

Voltage-gated Proton Channels Structure from Function



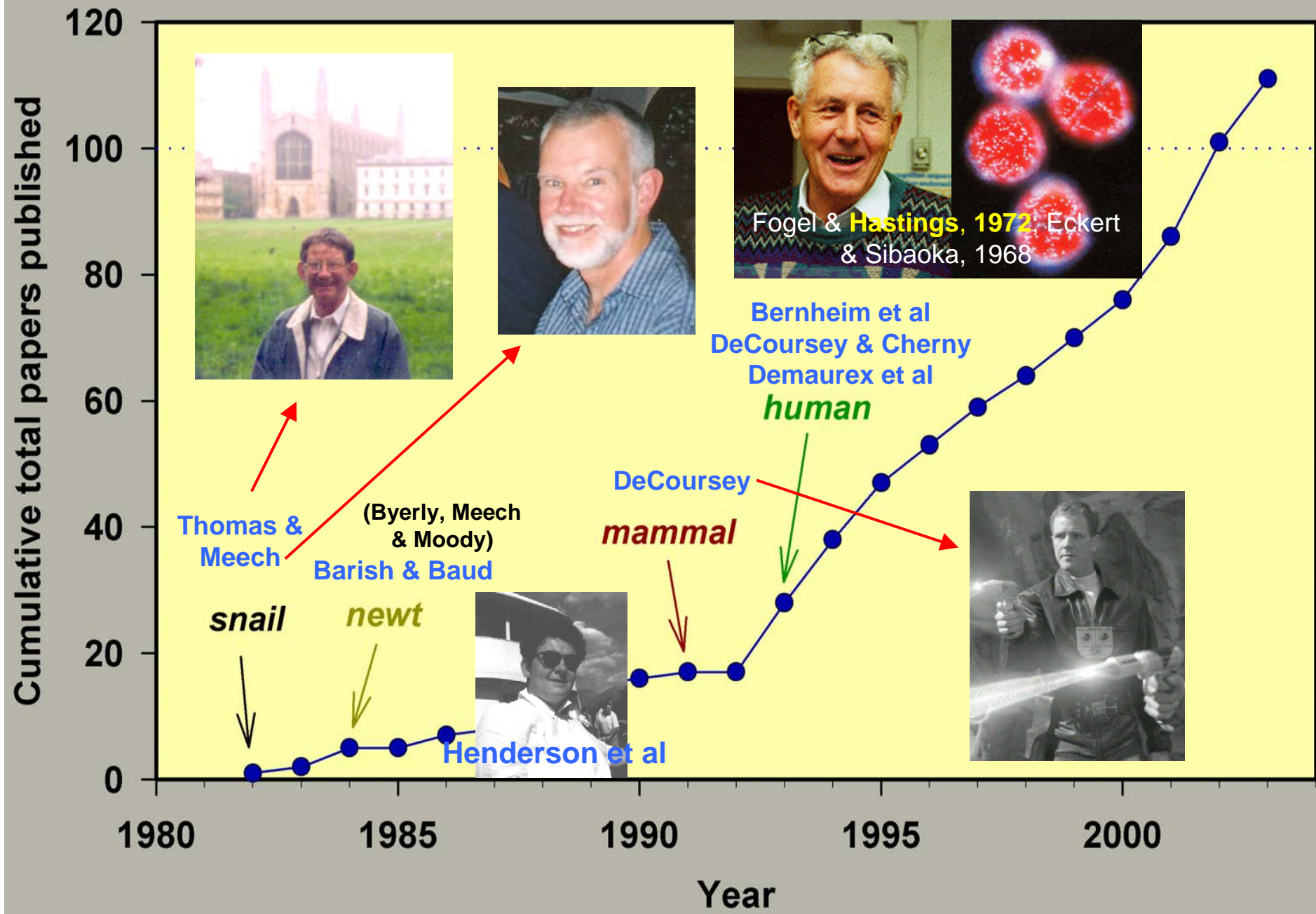
Proton Conduction Through Voltage-gated Proton Channels



Features Common to Biological Proton Channels

- Vladimir Cherny
- Deri Morgan
- Ricardo Murphy
- Tom DeCoursey
- Captain Proton

Voltage-clamp Studies of Proton Currents



Cells, Species With H⁺ Channels

Cells without H⁺ channels: COS-7

Neuron
Helix aspersa
Lynmaea stagnalis

Oocyte
Ambystoma
Rana esculenta

Epithelium
alveolar rat
lung A549 human
airway JME/CF15, Calu-3 human
cervical HeLa human
prostate PC-3 human
kidney HEK-293 human
renal proximal tubule *Rana pipiens*
ovary CHO hamster

Connective tissue
fibroblast 3T3 mouse

Skeletal muscle
myocyte human

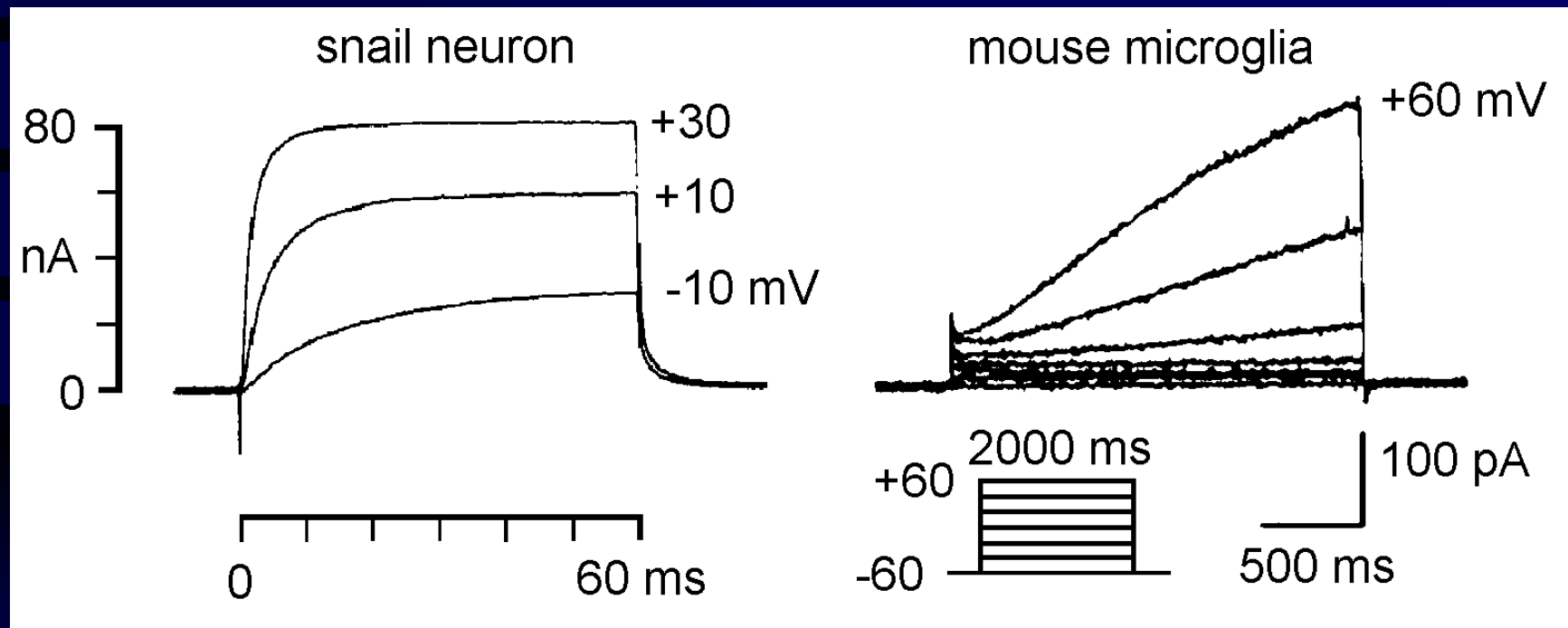
Lymphocyte
T lymphocyte human
B lymphocyte human
Jurkat E6-1 human

Granulocyte
neutrophil human
eosinophil human
basophil human
mast cell mouse
HL-60 human
PLB-985 human
K-562 human

Microglia
microglia mouse
microglia human
microglia rat
microglia BV-2 mouse
microglia GM1-R1 rat
microglia MLS-9 rat

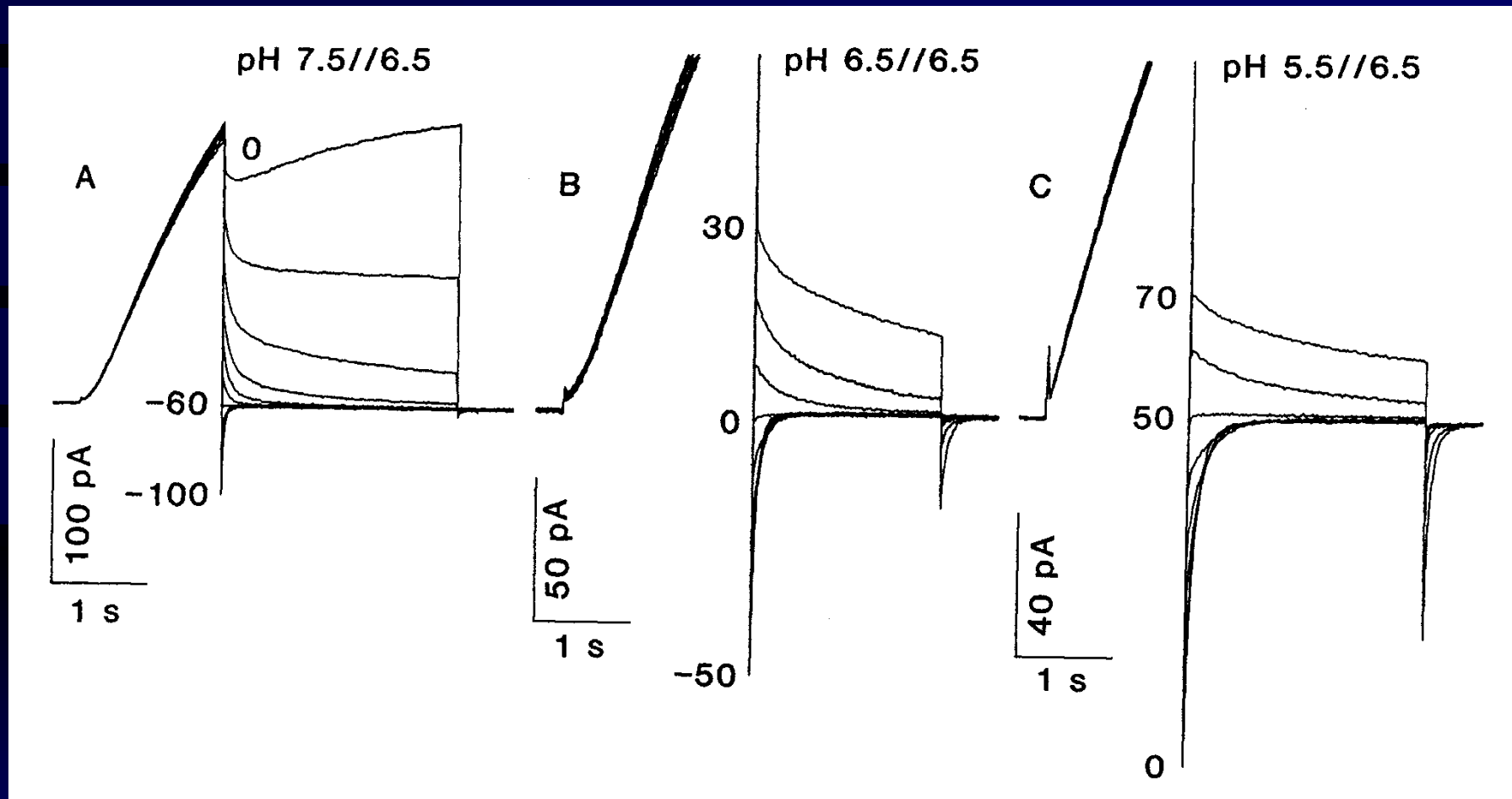
Macrophage
peritoneal mouse
alveolar rat
osteoclast rabbit
osteoclast mouse
osteoclast chicken
THP-1 monocyte human

Proton Currents in Snails and Mice: Similar, but Kinetics Differ



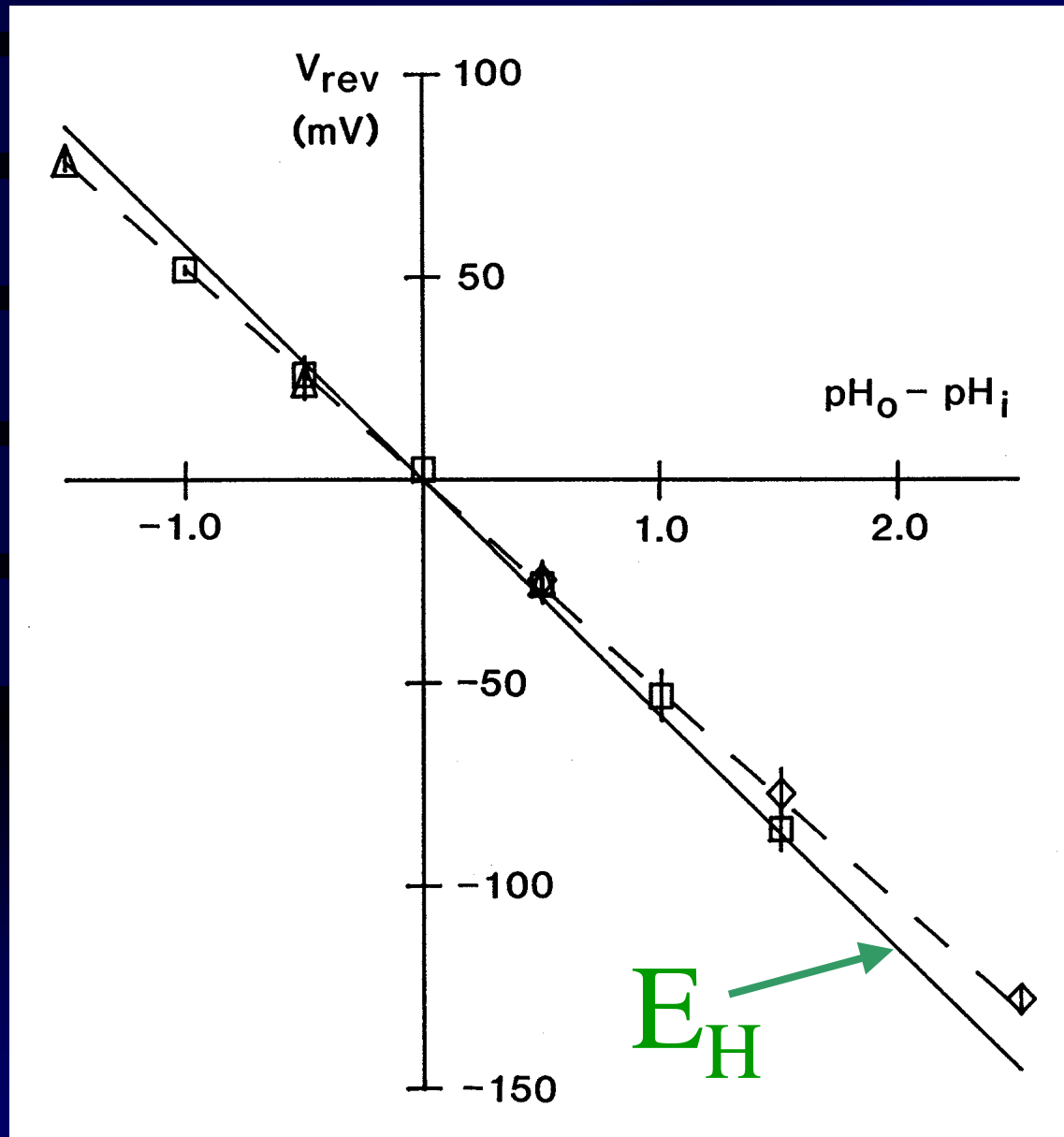
Eder & DeCoursey, 2001. *Progr. Neurobiol.* 64:277-305.

High Proton Selectivity



Cherny *et al*, 1995. *J. Gen. Physiol.* 105:861-896.

Reversal Potentials are Near the Nernst Potential, E_H



- H^+ channels are perfectly selective for H^+ and D^+

- No detectable permeation by any other ions

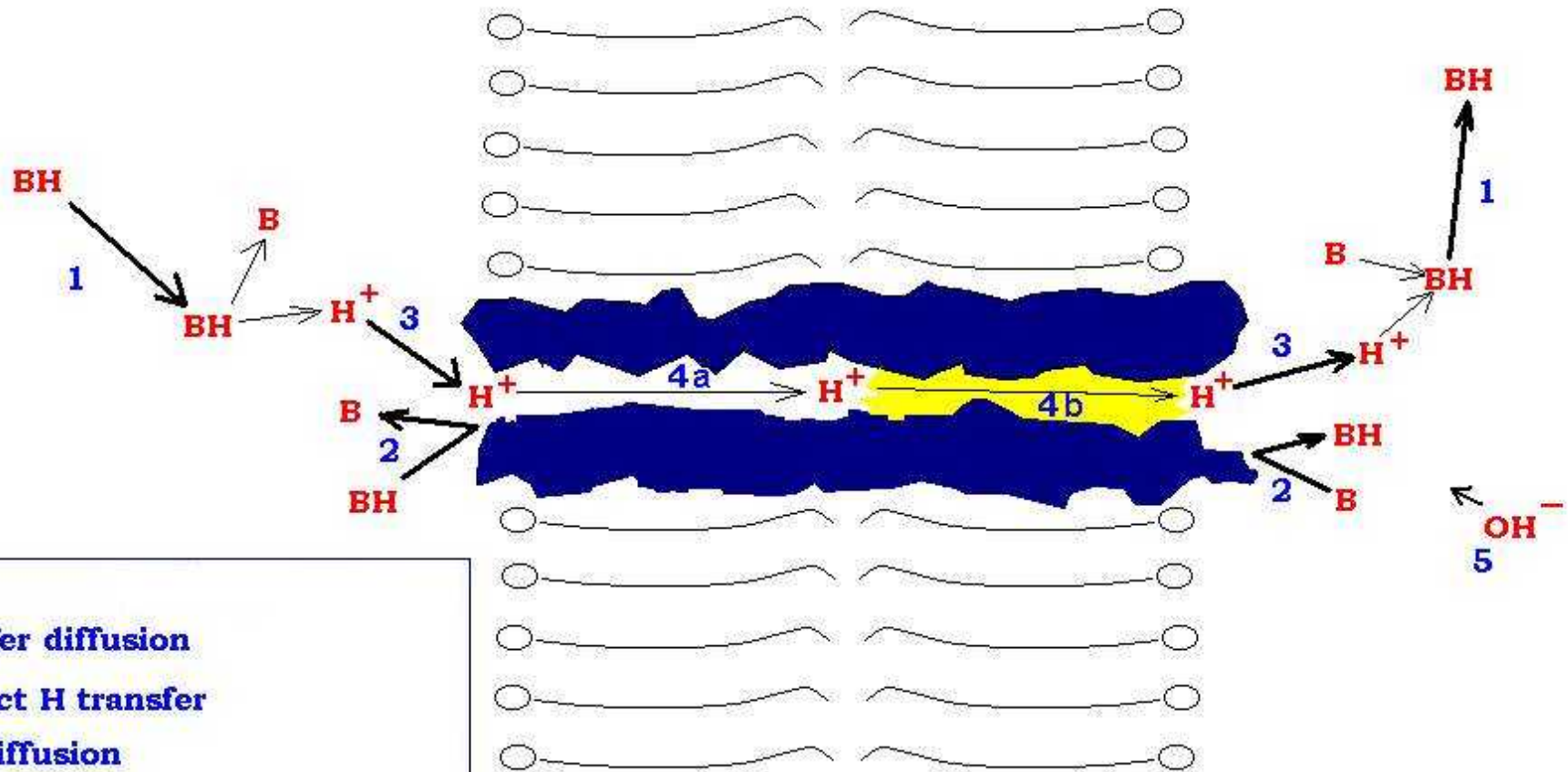
- Deviations from Nernst are due to imperfect control of pH

Cherny et al, 1995. *J. Gen. Physiol.* 105:861-896.

Selectivity of Ion Channels

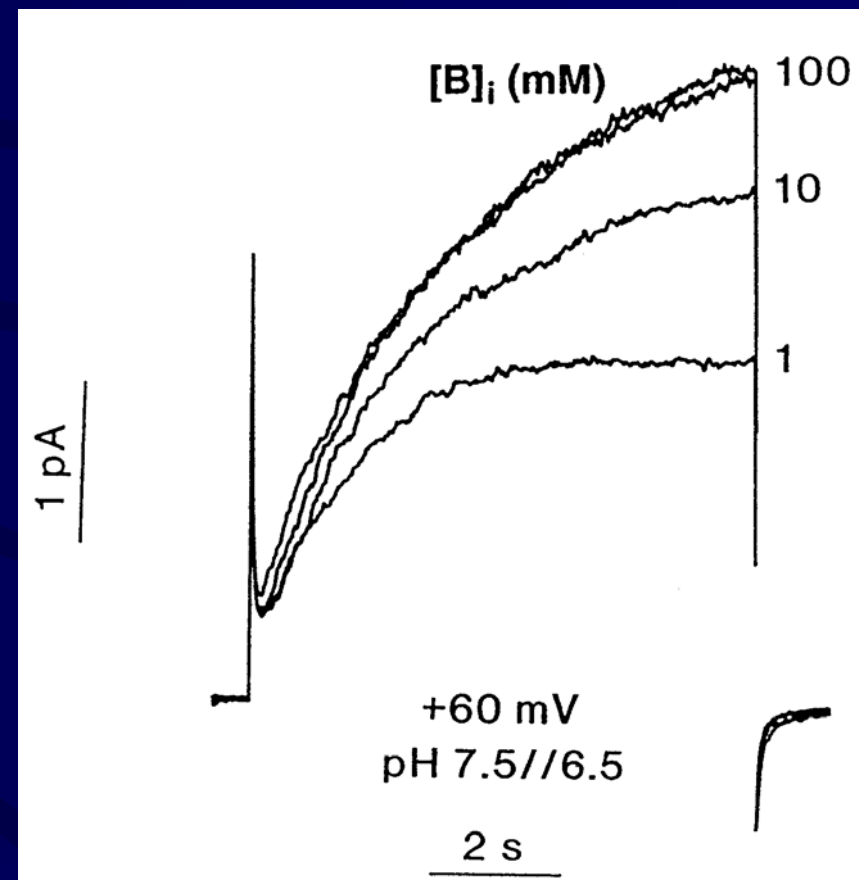
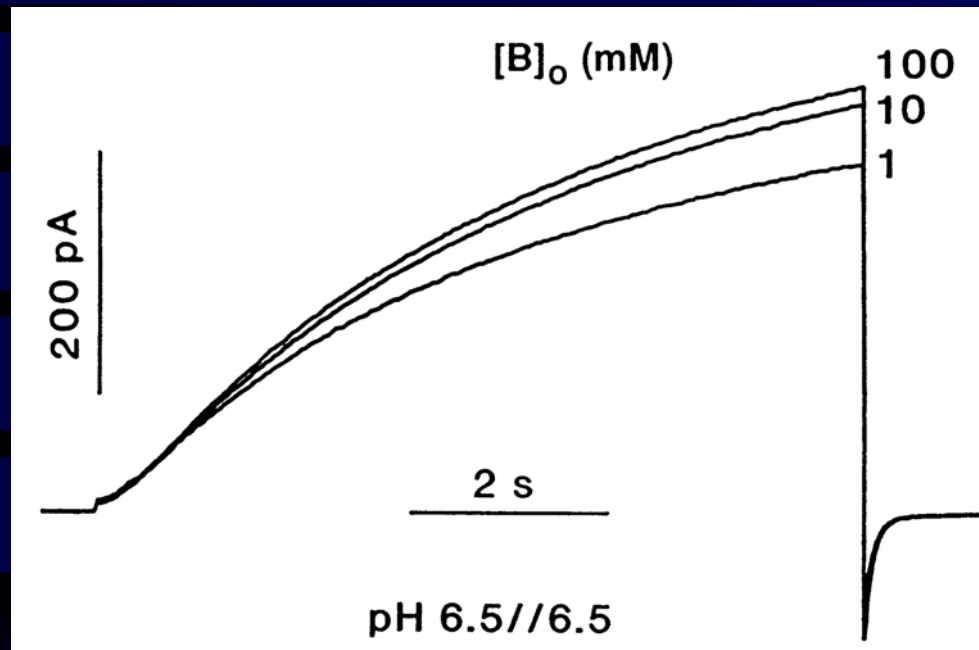
CHANNEL	PERMEANT IONS	SELECTIVITY
K ⁺ delayed rectifier	Tl > K > Rb > Cs > Na	$P_{Na}/P_K < 0.01$
KcsA	Tl > K > Rb > Cs > Na	$P_{Na}/P_K 0.006$
Maxi K ⁺ (Ca-activated)	Tl > K > Rb > Cs > Na	$P_{Na}/P_K < 0.001$
V-gated Ca ²⁺	Ba > Sr > Ca	$P_{Na}/P_{Ca} 0.0008$
V-gated Na ⁺	Na > Li > Tl > K	$P_K/P_{Na} 0.1$
Cl ⁻ (glycine-activated)	SCN > NO ₃ > I > Br > Cl > formate > acetate > F > phosphate	$P_{acetate}/P_{Cl} 0.5$
Gramicidin	H > NH ₄ > Cs > Rb > K > Na	$P_{Na}/P_K 0.25$
AchR	Guanidine > Cs > Rb > K > Na Mg > Ca > Ba > Sr	$P_{Na}/P_K 0.9$
Proton (V-gated)	H > D	$P_{Na}/P_H 0$

What is the Rate Limiting Step?

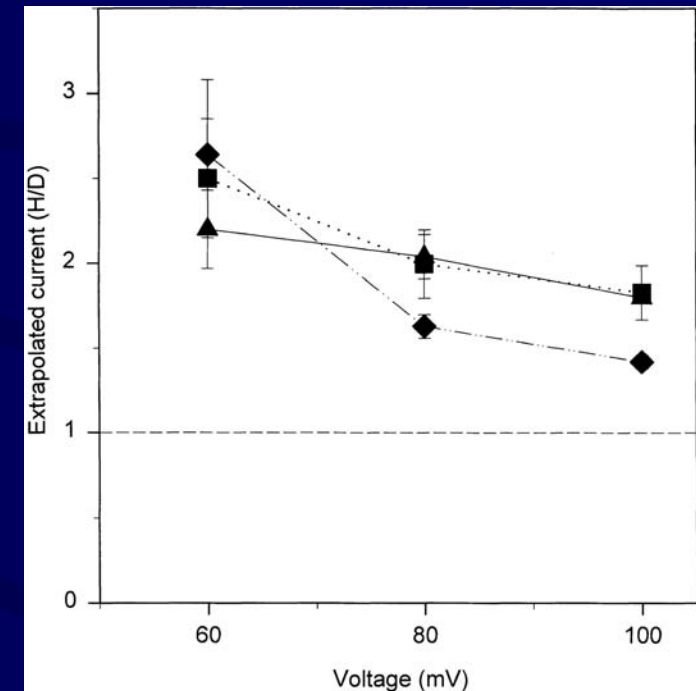
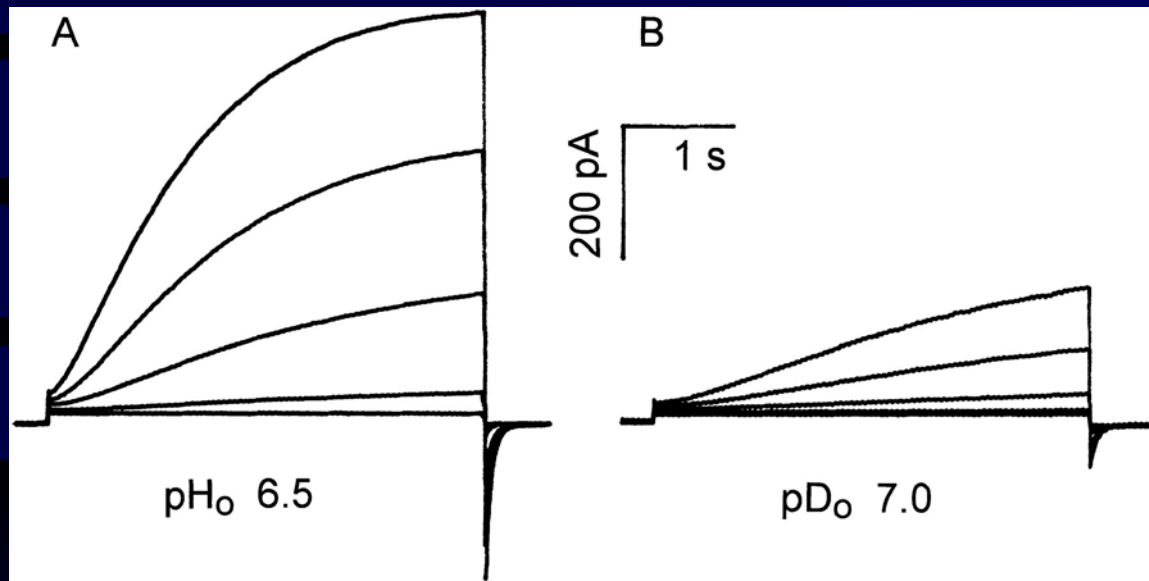


- 1 buffer diffusion
- 2 direct H transfer
- 3 H diffusion
- 4 H permeation
 - a- water wire
 - b- HBC
- 5 OH permeation

Direct Proton Transfer from Buffer is Not Rate Limiting



Strong Deuterium Isotope Effect: Permeation is **Not** Like Conduction in Gramicidin (a Water-filled Pore)



Isotope effect: H^+/D^+ Current

Proton Channel 1.7-1.9

Gramicidin 1.2-1.35

DeCoursey & Cherny. 1997. *J. Gen. Physiol.* 109:415-434.

Deuterium Isotope Effect on Conductance

TABLE I
Properties of H₂O and D₂O at 20°C

Property	D ₂ O/H ₂ O	Reference
Viscosity (shear)	1.245	Hardy and Cottington, 1949
Viscosity (volume)	1.09	Jarzynski and Davis, 1972
Mobility (conductance)	1.41 ^{-1*} 1.52 ^{-1†}	Lewis and Doody, 1933 Roberts and Northey, 1974
Dielectric constant	1.005	Schowen, 1977
Dielectric relaxation time	1.28 [§] , 1.29	Collie et al., 1948; Grant and Shack, 1969
Density	1.108	Schowen, 1977
Vapor pressure	1.15 ⁻¹	Brooks, 1937

TABLE IV
Comparison of D⁺ and H⁺ Flux through Other Membrane H⁺ Transporters

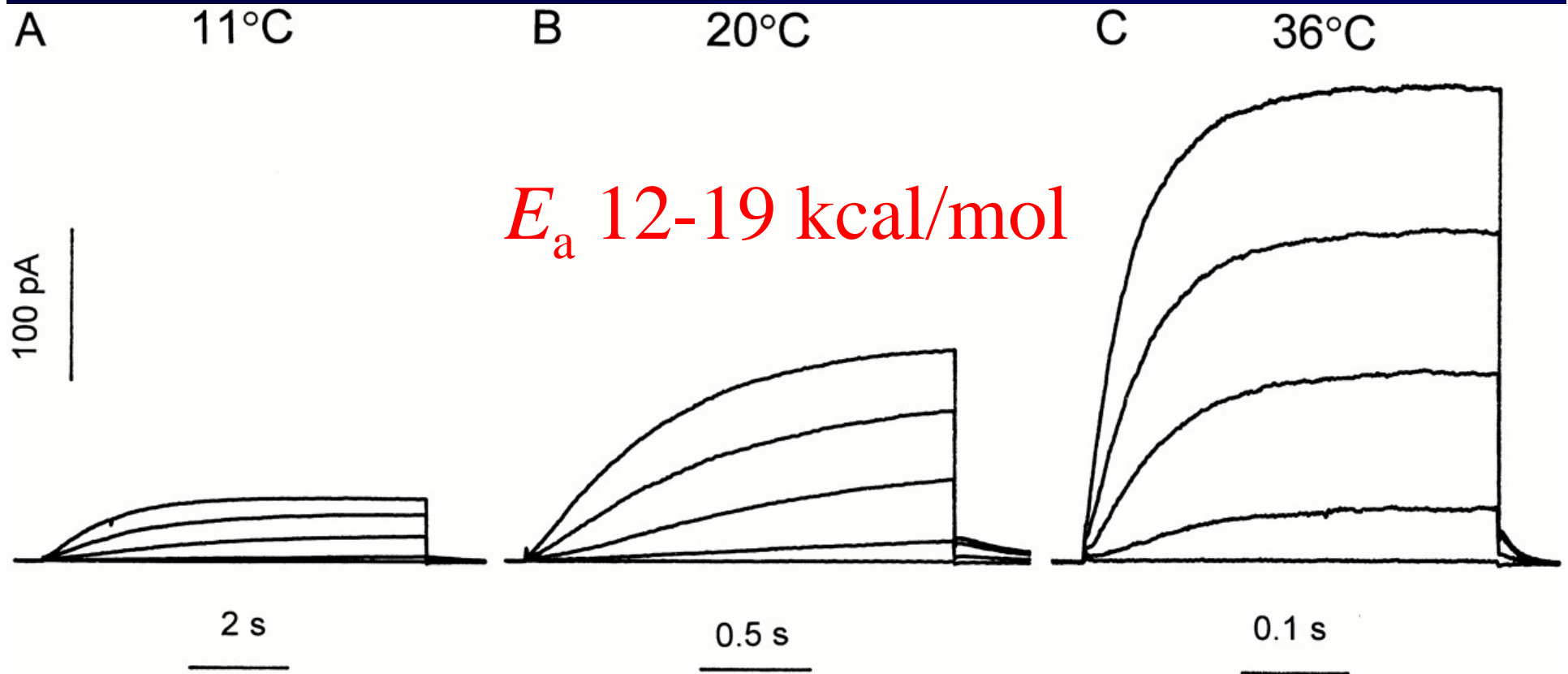
Transporter	Rate (H ₂ O/D ₂ O)	References
H ⁺ -ATPase (F ₀ component)	1.7	Althoff et al., 1989
H ⁺ -ATPase (intact) at 30°C	1.7–5.6	Kotyk and Dvoráková, 1992
Bacteriorhodopsin D85E mutant	2.1–4.7, 6.2, 2.8 1.2–6.7, 4.6, 1.7	Cao et al., 1995
Na ⁺ /H ⁺ antiport at 37°C	1.5*	Elsing et al., 1995
Bilayer permeability	~1	Perkins and Cafiso, 1986; Deamer, 1987; Gutknecht, 1987
Voltage-gated H ⁺ current	1.7–1.9	this study
M2 viral H ⁺ channel	~2	Mould et al, 2000

TABLE III
Deuterium Isotope Effects on Other Channels (temperature, °C)

Channel (permeant ion)	Conductance (H ₂ O/D ₂ O)	τ_{act} (D ₂ O/H ₂ O)	τ_{tail} (D ₂ O/H ₂ O)	τ_{inact} (D ₂ O/H ₂ O)	Reference
Na ⁺	1.22 (0–2)	1.4 (0–2)	1.39 (0–2)	1.86 (0–2)	Meves, 1974
Na ⁺	—	1.33 (9.5)	~1.0 (9.5)	>1 (95)	Alicata et al., 1990
Na ⁺	1.35 (2–4)	1.43 (2–4) 1.20 (12–14)	~1.0 (5–14)	1.5–2.6 (3–6) ~1.4 (11–14)	Schauf and Bullock, 1980, 1982
K ⁺	1.47 (2–4)	1.40 (2–4) 1.17 (12–14)			Schauf and Bullock, 1980
K ⁺ _{Ca}	1.18 (?)				Pottosin et al., 1993
gramicidin (Li ⁺ , Cs ⁺)	1.03, 1.16 (20)				Tredgold and Jones, 1979
gramicidin (K ⁺ , Rb ⁺ , Cs ⁺ , NH ₄ ⁺)	1.16 (25)				Andersen, 1983
gramicidin (H ⁺)	1.2–1.35 (22)				Akeson and Deamer, 1991
AchR	1.49 (12)		0.85 (12)		Lewis, 1985
cyclic nucleotide-gated	1.36 (20)				Root and MacKinnon, 1994
α -Toxin	1.13–1.2 (24)				Kasianowicz and Bezrukov, 1995
H ⁺ currents	1.9 (20)	~3 (20)	≤1.5 (20)	none	this study

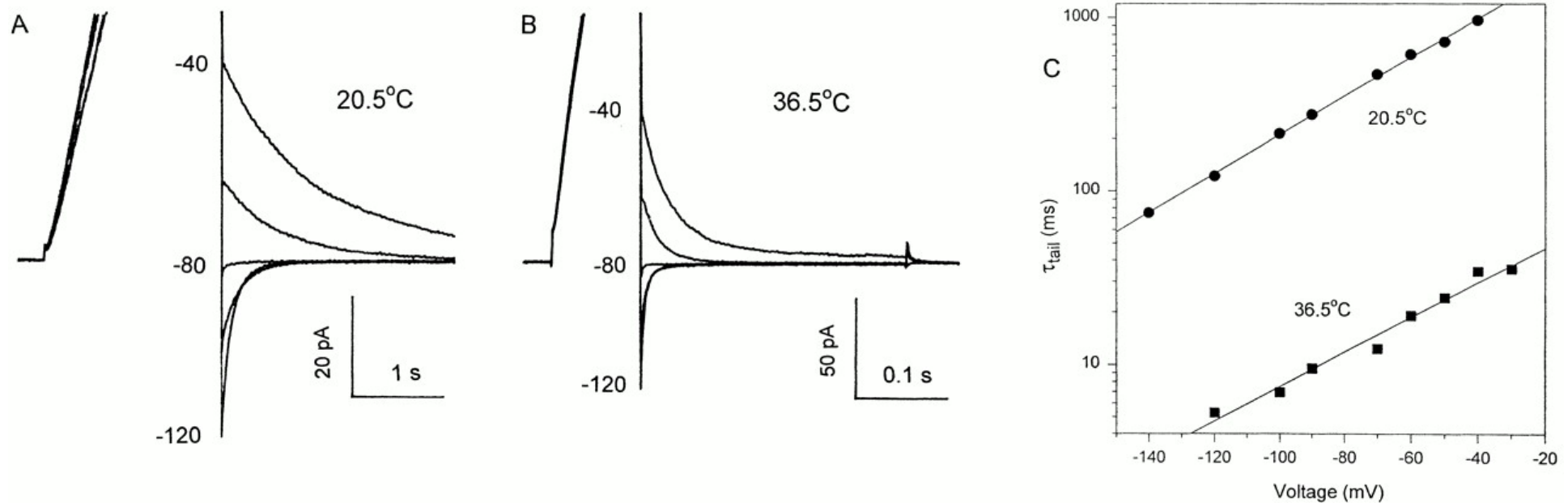
DeCoursey & Cherny
1997. *J. Gen. Physiol.*
109:415-434.

Strong Temperature Dependence of H⁺ Conductance: Permeation is Not Like Conduction in Water (2.6 kcal/mol)



Strong Temperature Dependence of Gating: Channel Opening Requires a Major Conformational Change (Proton Channels are Proteins)

E_a 32-38 kcal/mol



DeCoursey & Cherny. 1998. *J. Gen. Physiol.* 112:503-522.

Stronger Temperature Dependence than Other Channels

TABLE II
Q₁₀ Values for Ion Channel Permeation and Gating

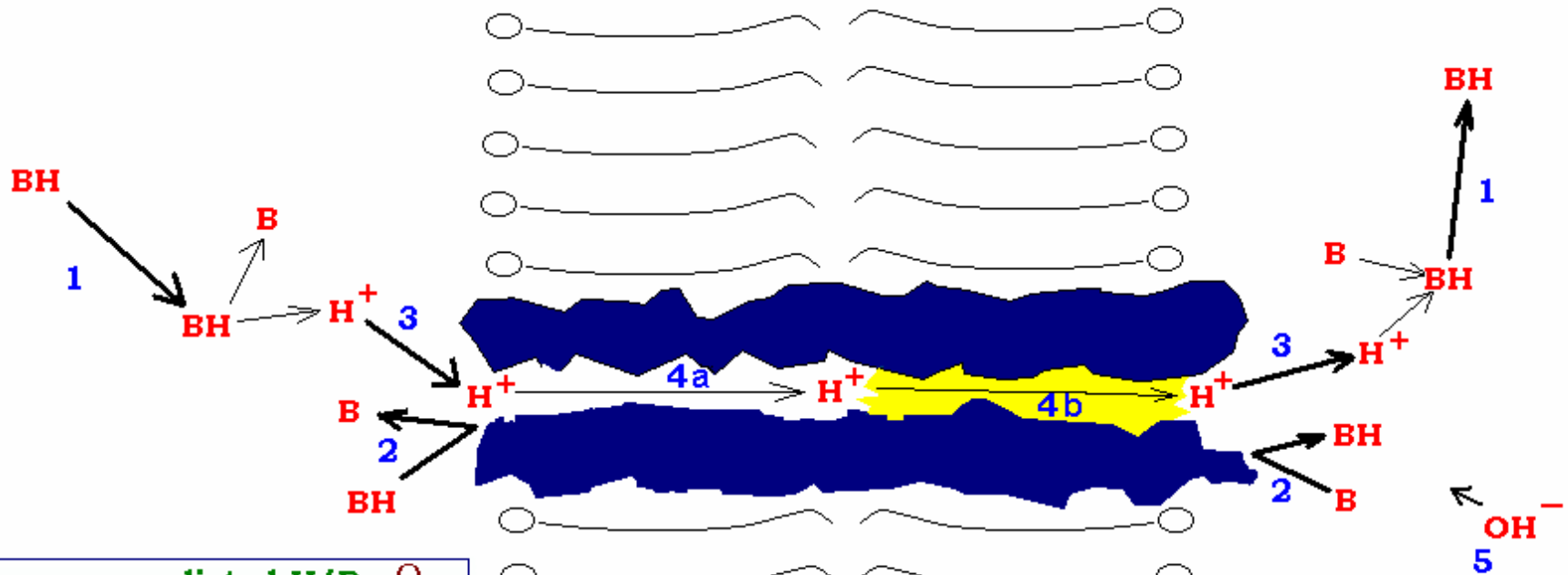
Channel	°C	Conductance	τ_{act}	τ_{tail}	τ_i	$\tau_{recovery}$	Reference
K _{IR} (frog muscle)	3.5–17.5	1.67	2.45	—	—	—	Almers, 1971
(tunicate oocyte)	5–20	1.44*	—	—	3.41 ^{V*}	—	Ohmori, 1978
(starfish oocyte)	1–26	1.62 ^H –5.8 ^L	—	—	—	—	Hagiwara and Yoshii, 1980
(guinea pig myocyte)	5–37	1.15	1.45	—	—	—	Martin et al., 1995
(cat cardiac myocyte)	5–37	1.22	1.82	—	—	—	Martin et al., 1995
K _V (<i>Xenopus laevis</i>)	5–20	1.2	3.2	2.8	—	—	Frankenhaeuser and Moore, 1963
(<i>Myxicola</i> axon)	5–14	—	2.40	—	3.02 ^B	1.70 ^B	Schauf and Bullock, 1982
(rat muscle)	1–37	1.42	2 ^H –6 ^L	1.7 ^H –11.5 ^L	—	—	Beam and Donaldson, 1983
(human T-cell)	27–37	1.56	3.31	7.09	3.87	9.81	Lee and Deutsch, 1990
(human T-cell)	20–25	1.18*	2.9	—	2.2	—	Pahapill and Schlichter, 1990
ShB K ⁺ peptide	5–20	—	—	—	5.0 ^π	<1.1 ^π	Murrell-Lagnado and Aldrich, 1993
<i>Shaker</i> K ⁺	5–20	1.51	3.14	—	7.2	1.57	Nobile et al., 1997
K _{Ca} (human RBC)	0–47	1.50	—	—	—	—	Grygorczyk, 1987
Na ⁺ (<i>X. laevis</i>)	5–20	1.3	1.8	1.7	—	—	Frankenhaeuser and Moore, 1963
(squid axon)	0–16	—	2.35	—	2.7 ^H –4.3 ^L	—	Kimura and Meves, 1979
(rabbit nerve)	0–25	1.7 ^H –4.7 ^L	—	—	3 ^H –33 ^L	—	Chiu et al., 1979
(neuroblastoma)	5.6–14	—	—	—	1.3 ^{α*}	1.3 ^{α*}	Yamamoto and Yeh, 1984
(frog muscle)	0–22	—	2.4	—	3.0 ^H –5.3 ^L	—	Kirsch and Sykes, 1987
(rabbit muscle)	0–22	—	2.3	—	3.4 ^H –9.1 ^L	—	Kirsch and Sykes, 1987
AchR (BC3H-1)	10–40	1.26 ^H –1.55 ^{L*}	3.6*	—	—	—	Dilger et al., 1991
Ca ²⁺ (muscle)	—	1.3–1.6*	~3.0	~3.0	—	—	McDonald et al., 1994
CIC-0	9–40	1.4*	—	2.2	~40	—	Pusch et al., 1997
Alamethicin	–1–32	1.28–1.31	2.9 ^H –9.0 ^L	2.04	—	—	Boheim and Kolb, 1978
Gramicidin (K ⁺ , Na ⁺)	3–23	1.38,* 1.34*	—	3.1*	—	—	Hladky and Haydon, 1972
Gramicidin (Na ⁺)	10–40	1.52*	3.09	2.62	—	—	Bamberg and Lauger, 1974
Gramicidin (H ⁺)	15–22	1.33*	—	—	—	—	Akeson and Deamer, 1991
H ⁺ (snail neuron)	10–25	2.1	—	—	—	—	Byerly and Suen, 1989
(mammalian cells)	5–44	2.8 ^H , 5.3 ^L	6–9	6–8	—	—	This study

Large E_a Indicates Complex Conduction Mechanism

TABLE IV
Q₁₀ Values for Other H⁺ (or Non-H⁺) Transporters*

Transporter	Range (°C)	Q ₁₀	Reference
Passive currents			
H ⁺ conductivity, water	18–25	1.17	Robinson and Stokes, 1959
H ⁺ /OH ⁻ permeability, renal	25–40	1.30	Ives, 1985
Gramicidin: H ⁺ current	15–22	1.33	Akeson and Deamer, 1991
Gramicidin: Na ⁺ current*	0–25	1.56*	Althoff et al., 1989
CF ₀ H ⁺ channel conductance	0–25	1.89	Althoff et al., 1989
Water channel H ₂ O flux*	4–37	1.24	Dempster et al., 1992
Carriers			
Na ⁺ -H ⁺ antiport	25–37	1.22	Graber et al., 1992
	25–40	1.85	Ives, 1985
Nonactin	42–48	4.23	Krasne et al., 1971
Valinomycin*	0–25	2.61*	Althoff et al., 1989
Na ⁺ glutamate co-transport	8–18	1.95	Schwartz and Tachibana, 1990
Malonate flux (mitochondrial anion channel)*	5–45	1.3–13	Lie et al., 1996
Glucose facilitated diffusion (protozoan)*	20–33	2.33*	Wille et al., 1996
Intramolecular H ⁺ transfer, carbonic anhydrase II	5–30	1.6	Ghannam et al., 1986
Proton shuttle on K ⁺ channel voltage sensor	16–30	2.6	Starace et al., 1997
Pumps (turnover rate)			
H ⁺ -ATPase	13–40	2.1	Bidani et al., 1994
MotA H ⁺ flux	16–32	2.1–2.5	Meister et al., 1987; Blair and Berg, 1990
Bacteriorhodopsin	5–40	1.33–3.37†	Cao et al., 1995
Na,K-ATPase*	5–37	3.0–5.7*	Ellory and Willis, 1982
Na,K-ATPase*	5–35	2.4 ^H -5.3 ^L *	Hakao and Gadsby, 1986; Apell, 1997

What is the Rate Limiting Step?



	predicted H/D	Q_{10}
1 buffer diffusion	1.25	1.3
2 direct H transfer	?	?
3 H diffusion	1.41	1.17
4 H permeation		
a- water wire	1.35	1.33
b- HBC	?	?
5 OH permeation	1.03	1.4

OBSERVED 1.9 2-5

Unusual Properties of Voltage-gated Proton Channels (*vs.* Other Ion Channels)

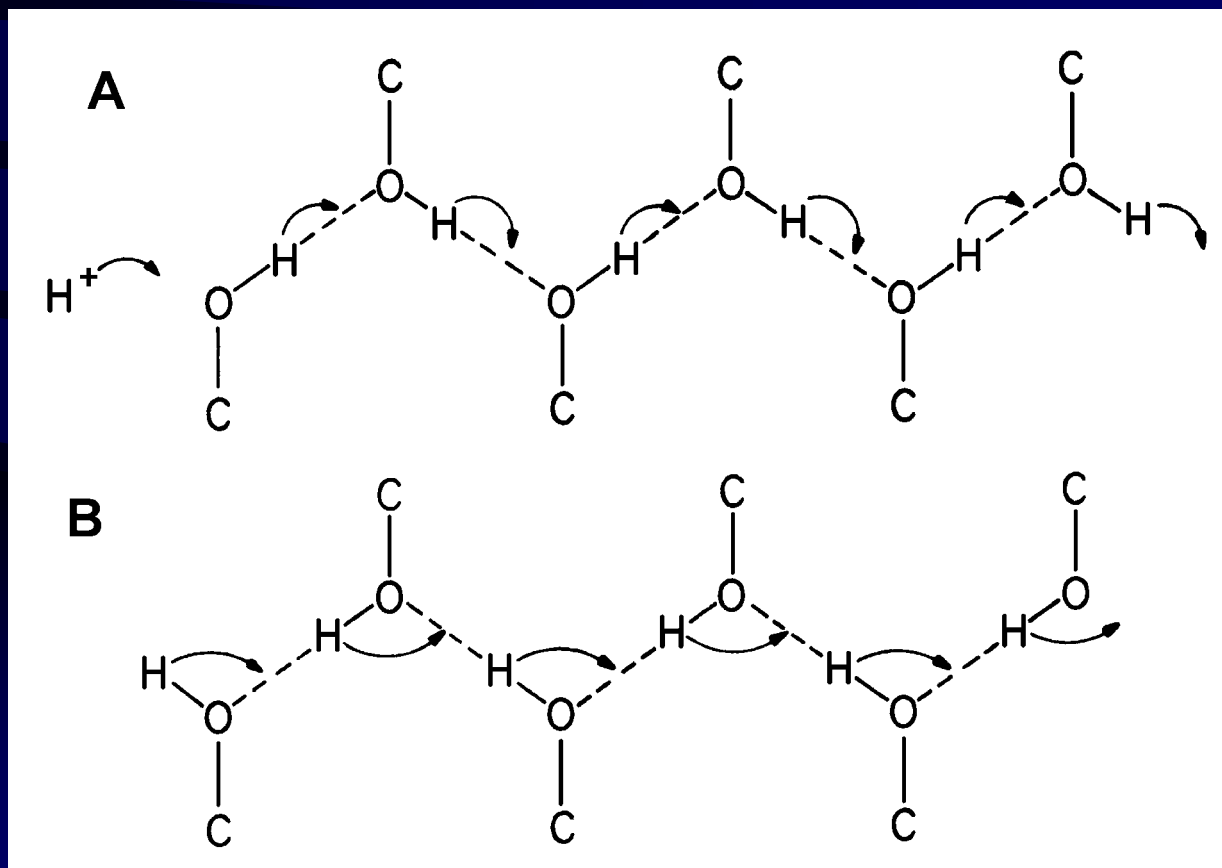
- Perfect selectivity
- Large isotope effect (conductance)
- Strong temperature dependence

Explanations

- Not a real ion channel?
- Unique conduction mechanism?
 - Not a water-filled pore!

Hydrogen Bonded Chain (HBC) Conduction

Nagle & Morowitz. 1978. *Proc. Natl. Acad. Sci. USA*. 75:298-302.



Hydrogen-Bonded Chains

Proposed Proton Pathways through Various Molecules

- Generic ion channel $\text{H}_2\text{O} - (\text{H}_2\text{O})_n - \text{H}_2\text{O}$
- K^+ channel voltage sensor $(\text{H}_2\text{O})_n - \text{His}^{371} - (\text{H}_2\text{O})_n$
- M_2 $\text{H}_2\text{O} - (\text{H}_2\text{O})_n - \text{His}^{37} - \text{H}_2\text{O}$
- F_o (residues that form channel) $\text{H}_2\text{O} - \text{Asn} - \text{Ser} - \text{Glu} - \text{His} - \text{Asp} - \text{Asn} - \text{Asn} - \text{Gln} - \text{Arg} - \text{Asp}^{61}$
- F_o (possible proton pathway) $(\text{H}_2\text{O})_n - \text{Asp}^{61} - (\text{H}_2\text{O})_n$
- MotA-MotB $(\text{H}_2\text{O})_n - \text{Asp}^{32} - (\text{H}_2\text{O})_n$
- bR minimal pathway $(\text{H}_2\text{O})_n - \text{Asp} - (\text{H}_2\text{O})_n - \text{Retinal Schiff base} - \text{Asp} - (\text{H}_2\text{O})_n$
- bR cytoplasmic pathway $\text{Asp} - (\text{H}_2\text{O})_n - \text{Retinal Schiff base}$
- bR extracellular pathway $\text{Retinal} - \text{Asp} - (\text{H}_2\text{O})_2 - \text{Arg} - \text{H}_2\text{O} - \text{Glu} - \text{H}_2\text{O} - \text{Glu}$
- BRC - 1 $\text{H}_2\text{O} - \text{His} - (\text{H}_2\text{O})_n - \text{Asp} - \text{H}_2\text{O} - \text{Asp} - \text{Ser} - \text{Q}_B$
- BRC - 2 $\text{H}_2\text{O} - \text{His} - (\text{H}_2\text{O})_n - \text{Asp} - \text{H}_2\text{O} - \text{Asp} - (\text{H}_2\text{O})_2 - \text{Glu} - \text{H}_2\text{O} - \text{Q}_B$
- Cytochrome *c* oxidase (D) $\text{Asp}^{132} - \text{H}_2\text{O} - (\text{H}_2\text{O})_n - \text{H}_2\text{O} - \text{Glu} - (\text{H}_2\text{O})_n - \text{binuclear center/exit path}$
- Cytochrome *c* oxidase (K) $\text{Glu}^{101} - \text{Ser} - \text{H}_2\text{O} - \text{Lys}^{362} - \text{Thr} - \text{H}_2\text{O} - \text{Tyr} - \text{binuclear center}$
- CA II $\text{BH} - \text{His}^{64} - \text{H}_2\text{O} - (\text{H}_2\text{O})_n - \text{H}_2\text{O} - \text{E-Zn-OH}^-$
- CA III $\text{BH} - \text{H}_2\text{O} - \text{H}_2\text{O} - (\text{H}_2\text{O})_n - \text{H}_2\text{O} - \text{E-Zn-OH}^-$
- UCP1 $\text{Asp} - \text{FA-COO}^- - (\text{FA-COO}^-)_n - \text{Asp} - \text{His} - \text{His} - \text{H}_2\text{O}$

Nothing Blocks Proton Channels

TABLE 6. *Drugs that do not block H⁺ currents*

Compound	Concentration	I _H Reduction, %	Reference Nos.
Amantadine	0.1-1 mM	Via ↑ pH _i ?	238
Amiloride	200 μM	0	710
Amiloride	100 μM	0	642
Amiloride	100 μM	weak, V dependent	237
Apamin	300 nM	0	642
4-AP	10 mM	67	134
4-AP	1 mM	50	762
4-AP	10 mM	Via ↑ pH _i ?	676
4-AP	1 mM	50	281
4-AP	5 mM	Strong	473
A9C	1 mM	0	238
Bafilomycin A	200 nM	0	709,710
Ba ²⁺	1 mM	0	574
Bromophenacyl bromide	≤1 mM*	0	V. Cherny and T. DeCoursey, unpublished data
CCmP	20 μM	0	676
Charybdotoxin	100 nM	0	238
Cinnamate	?	0	85
D600	100 μg/ml	V dependent	676
DCCD	100 μM	0	676
DCCD	200 μM	0	238
Diethylpyrocarbonate	1 mM	0	642
DIDS	100 μM	0	710
DPI	1 μM	0	248
DPI	3 μM	0	246
Dimethylamiloride	10 μM	0	237
Mefenamic acid	100 μM	0	238
N-ethylmaleimide	2 mM	0	642
Nicardipine	10 μM	Small	1007
Nifedipine	1 μM	0	85
Noxiustoxin	100 nM	0	238
Oligomycin	10 μg/ml	0	676
Phencyclidine	200 μM	0	238
Rimantadine	0.1-1 mM	Via ↑ pH _i ?	238
SITS	20 μM	0	676
Strophanthidin	2 mM	0	238
TEA ⁺	10 mM	35	85
TEA ⁺	52 mM	34	134
TEA ⁺	Isotonic	0	473
TEA ⁺	5 mM	5	762
TEA ⁺	1 mM	29	281
TEA ⁺	50 mM	V dependent	676
Tetrapentylammonium	1 mM	0	136
Tetrapentylammonium	10 mM	0	499
Venturicidin	10 μM	0	238

DeCoursey, 2003. *Physiol. Rev.* 83:475-579.

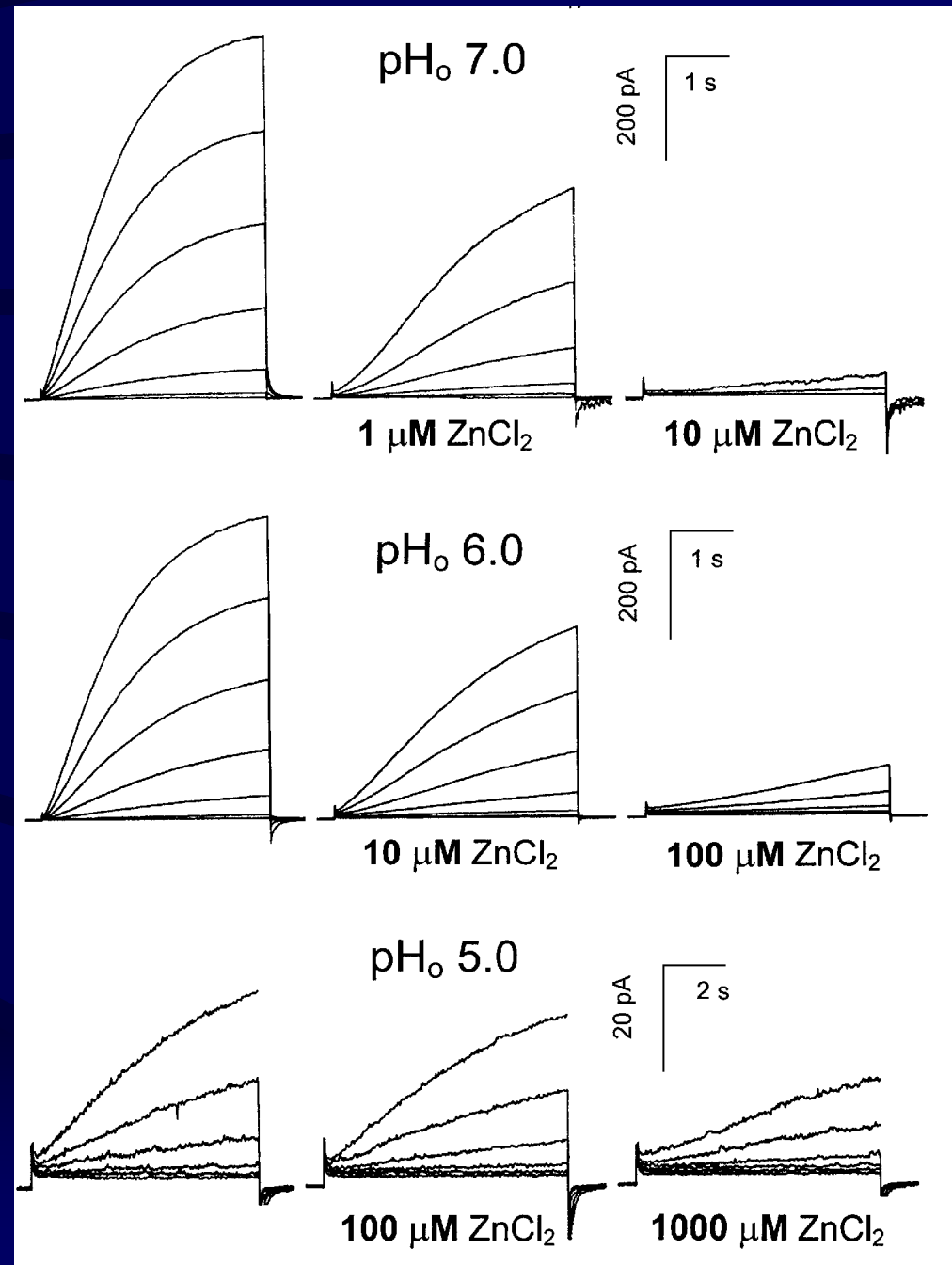
Nothing blocks H⁺
channels!

Zn²⁺ inhibits H⁺
currents.

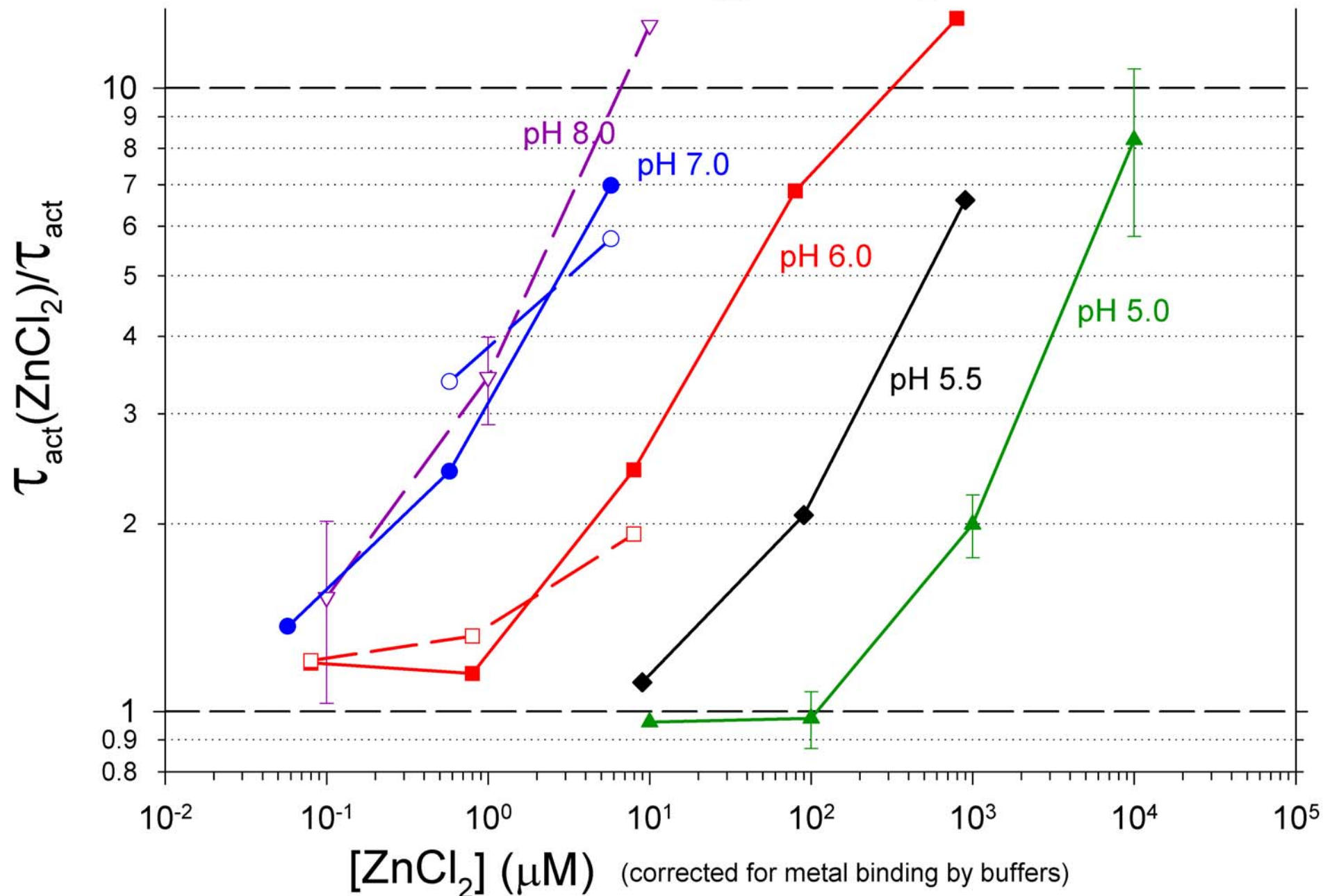
Low pH_o attenuates
Zn²⁺ effects.

**Hypothesis: Zn²⁺ and
H⁺ compete for an
external receptor.**

Cherny & DeCoursey, 1999.
J. Gen. Physiol. 114:819-838

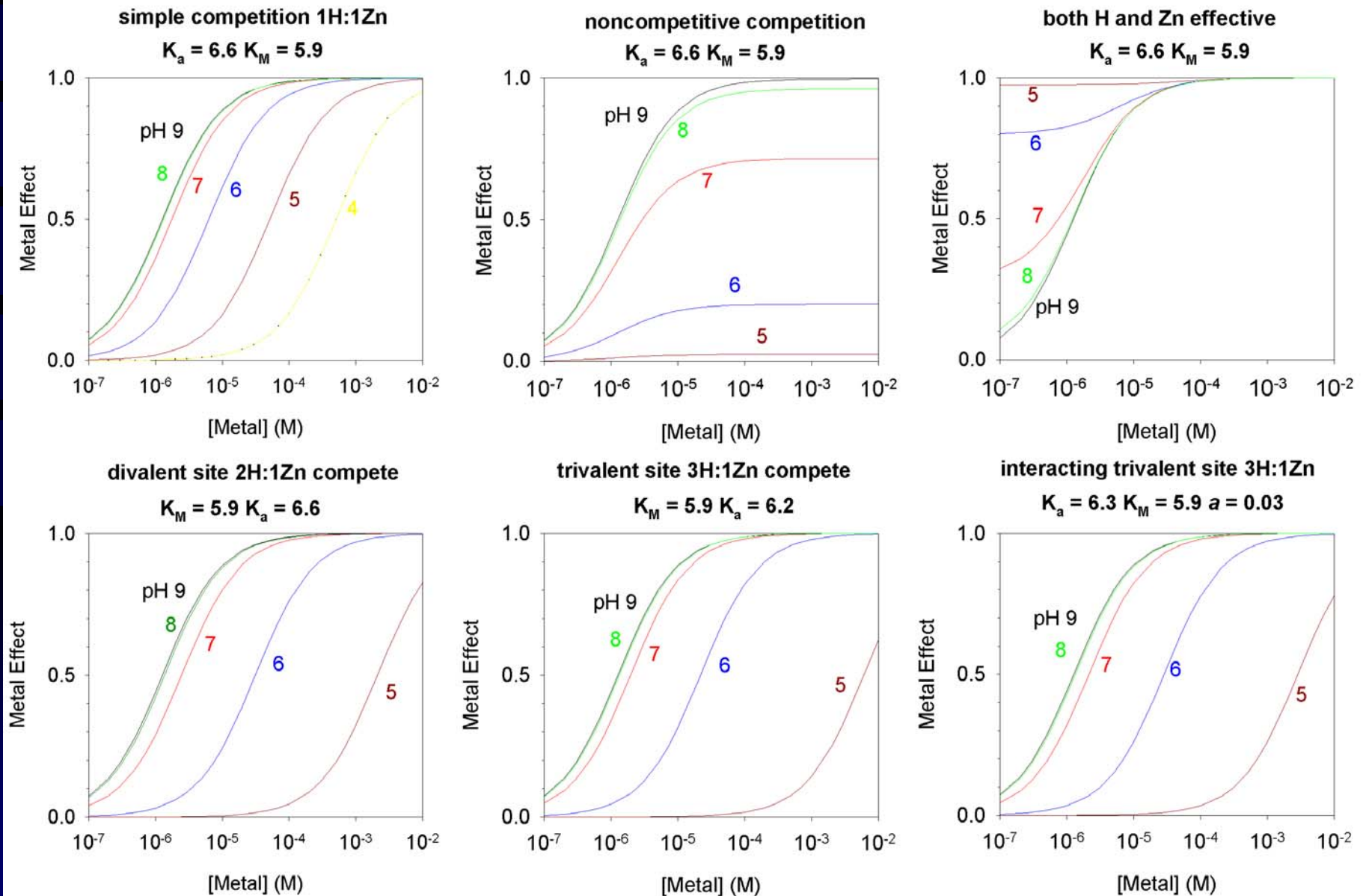


Slowing of τ_{act} by $ZnCl_2$

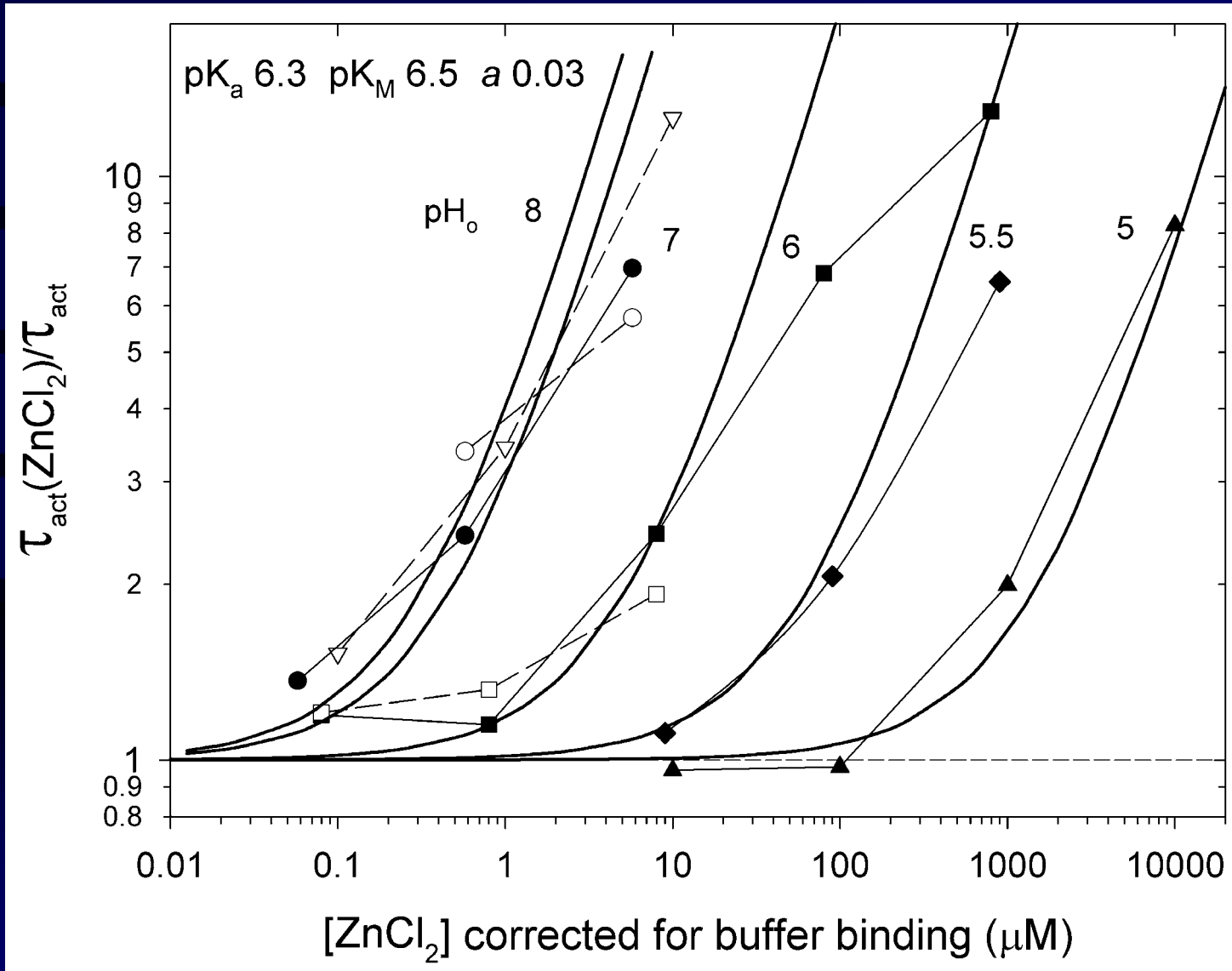


Various Models of Competition

Cherny & DeCoursey, 1999. *J. Gen. Physiol.* 114:819-838

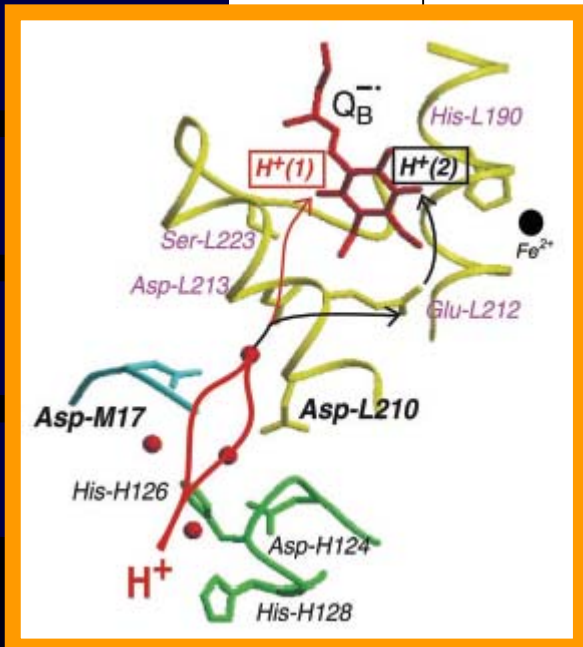


The Zinc Receptor May Comprise Three His

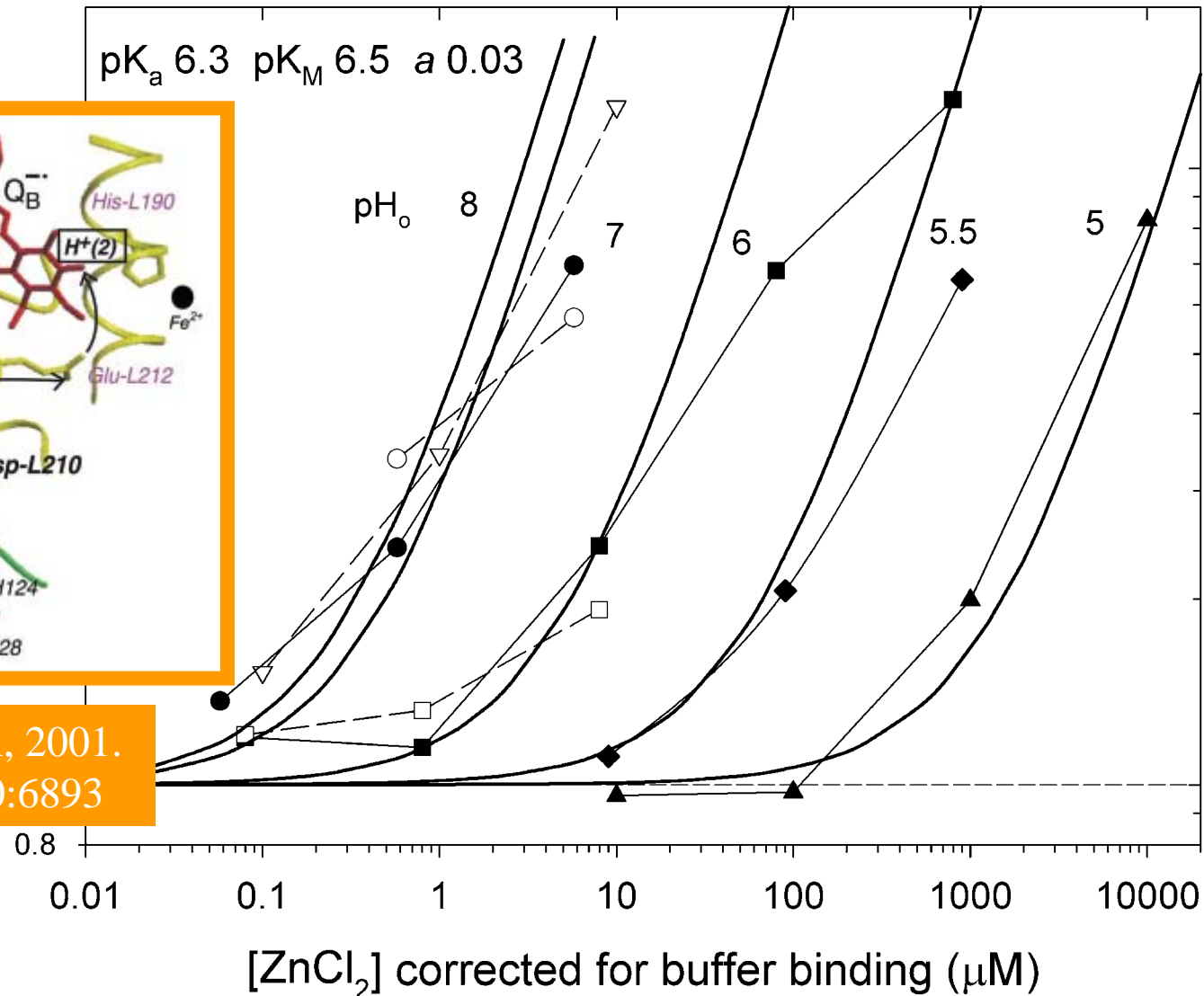


Cherny & DeCoursey, 1999.
J. Gen. Physiol. 114:819-838

The Zinc Receptor May Comprise Three His

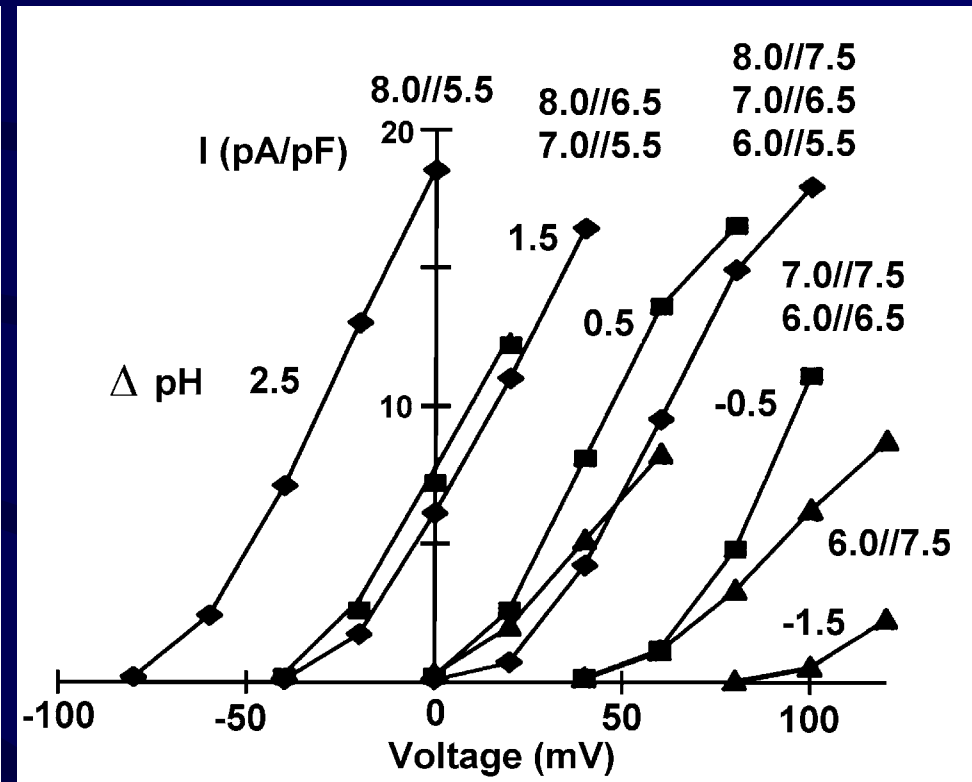
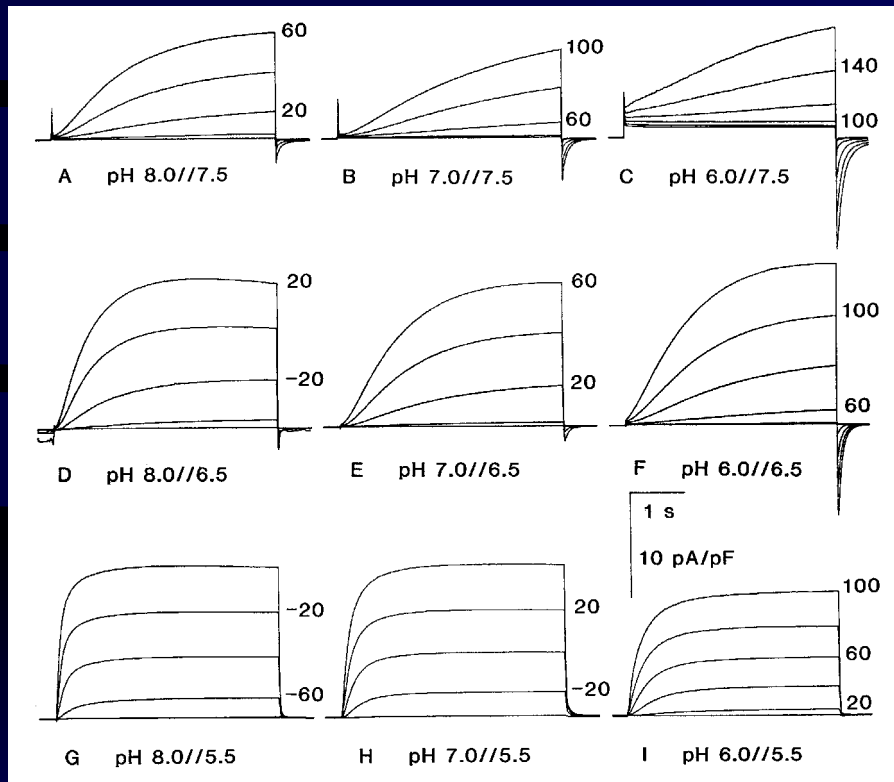


Paddock et al, 2001.
Biochem. 40:6893



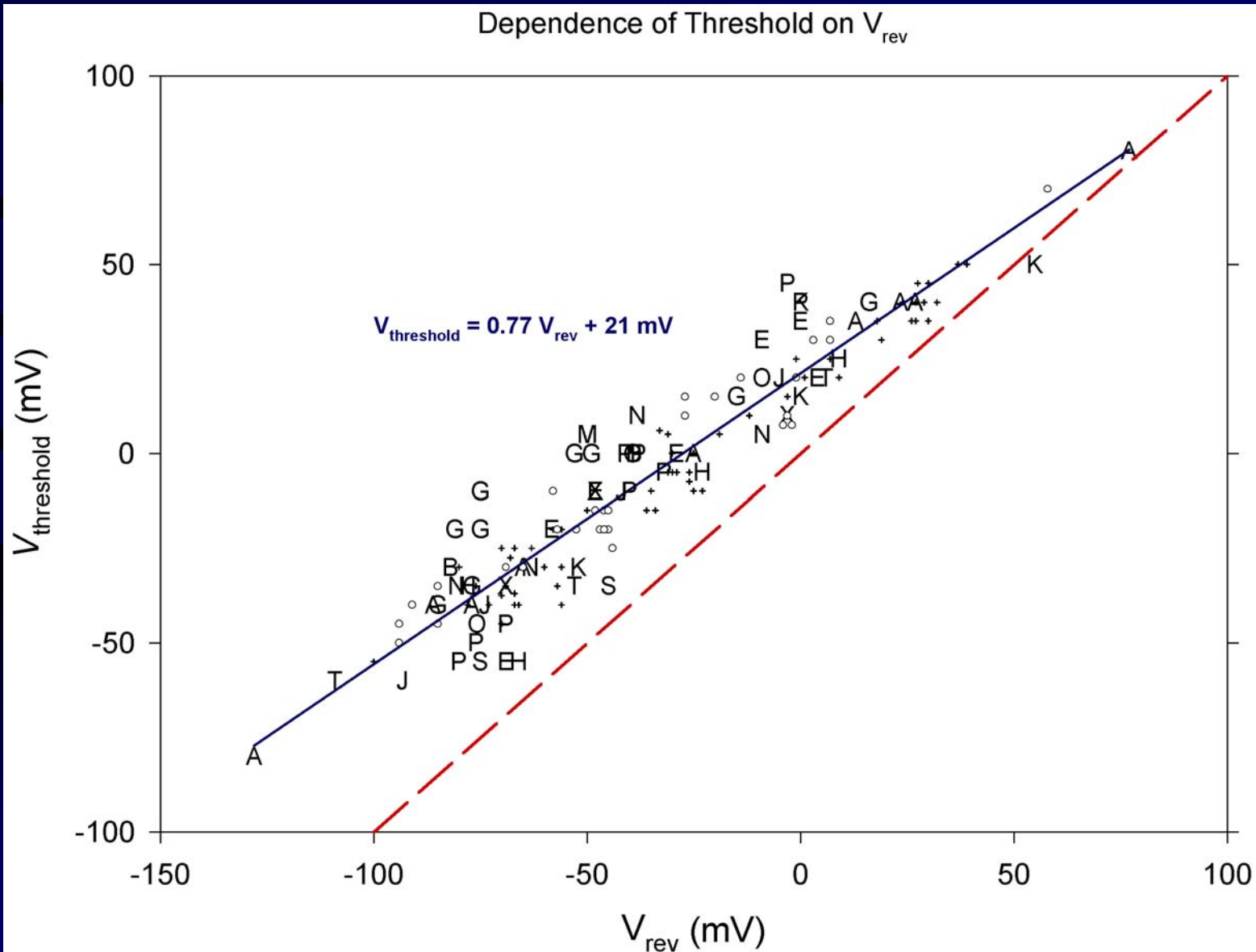
Cherny & DeCoursey, 1999.
J. Gen. Physiol. 114:819-838

Proton Channel Gating Depends on Voltage and ΔpH

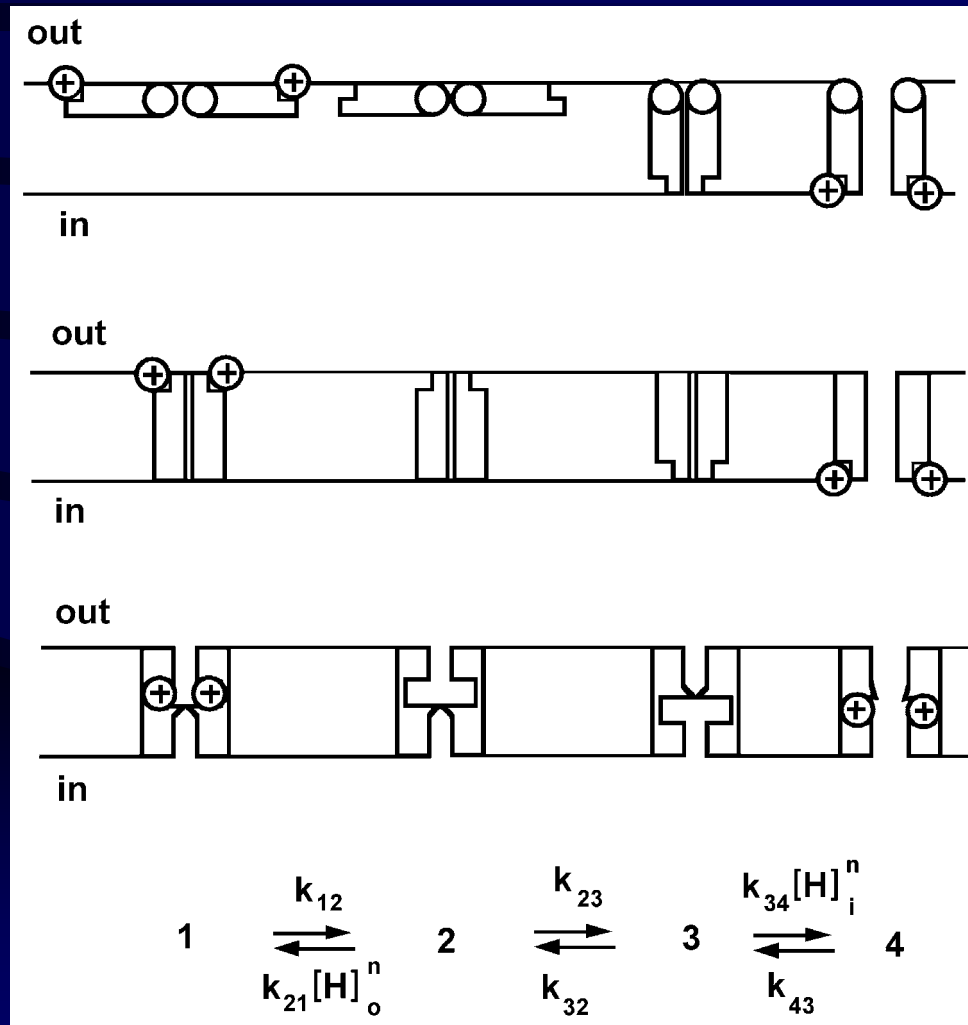


Cherny et al, 1995. *J. Gen. Physiol.* 105:861-896.

H⁺ Channels Open Only When the Electrochemical Gradient is Outward (H⁺ Channels Extrude Acid)



Model of pH- and Voltage-Dependent Gating

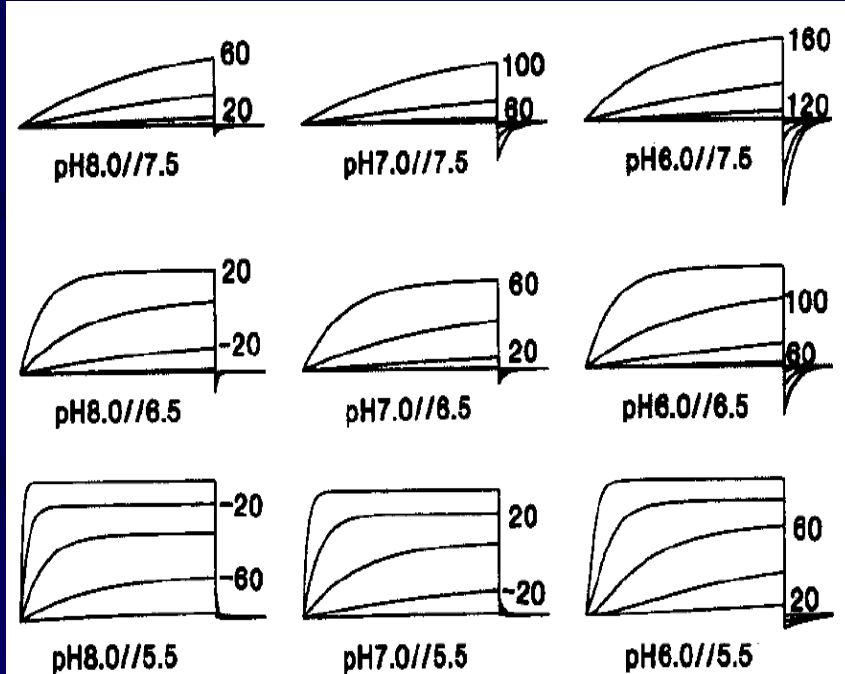
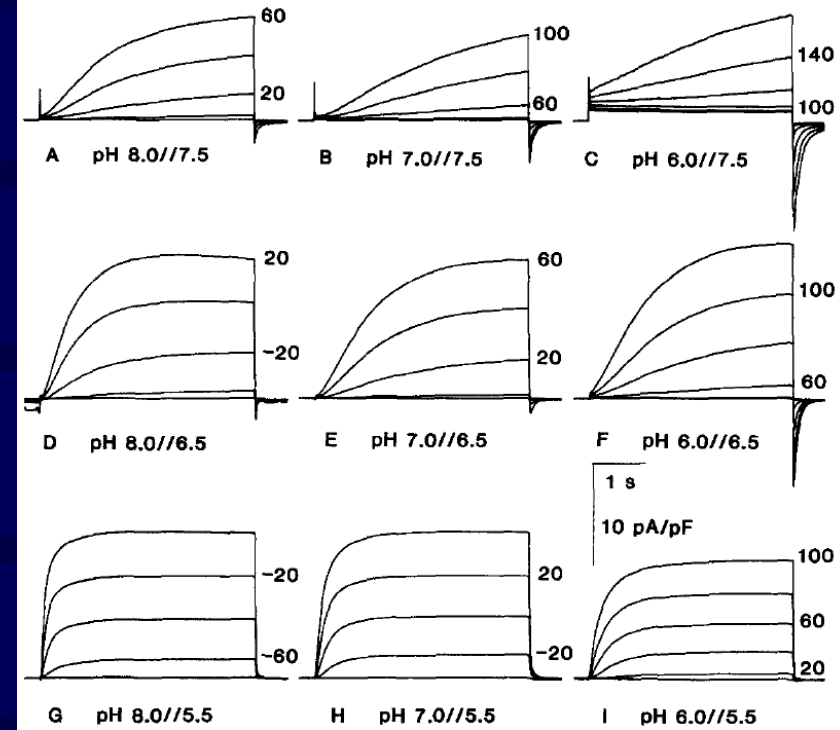


Cherny et al. 1995. *J. Gen. Physiol.* 105:861-896.

Model Reproduces Main Features of pH and Voltage Dependent Gating

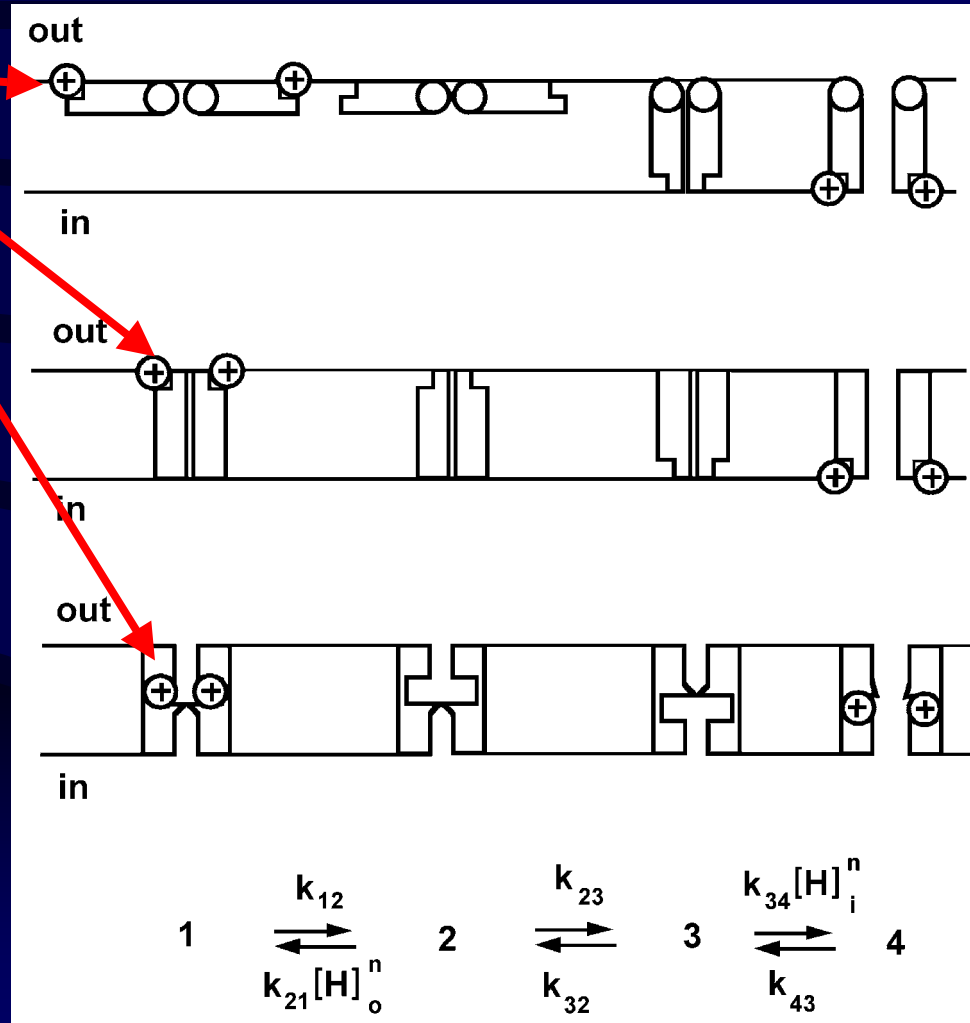
DATA

MODEL



External Zinc and Protons Have Similar Effects

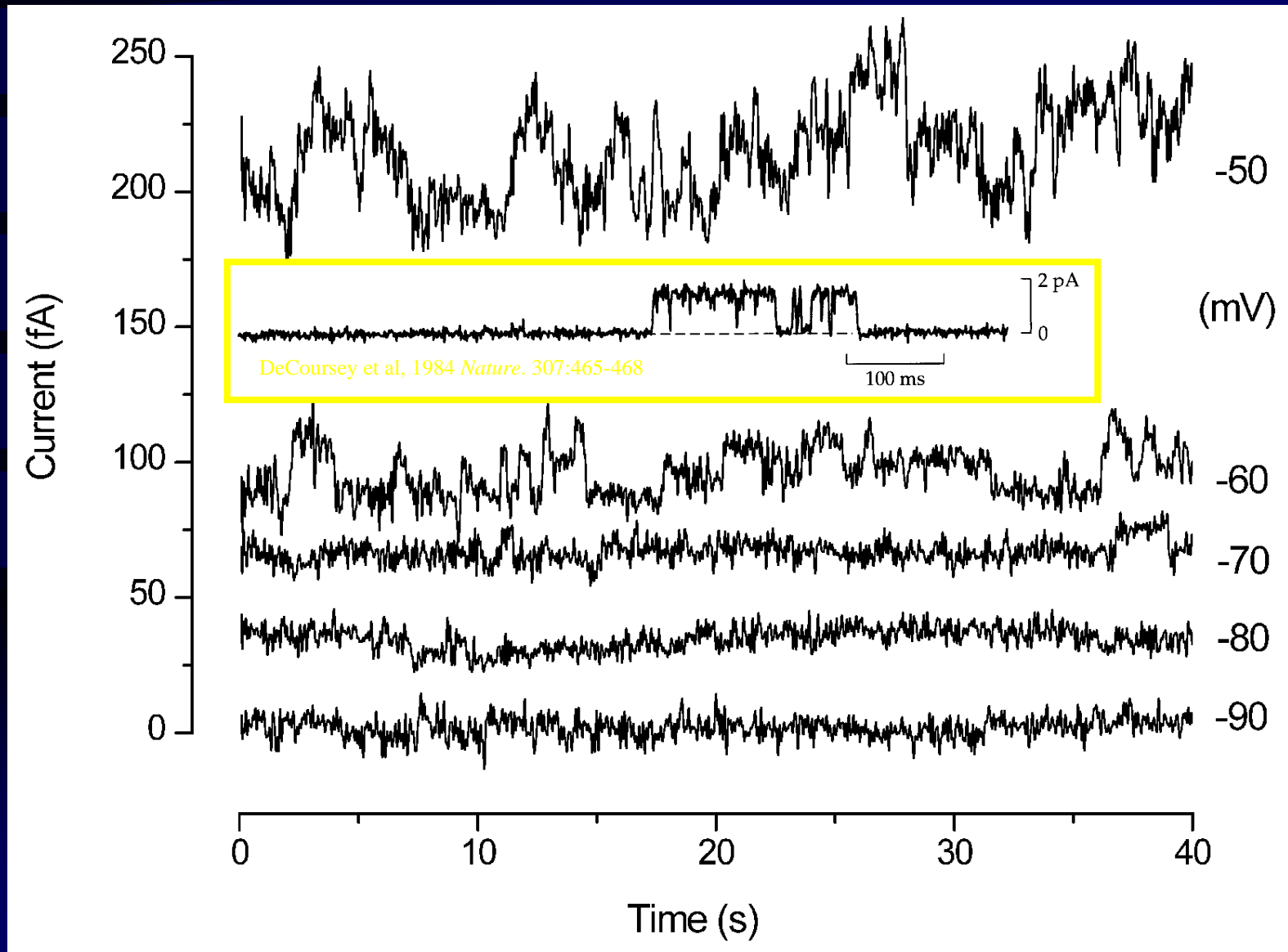
Are the external sites HIS?



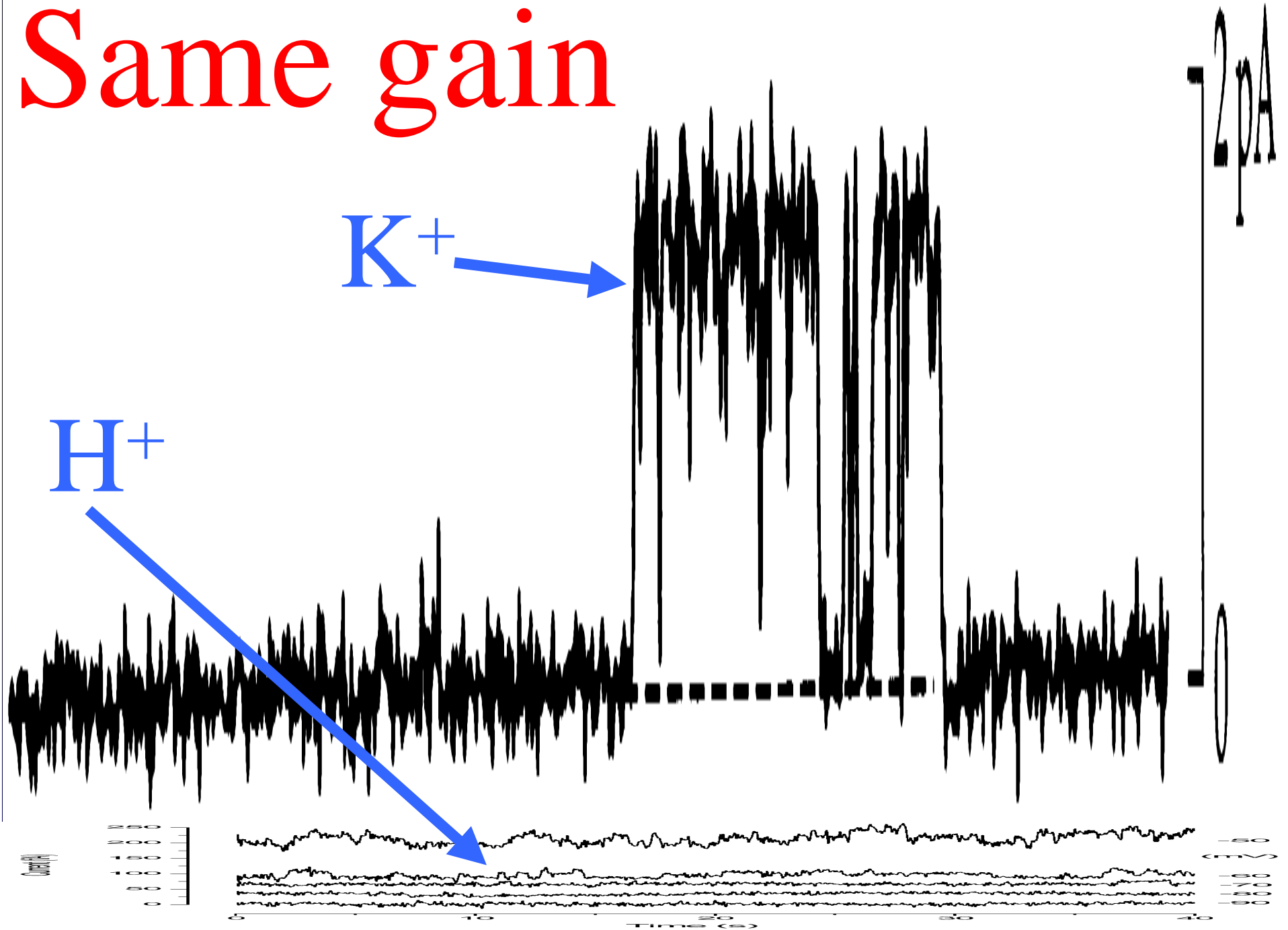
Cherny et al. 1995. *J. Gen. Physiol.* 105:861-896.

Single H⁺ Channel Currents Are Really Small

Cherny et al. (2003). *J. Gen. Physiol.* 121:615-628.

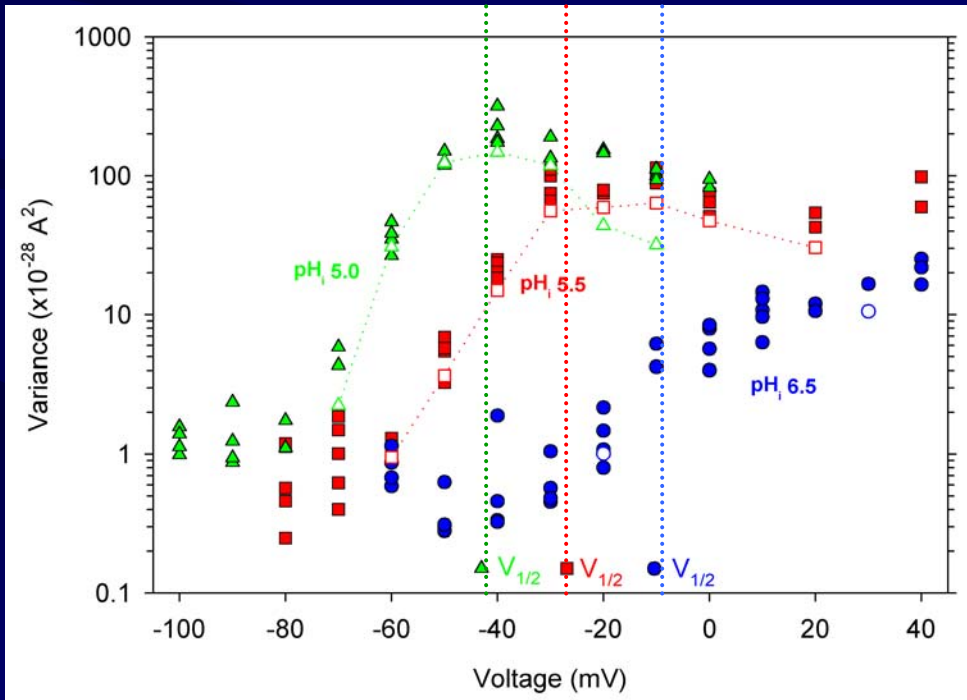
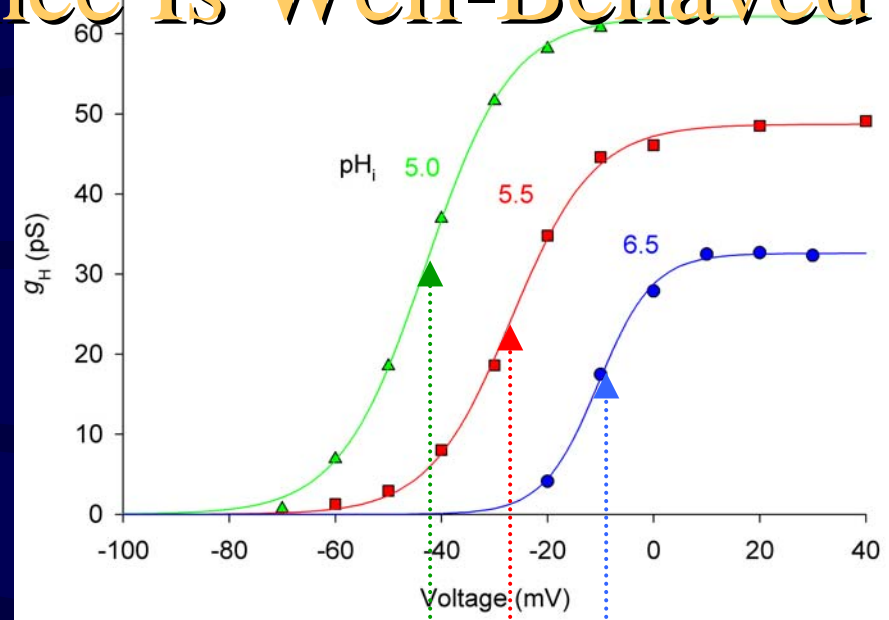
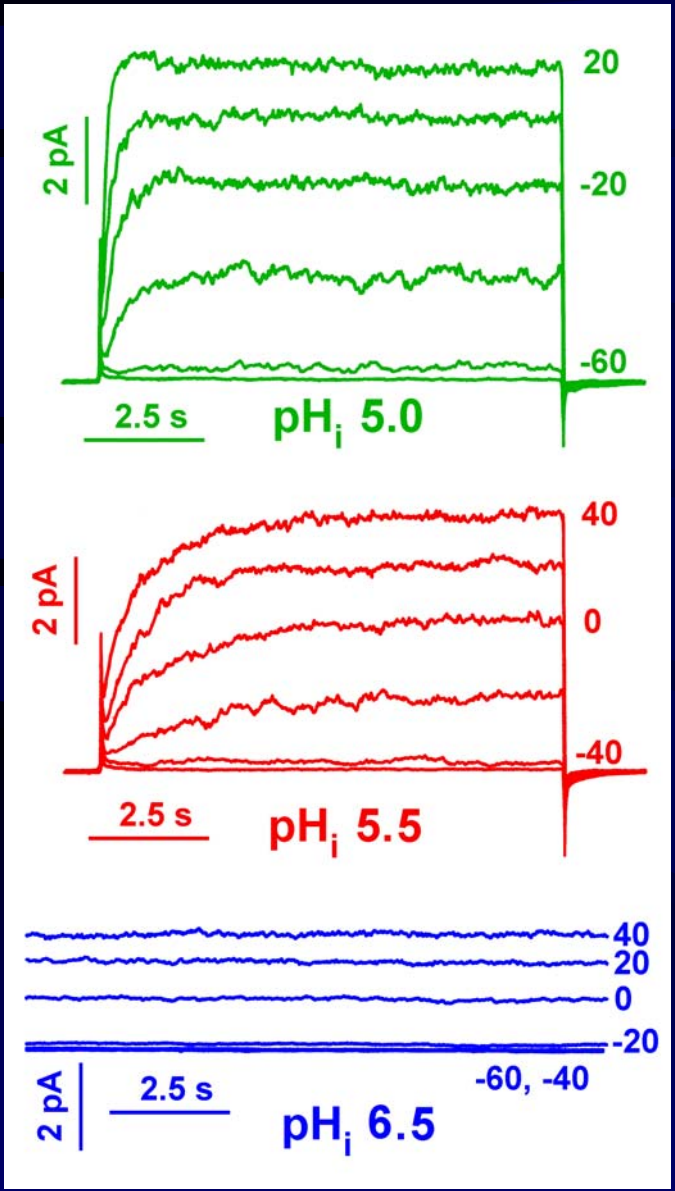


Same gain

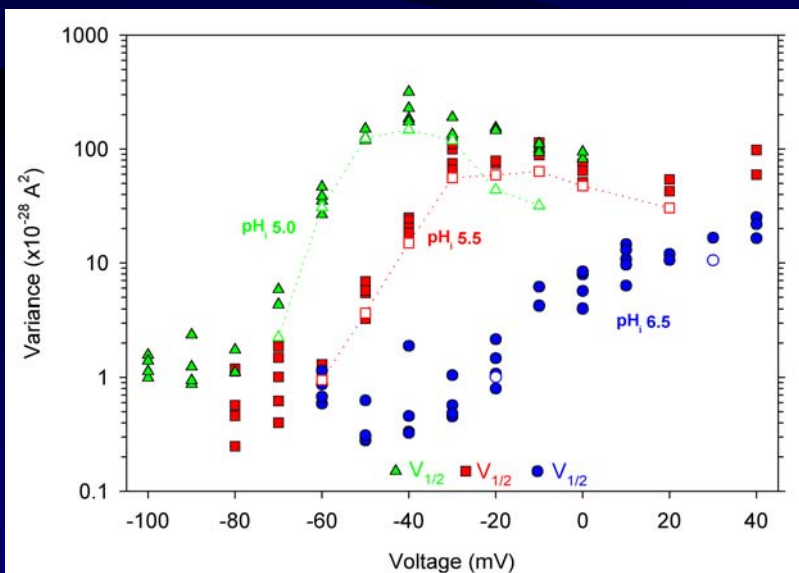
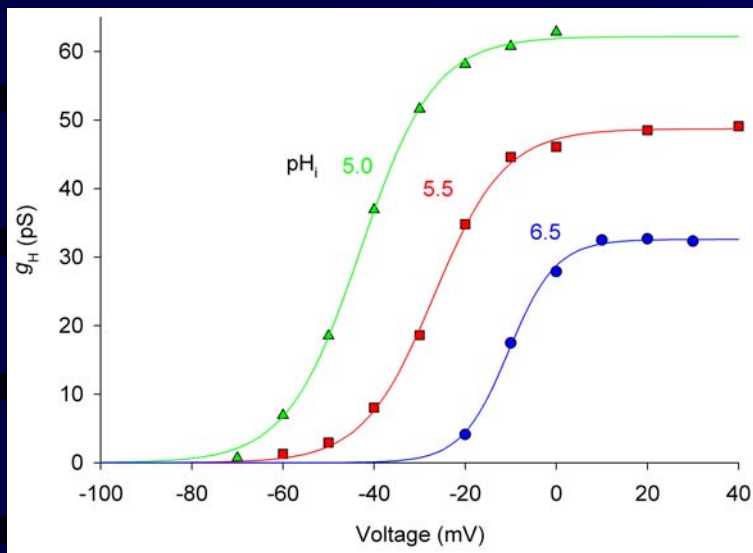


H⁺ Current Variance Is Well-Behaved

Cherny et al. (2003). *J. Gen. Physiol.* 121:615-628.

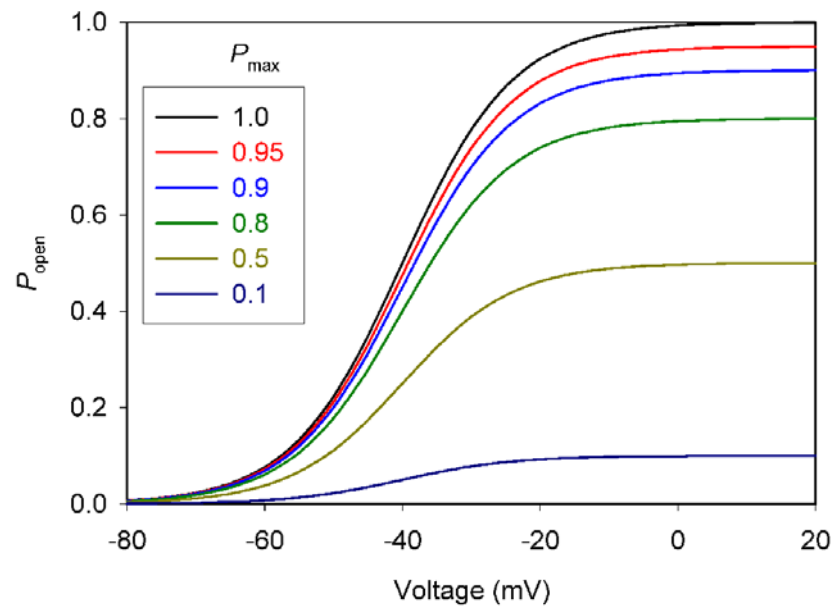


H⁺ Current Variance Is Well-Behaved

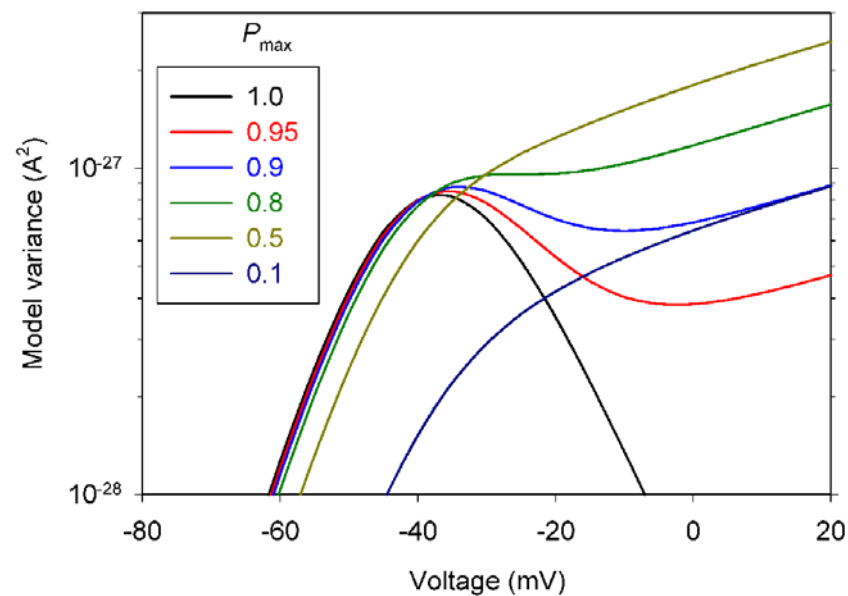


Model P_{open} -V Relationships

$V_{1/2} = -40$ mV $k = 8$ mV $V_{rev} = -120$ mV

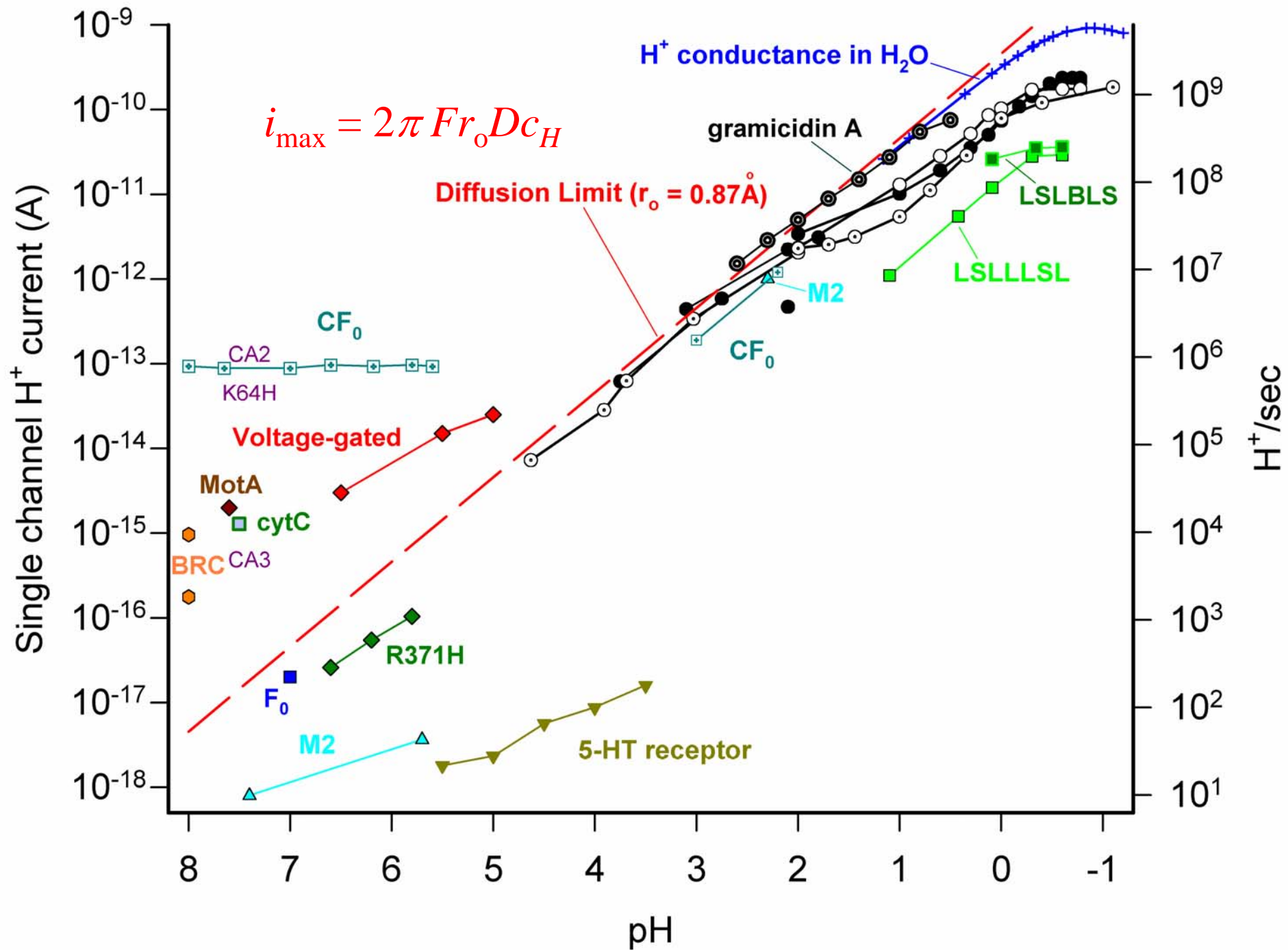


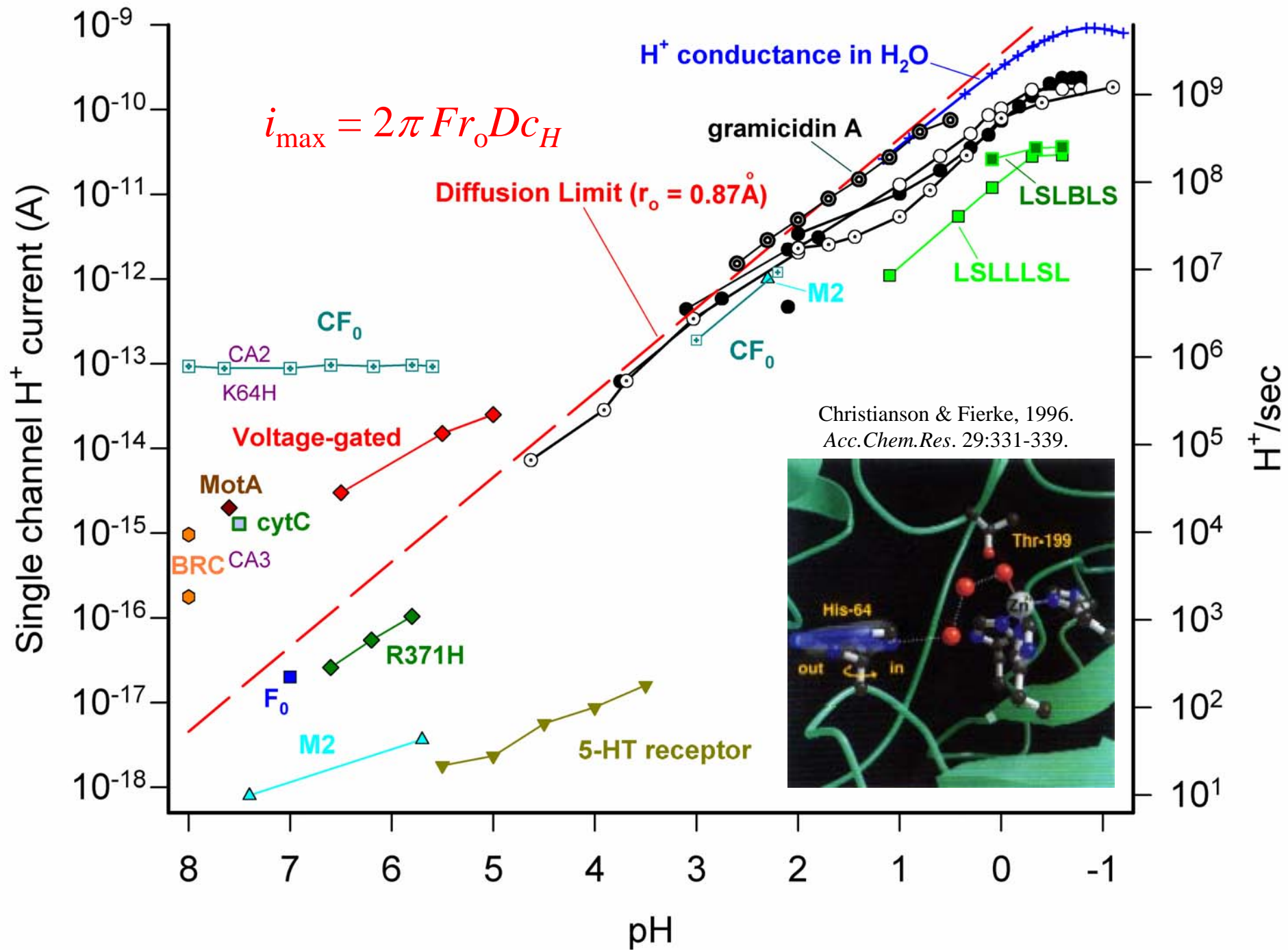
Model Variance: 200 channels @ 50 fS



Conductance of Ion Channels

CHANNEL	γ (pS)	[ion] (mM)	SELECTIVITY
K ⁺ delayed rectifier	12	140	$P_{Na}/P_K < 0.01$
KcsA	80	200	$P_{Na}/P_K 0.006$
Maxi K ⁺ (Ca-activated)	250	140	$P_{Na}/P_K < 0.001$
V-gated Ca ²⁺	10	110	$P_{Na}/P_{Ca} 0.0008$
V-gated Na ⁺	15	160	$P_K/P_{Na} 0.1$
Gramicidin	12	100	$P_{Na}/P_K 0.25$
AchR	25	160	$P_{Na}/P_K 0.9$
Proton (V-gated)	0.015	0.00006	$P_{Na}/P_H 0$





Vladimir Cherny



Tatiana Iastrebova

The DeCoursey Lab

Ricardo Murphy



Larry Thomas



Deri Morgan



Deri Morgan, otherwise known as "The Welsh Windmill," wins his bout at the Golden Gloves tournament.

Captain Proton



Tom DeCoursey

