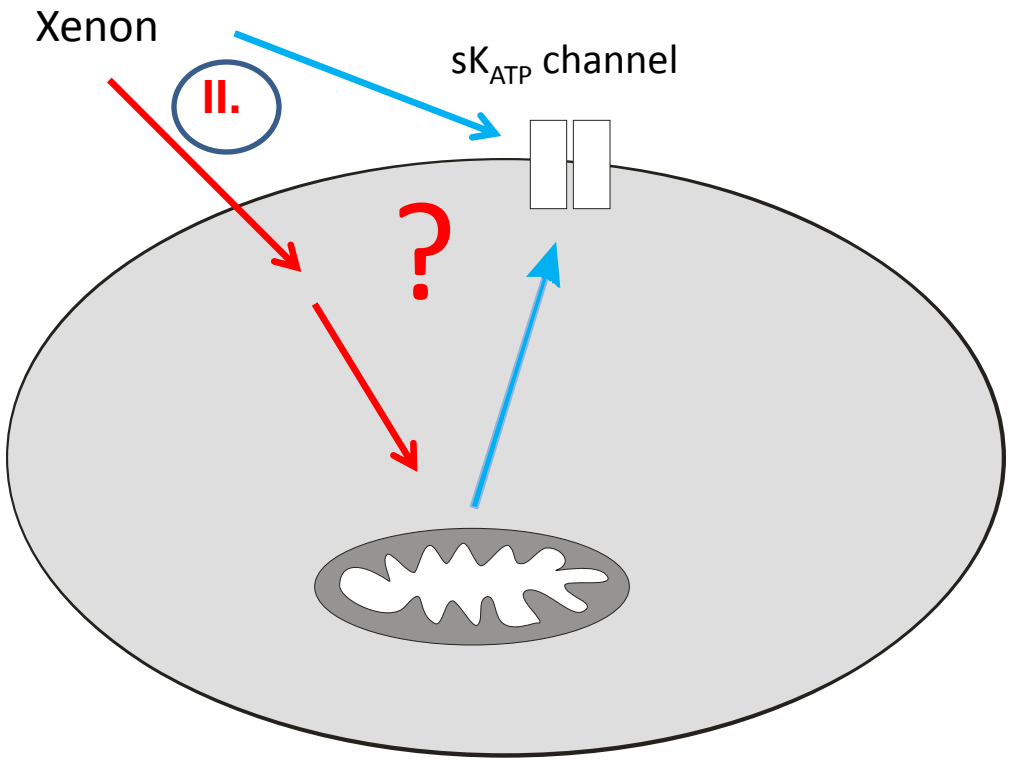
Summary: Electrophysiology

- mK_{ATP} specific drugs do not affect activity of sK_{ATP} channels in whole-cell recordings from HEK cells
- Sevoflurane is a weak sK_{ATP} channel inhibitor
- Xenon is an opener of sK_{ATP} channels

Bantel et al., 2009 *Anesthesiology* **110**: 986-995

Direct or Indirect????
SUR1 or Kir6.2????





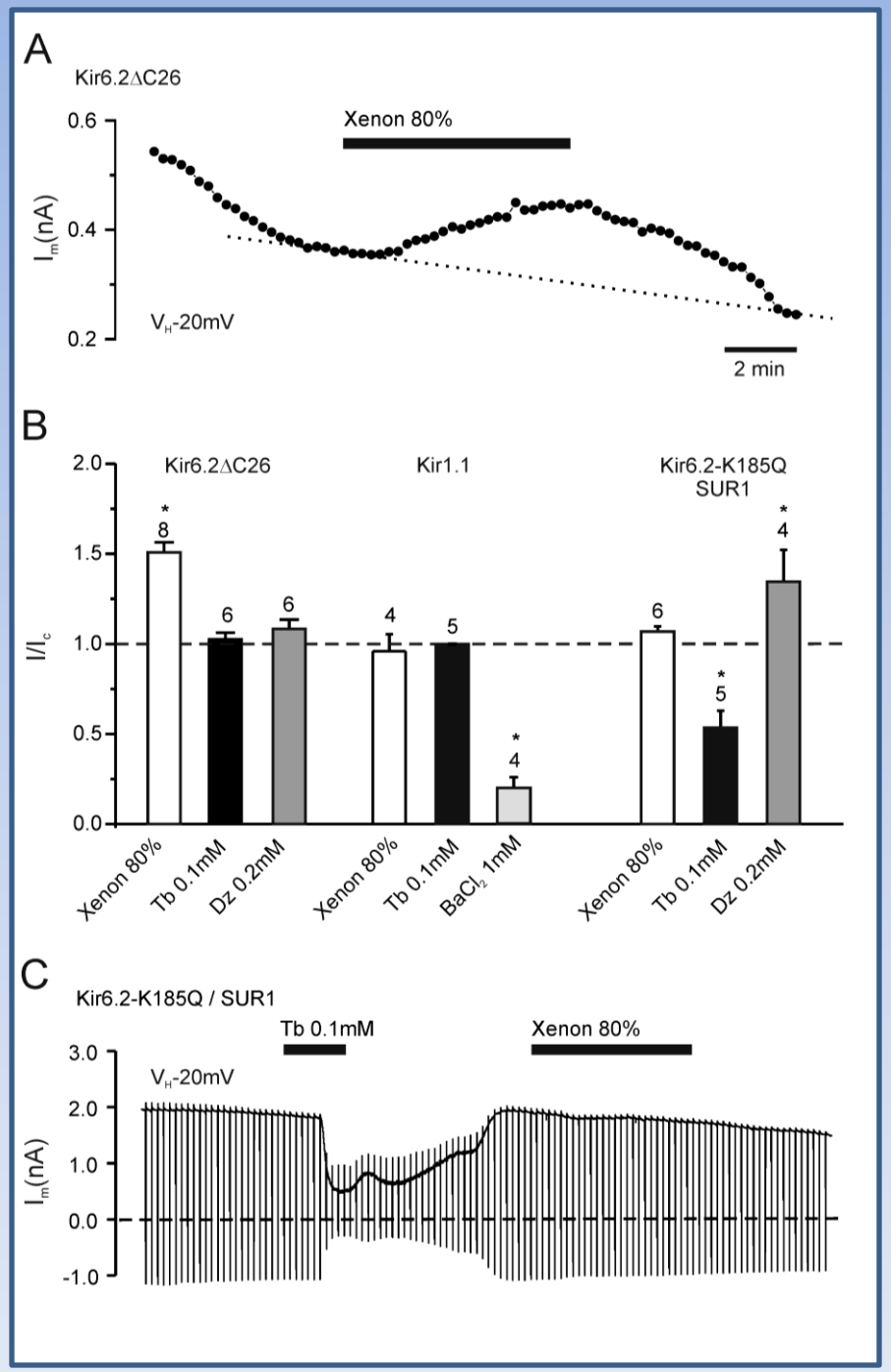
I. Truncated Kir6.2ΔC26

II. Macropatch:

wild type vs Kir6.2ΔC26

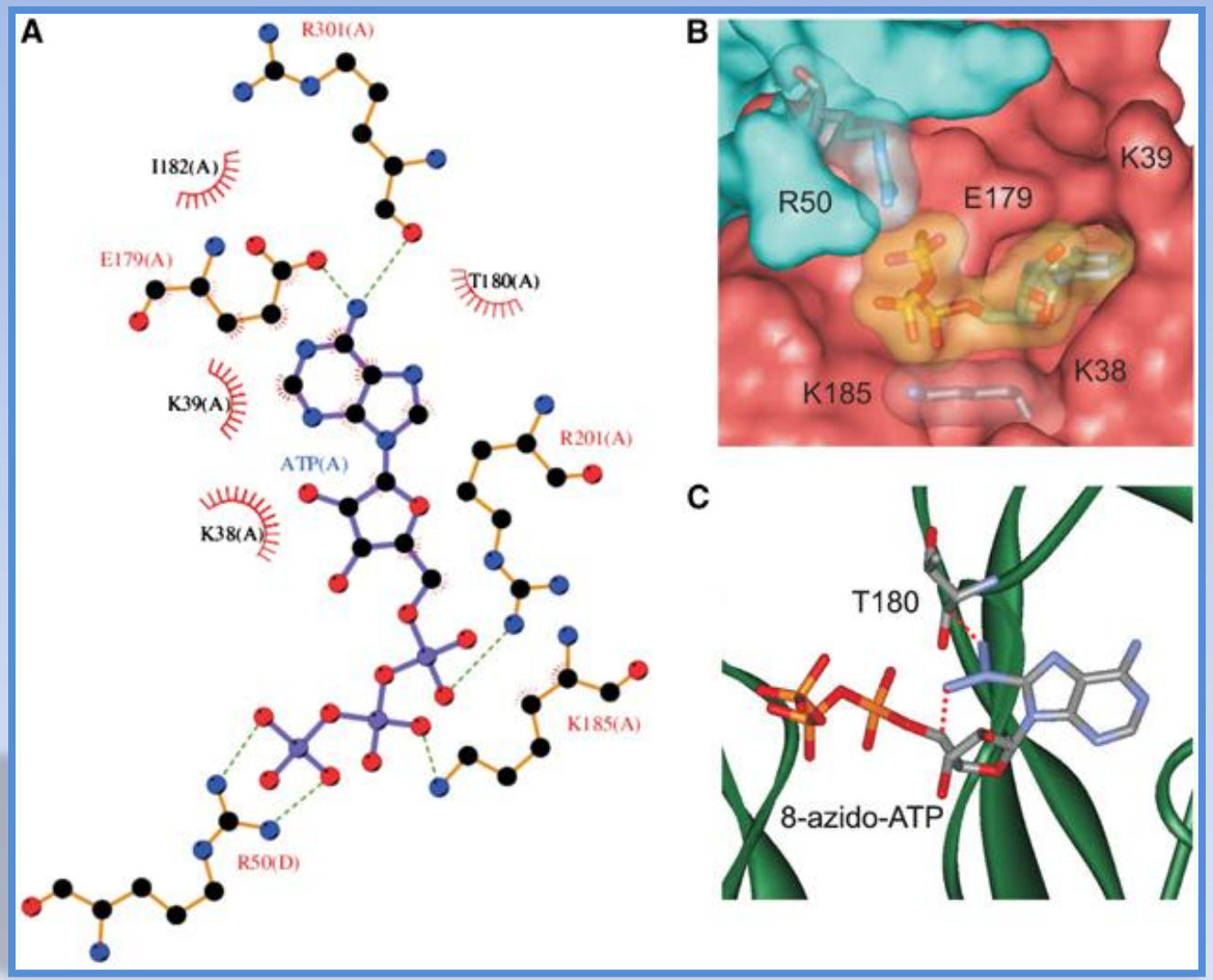
Xenon activates Kir6.2ΔC26 but not Kir1.1

Xenon does not activate the ATP-insensitive mutant Kir6.2-K185Q



Bantel *et al.* (2010)
Anesthesiology **112**:623-630.

The proposed binding site for ATP on Kir6.2



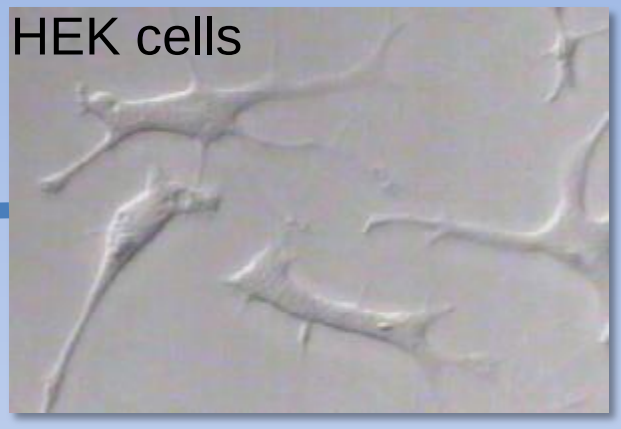
Trapp et al., (2003) *EMBO J* **22:**2903-2912.
Antcliff et al., (2005) *EMBO J* **24**:229-239.

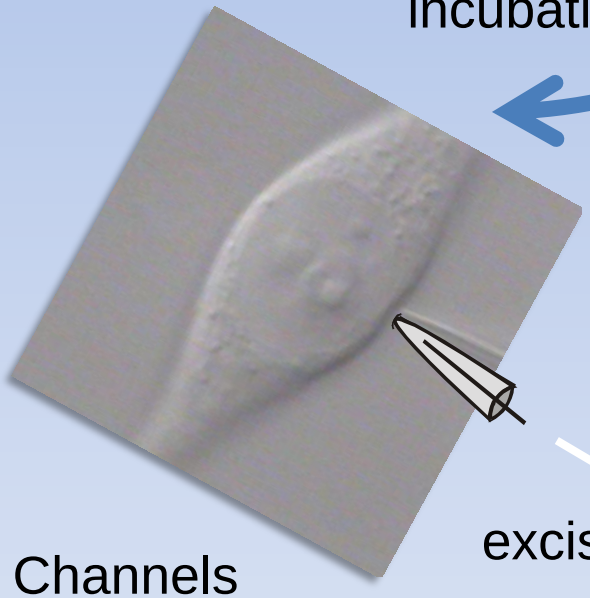
HEK293 cell expression system

cDNA plasmids

Transfection

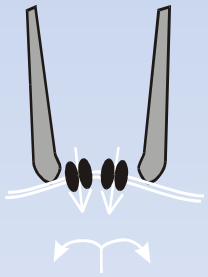
1-4 days
incubation



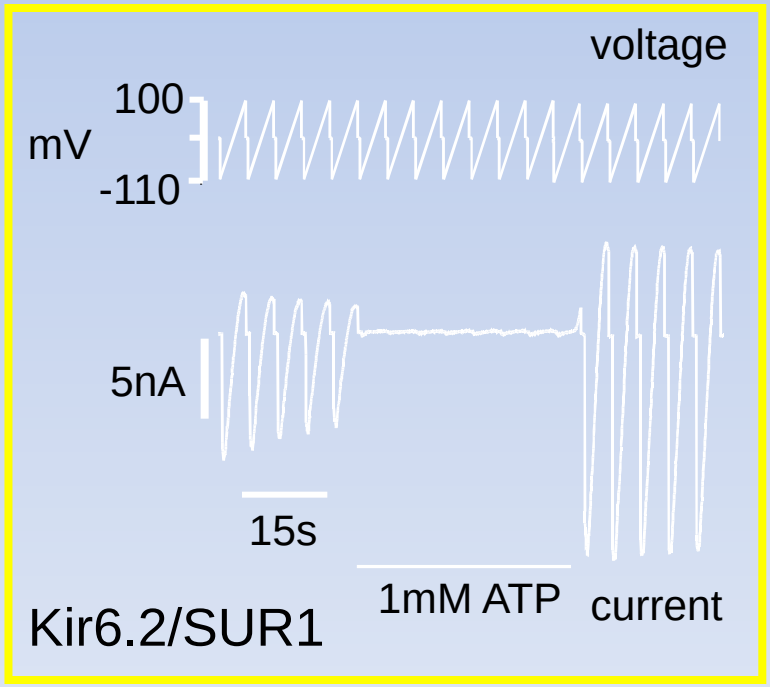


Channels
expressed

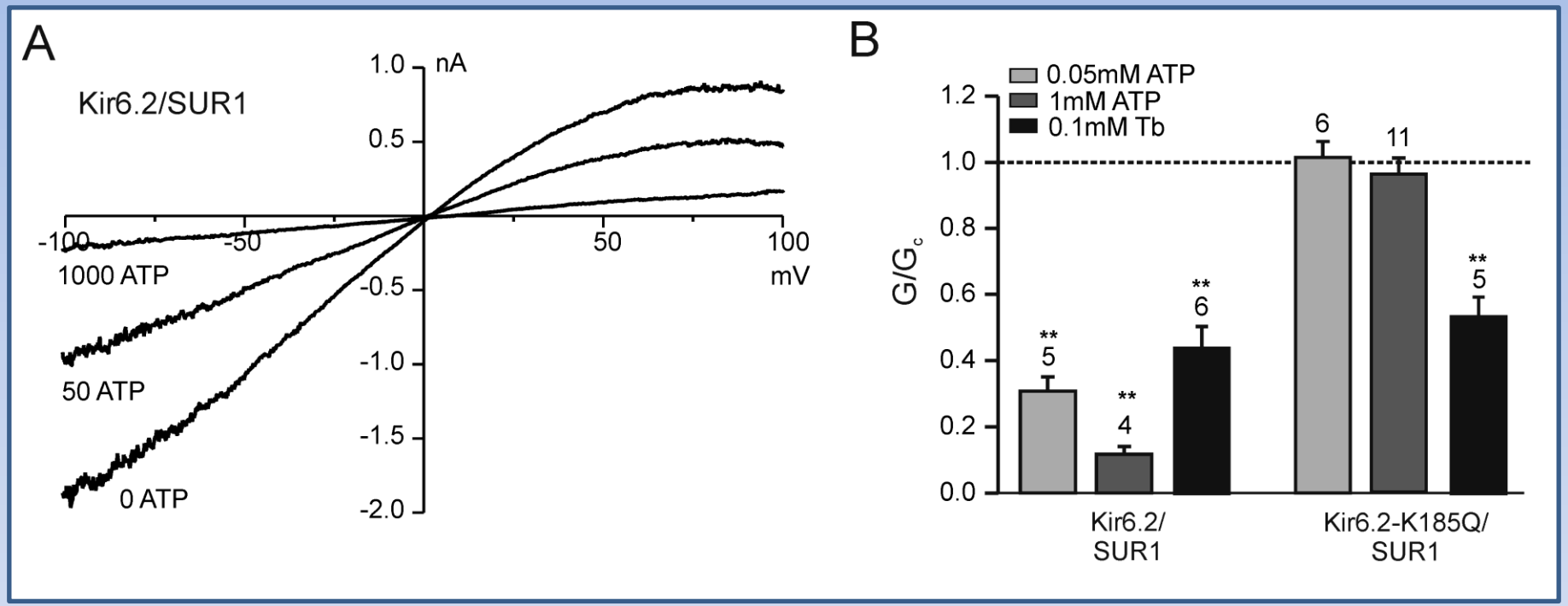
excision

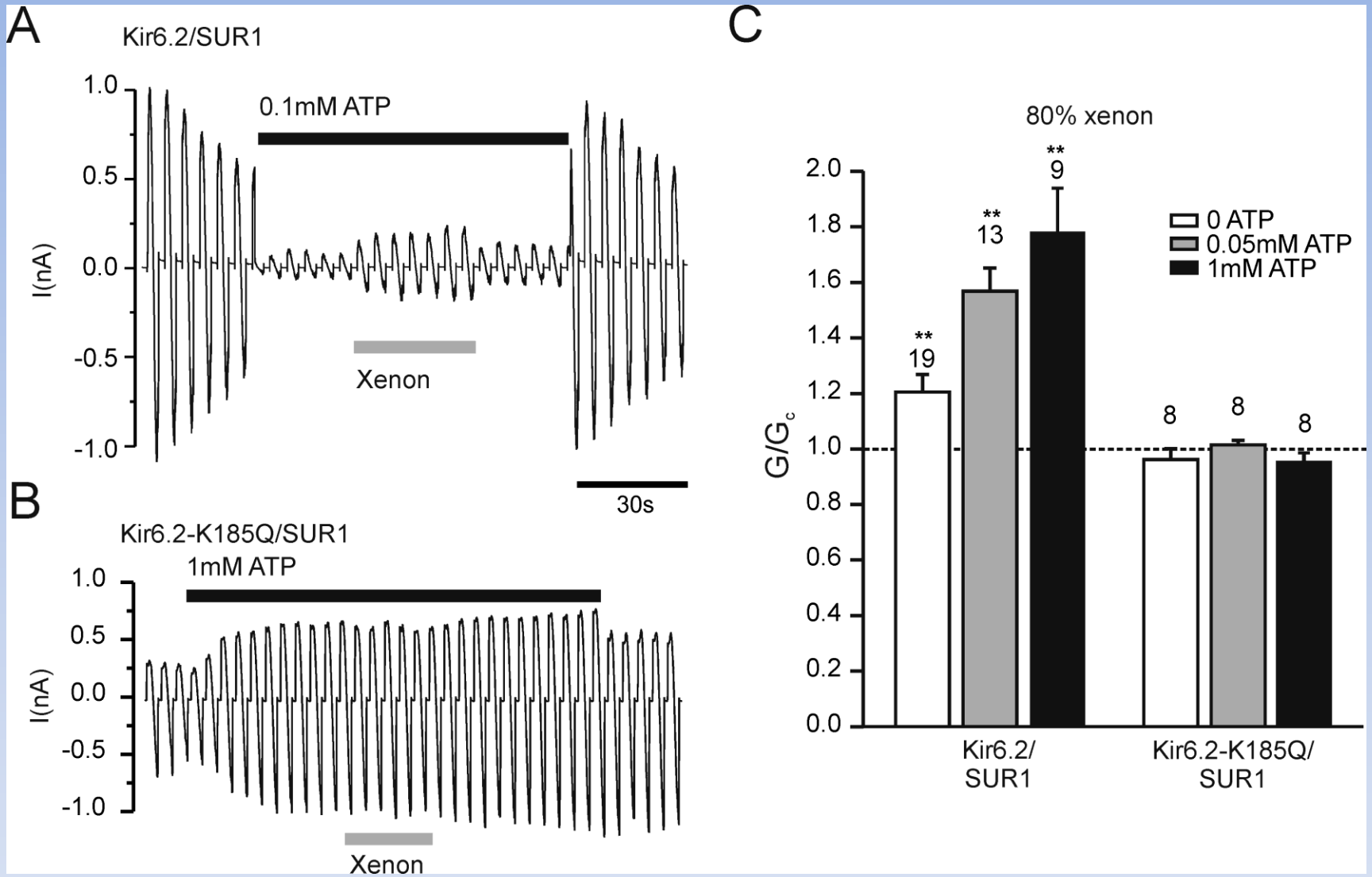


perfusion



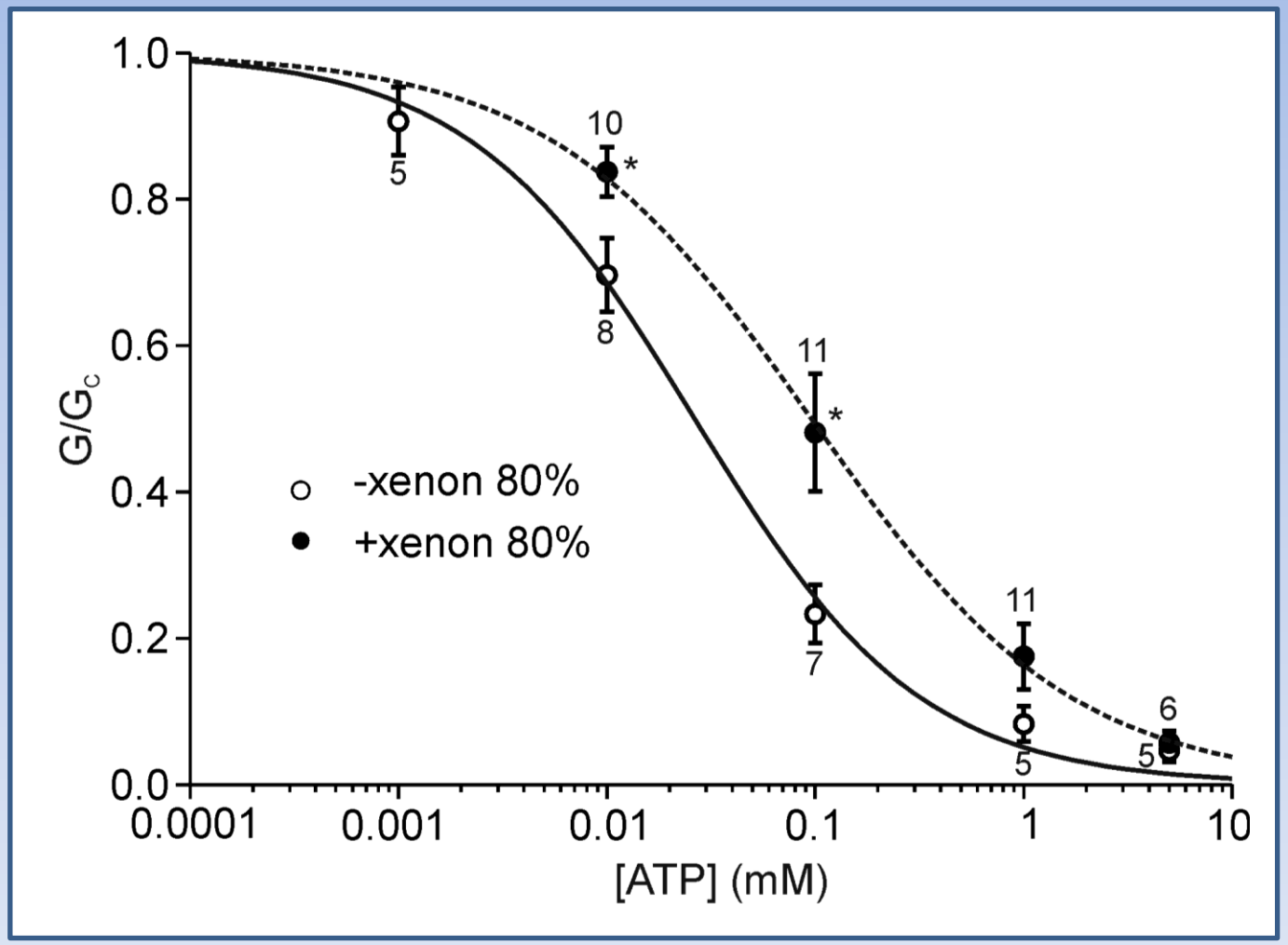
Inside-out macropatches: symmetrical [K^+]





Xenon activates wildtype, but not ATP-insensitive mutant, K_{ATP} channels

Xenon shifts the K_i for ATP-inhibition of Kir6.2/SUR1 to an approximately 4-fold higher concentration



Xenon

- Xenon is an opener of sK_{ATP} channels
- Xenon acts on the Kir6.2 subunit
- Xenon shifts the ATP-sensitivity of the channel

→ Xenon is a novel BBB-permeant K-channel opener