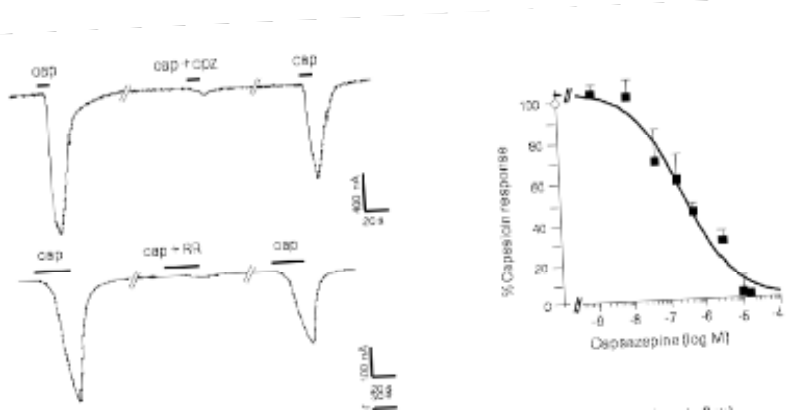
FIG. 1


cap
cap + CDZ
cap
cap
cap + RR
cap
% Capsaicin response
Capsazepine (log M)

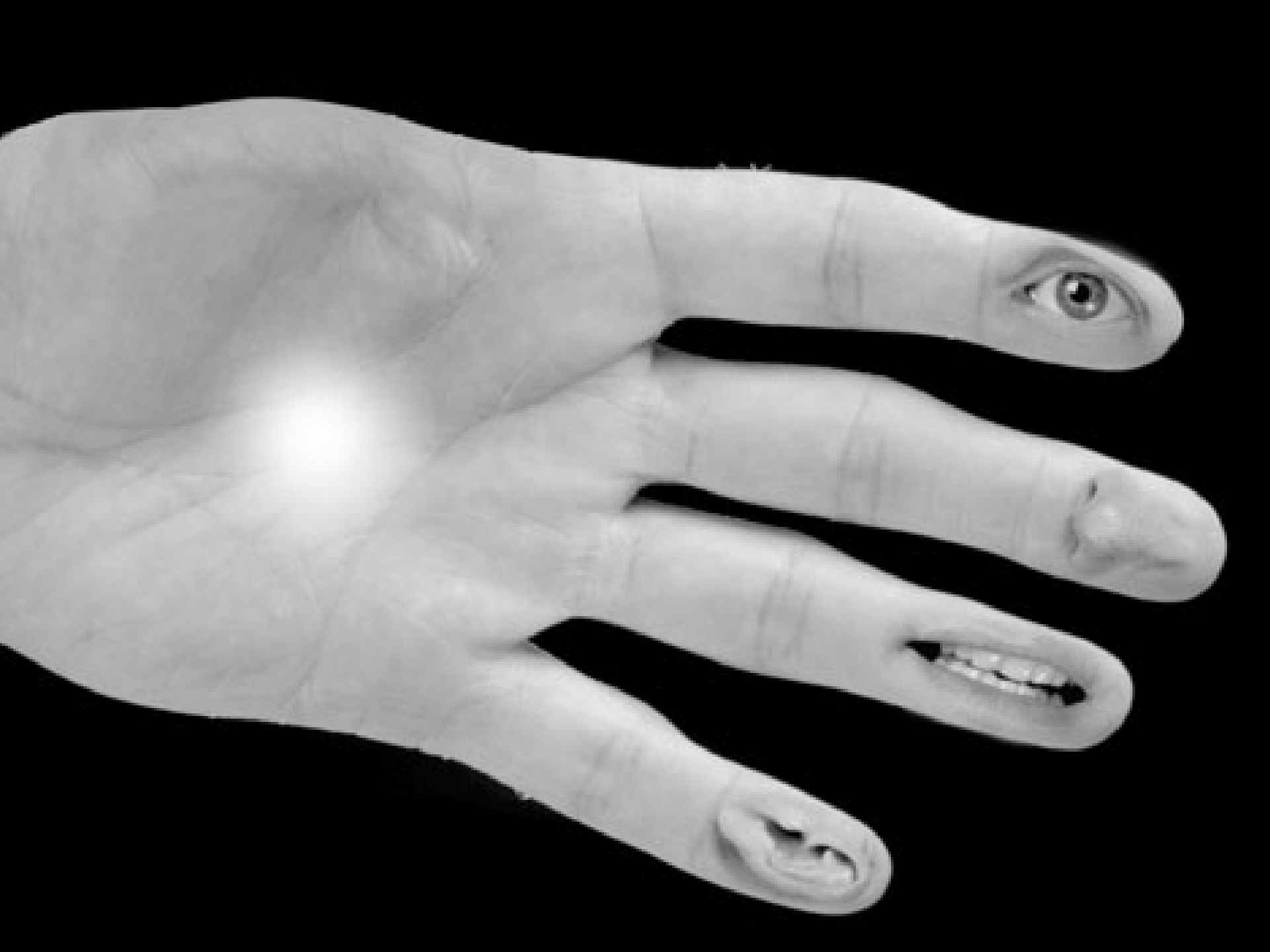
What a Long, Strange Trp It's Been

9.013: Cell. and Mol. Neurobiology

Nathan Wilson

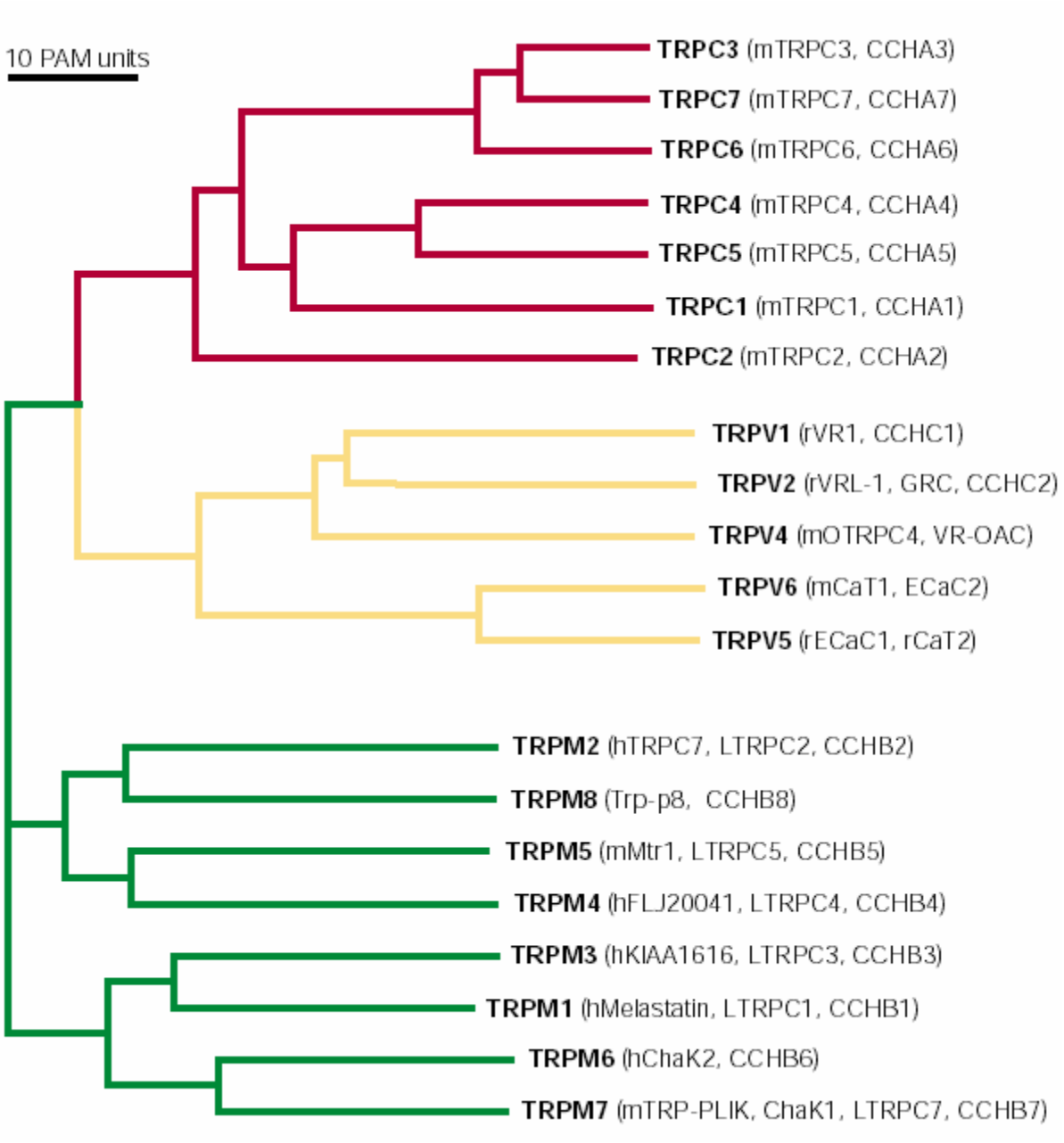
February 9, 2004

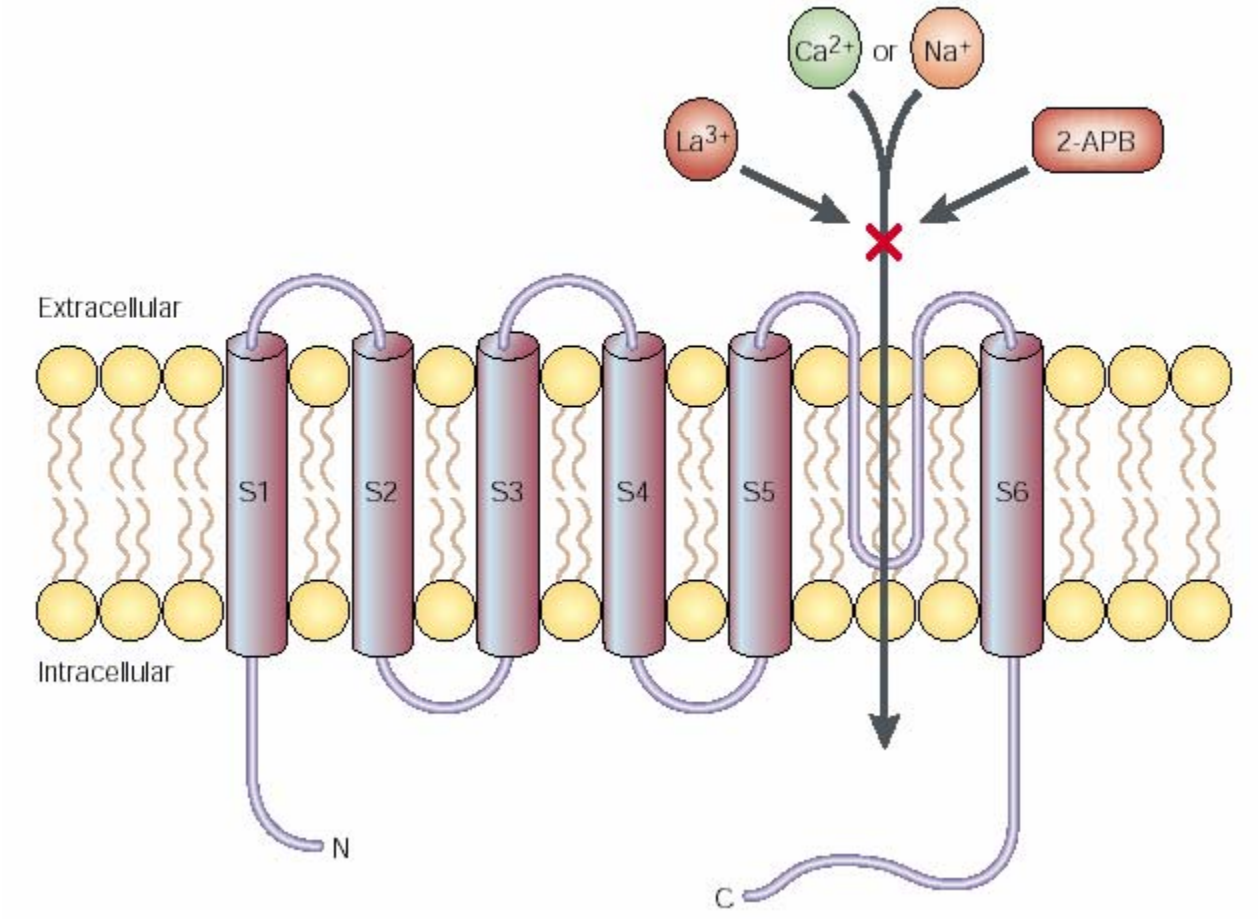

VR

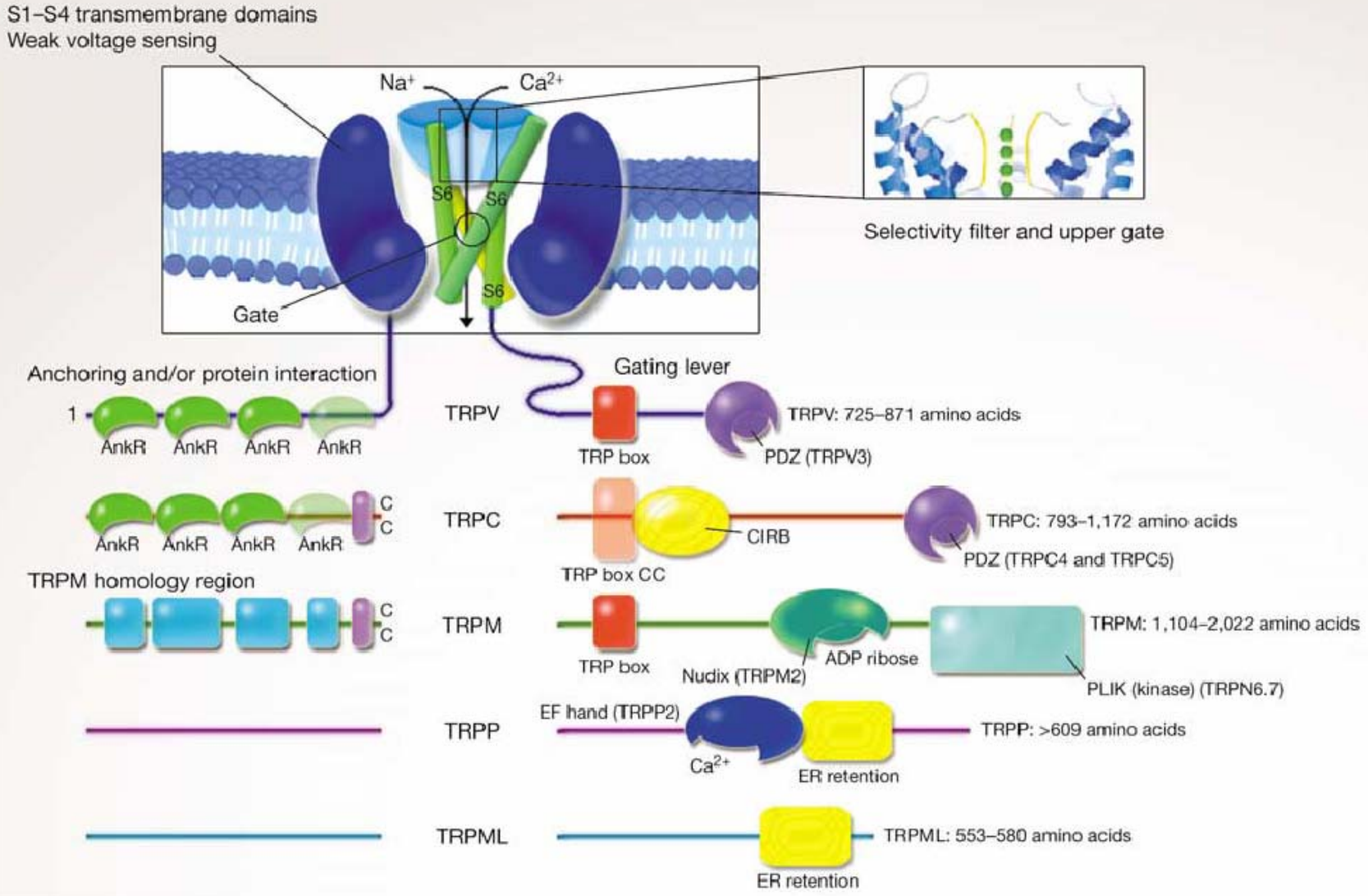


“Human Vision”
Visible Light Illumination

“Honeybee Vision”
Ultraviolet Illumination

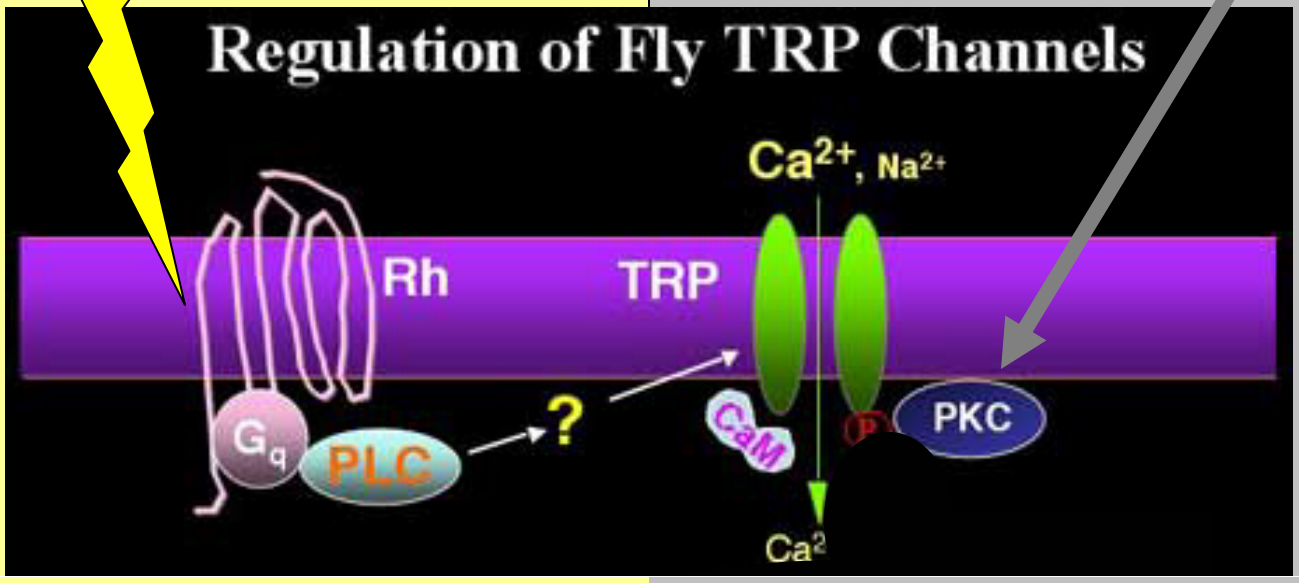

10 PAM units
TRPC3 (mTRPC3, CCHA3)
TRPC7 (mTRPC7, CCHA7)
TRPC6 (mTRPC6, CCHA6)
TRPC4 (mTRPC4, CCHA4)
TRPC5 (mTRPC5, CCHA5)
TRPC1 (mTRPC1, CCHA1)
TRPC2 (mTRPC2, CCHA2)
TRPV1 (rVR1, CCHC1)
TRPV2 (rVRL-1, GRC, CCHC2)
TRPV4 (mOTRPC4, VR-OAC)
TRPV6 (mCaT1, ECaC2)
TRPV5 (rECaC1, rCaT2)
TRPM2 (hTRPC7, LTRPC2, CCHB2)
TRPM8 (Trp-p8, CCHB8)
TRPM5 (mMtr1, LTRPC5, CCHB5)
TRPM4 (hFLJ20041, LTRPC4, CCHB4)
TRPM3 (hKIAA1616, LTRPC3, CCHB3)
TRPM1 (hMelastatin, LTRPC1, CCHB1)
TRPM6 (hChaK2, CCHB6)
TRPM7 (mTRP-PLIK, ChaK1, LTRPC7, CCHB7)


Ca^{2+}
or
Na^{+}
La^{3+}
2-APB
Extracellular
S1
S2
S3
S4
S5
S6
Intracellular
N
C


S1–S4 transmembrane domains
Weak voltage sensing
Na+
Ca2+
S6
S6
S6
Gate
Selectivity filter and upper gate
Anchoring and/or protein interaction
Gating lever
1
AnkR
AnkR
AnkR
AnkR
TRPV
TRP box
PDZ (TRPV3)
TRPV: 725–871 amino acids
C
C
AnkR
AnkR
AnkR
AnkR
TRPC
CIRB
TRP box CC
PDZ (TRPC4 and TRPC5)
TRPC: 793–1,172 amino acids
TRPM homology region
C
C
TRPM
TRP box
Nudix (TRPM2)
ADP ribose
PLIK (kinase) (TRPN6.7)
TRPM: 1,104–2,022 amino acids
TRPP
EF hand (TRPP2)
Ca2+
ER retention
TRPP: >609 amino acids
TRPML
ER retention
TRPML: 553–580 amino acids

hv

dark

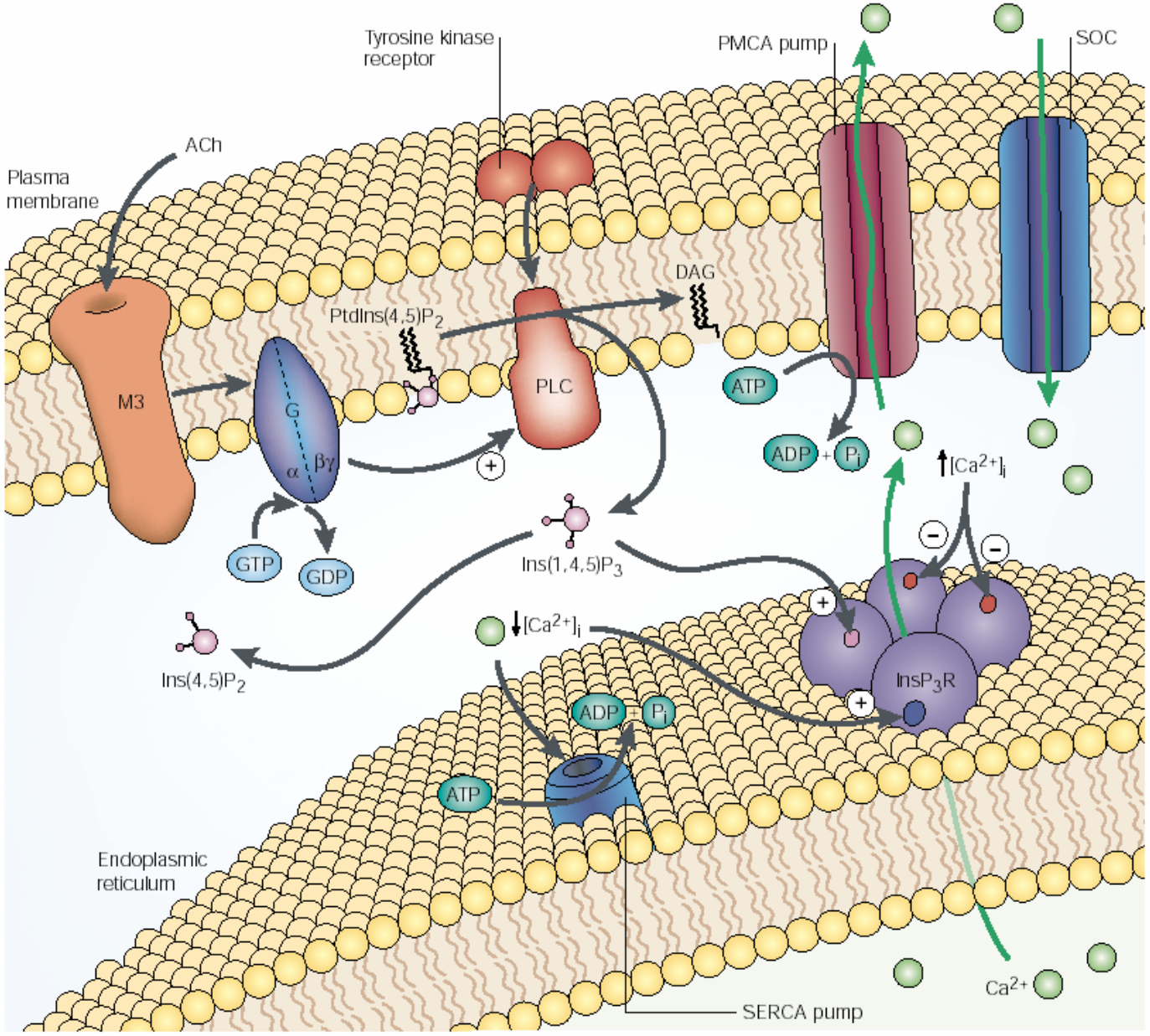


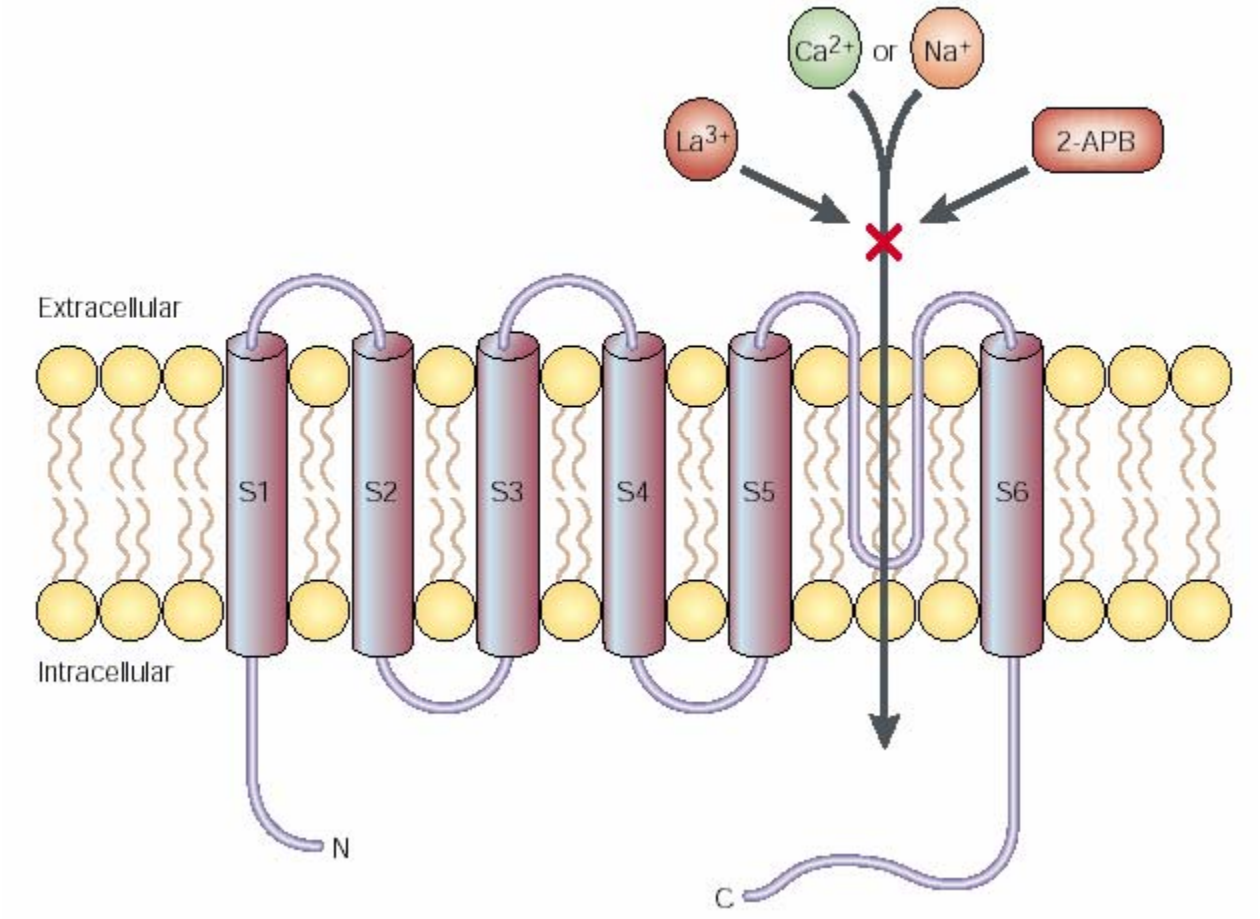
Rhodopsin activates G protein (Gqα) which activates PLC

PLC breaks down PIP2 → IP3 & DAG which activate TRP which let in Ca++

Ca++ activates PKC which deactivates TRP channel

Based on Tsunoda, 1997


Tyrosine kinase receptor
PMCA pump
SOC
ACh
Plasma membrane
DAG
PtdIns(4,5)P2
M3
G
α
βγ
PLC
ATP
ADP
Pi
[Ca2+]i
GTP
GDP
Ins(1,4,5)P3
Ins(4,5)P2
InsP3R
Endoplasmic reticulum
Ca2+
SERCA pump

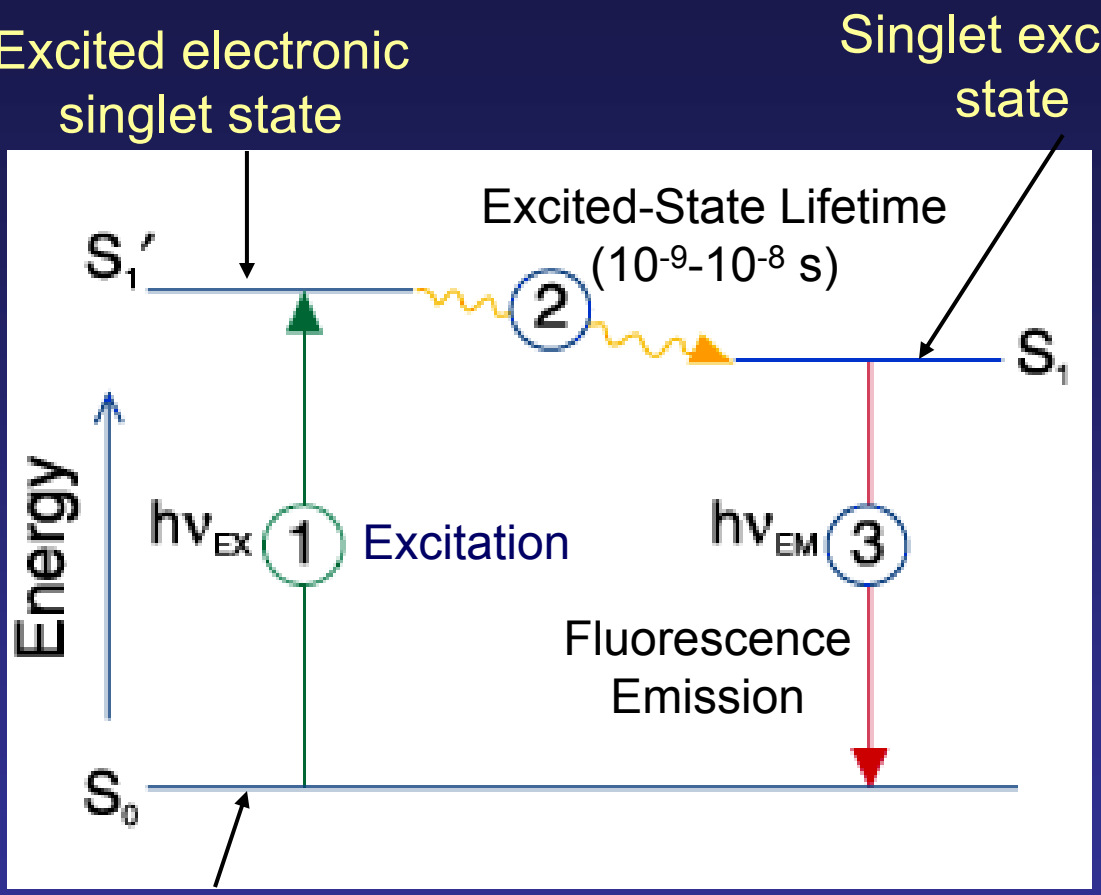

Ca^{2+}
or
Na^{+}
La^{3+}
2-APB
Extracellular
S1
S2
S3
S4
S5
S6
Intracellular
N
C

Previously Known

- Locus of capsaicin sensitivity:
  Dorsal root ganglia

- Action of putative capsaicin receptor:
  trigger calcium influx into its cells.

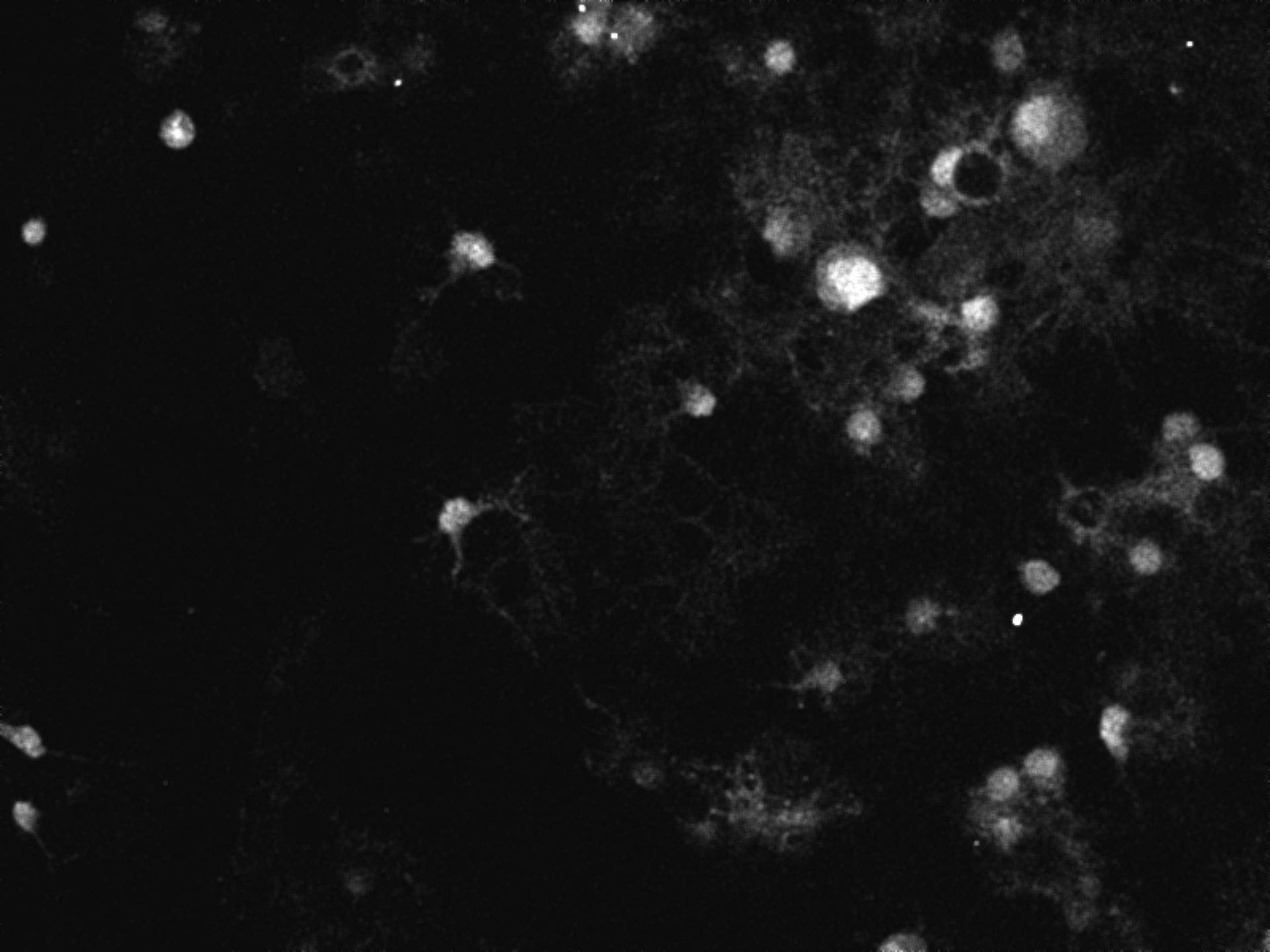
JABLONSKI DIAGRAM



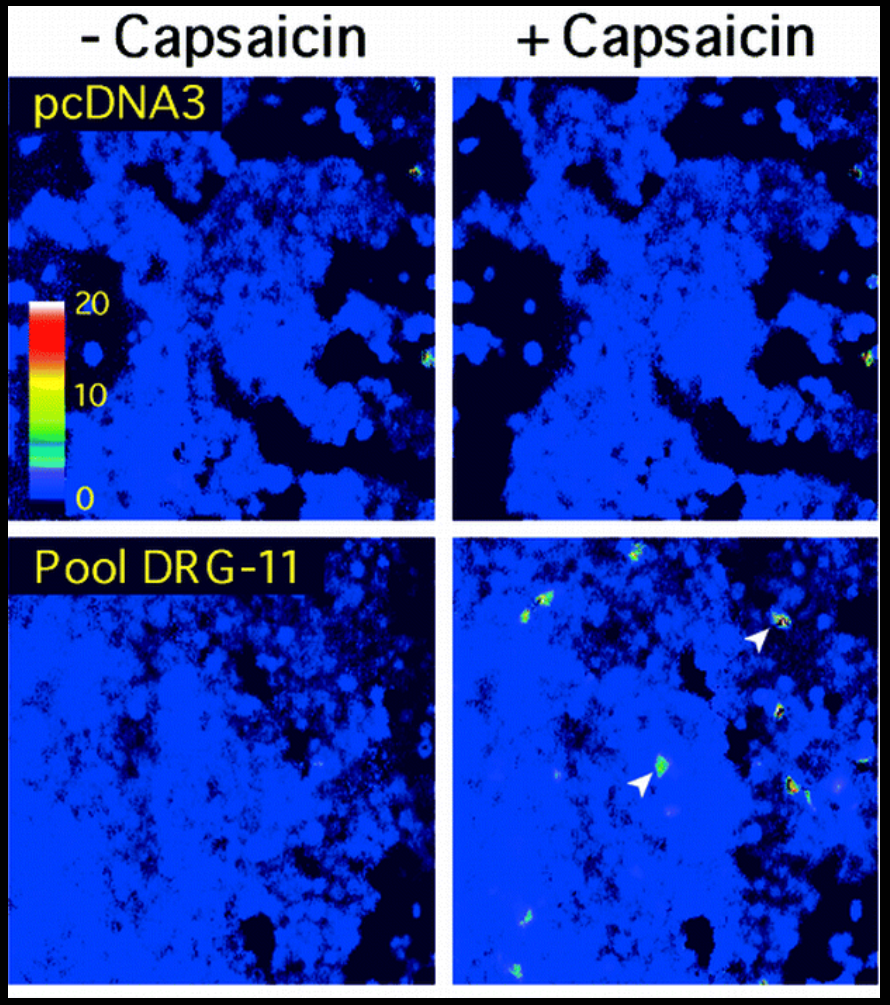
Stokes shift
$h\upsilon_{EX} - h\upsilon_{EM}$

FLUORESCENCE QUANTUM YIELD

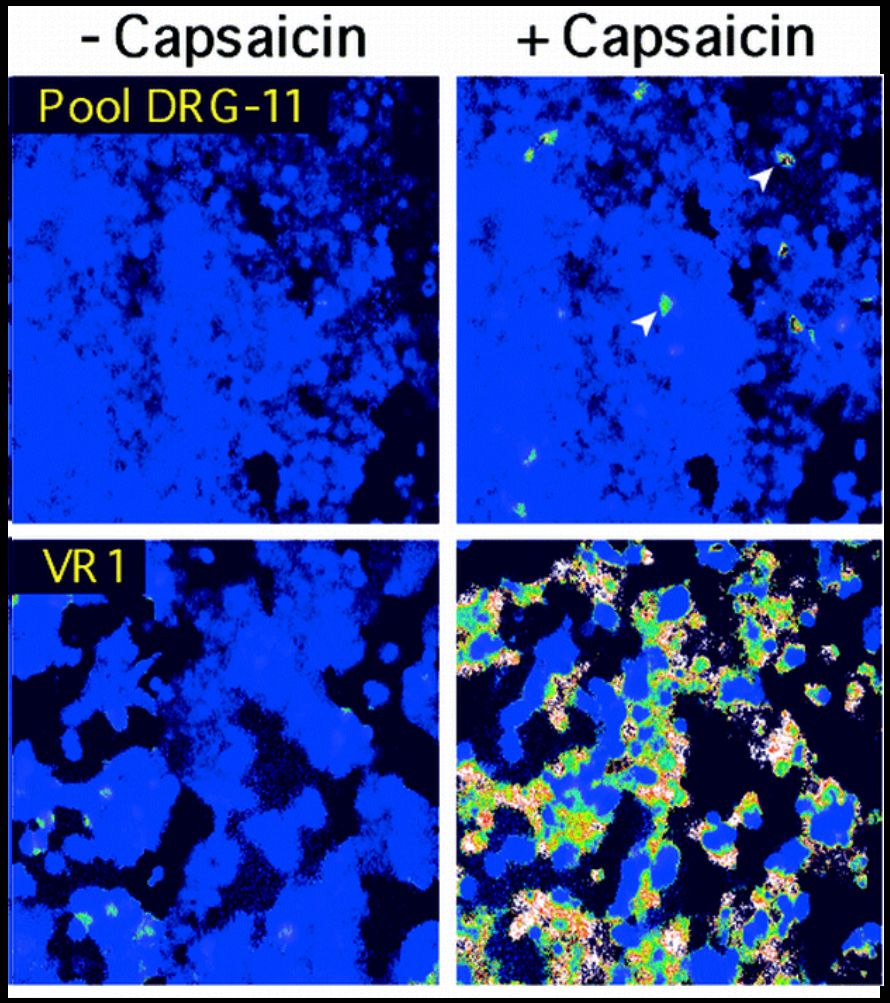
$$\frac{\text{\# fluorescence photons emitted (Stage 3)}}{\text{\# fluorescence photons absorbed (Stage 1)}}$$



Search for the Capsaicin Receptor: Expression Cloning



Search for the Capsaicin Receptor: Expression Cloning



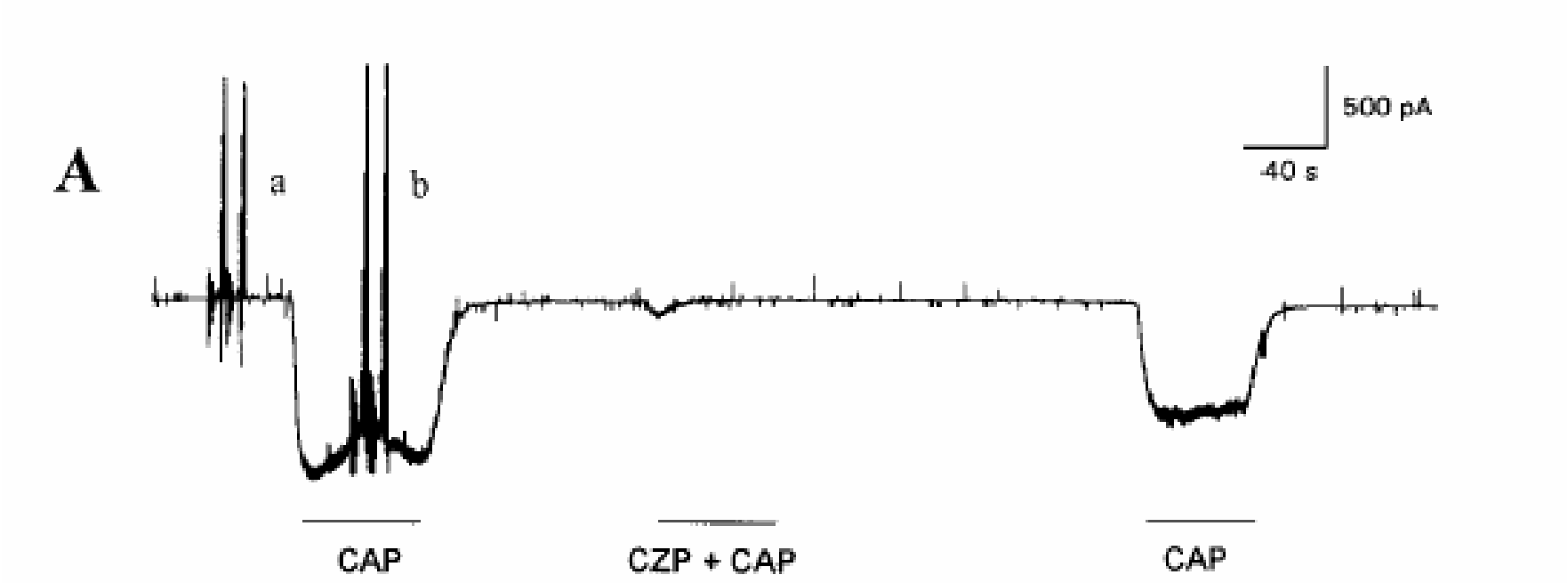
Does it Work the Same? Previously Known

- Nature of receptor channel:
  Non-selective cation channel

- Agonists of receptor:
  include capsaicin and resiniferatoxin.

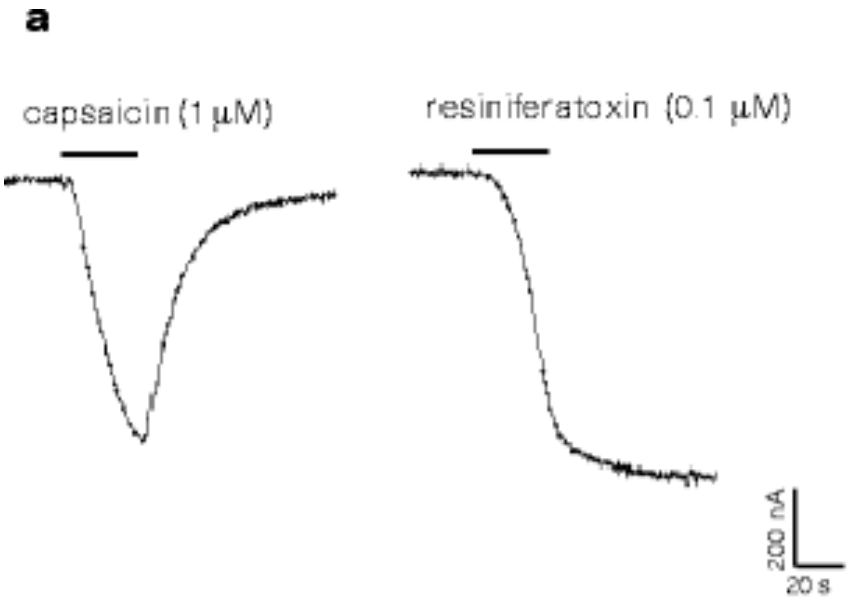
- Antagonists of receptor:
  include capsazepine and rubidium red.

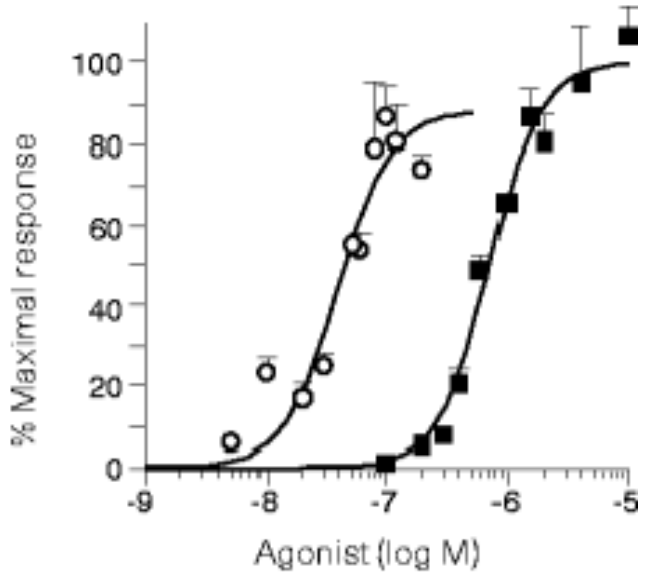
Does it work the same? Does expression of the isolated protein confers capsaicin sensitivity?



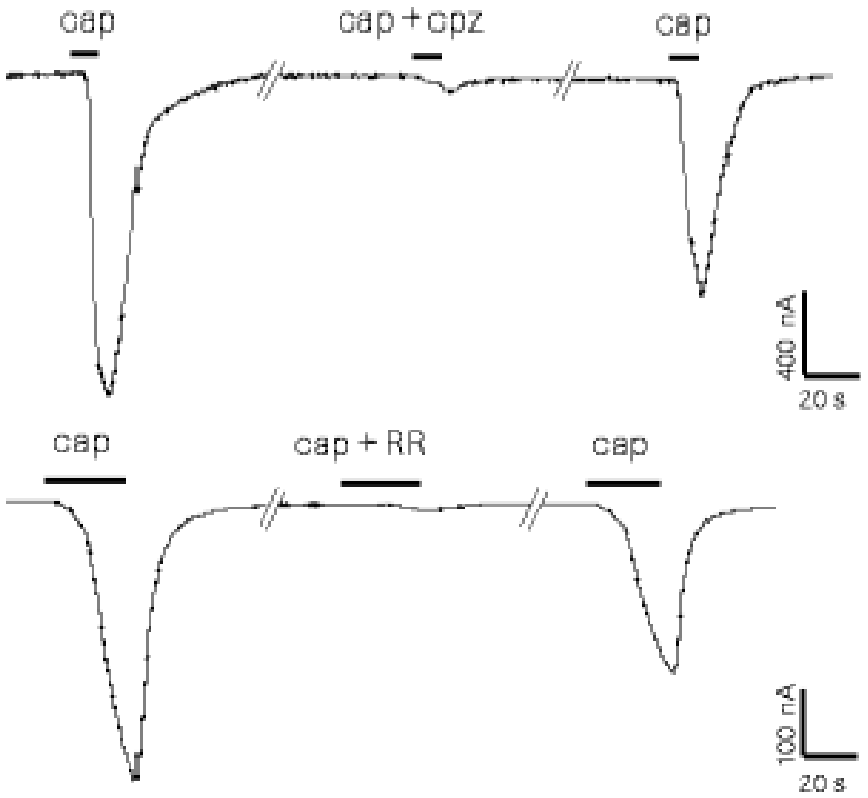
(Oh et al., J. Neurosci., 1996)

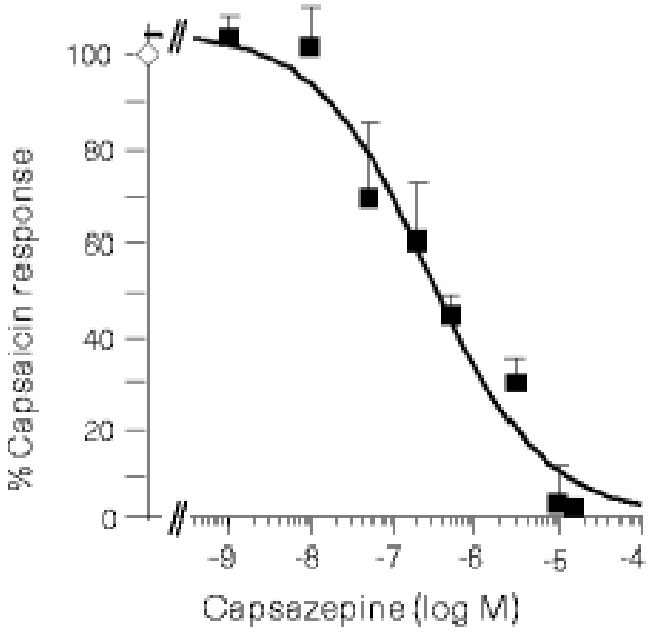
Does it work the same? Expression of the isolated protein confers capsaicin sensitivity



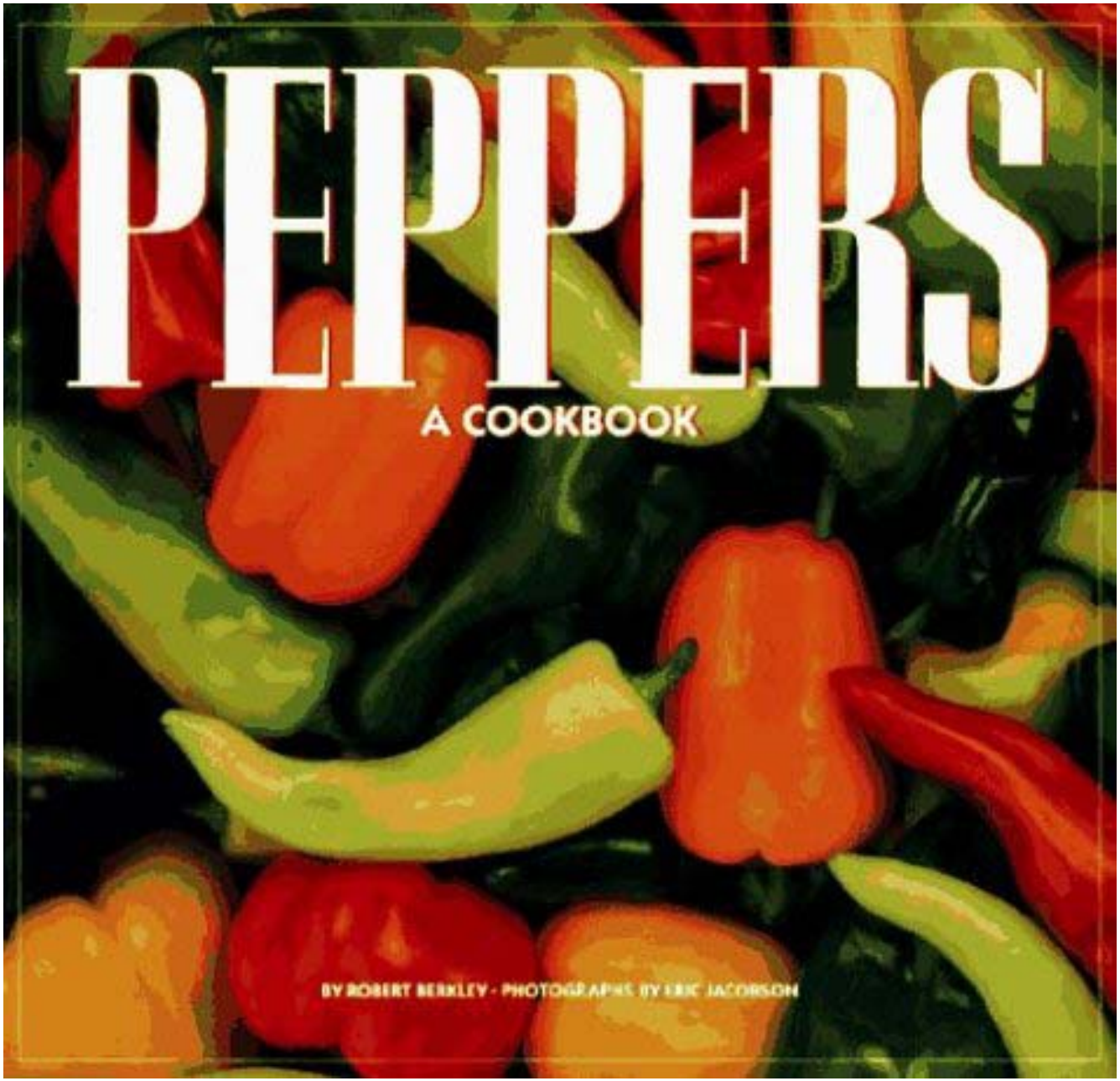


Does it work the same? Expression of the isolated protein confers **antagonist** sensitivity

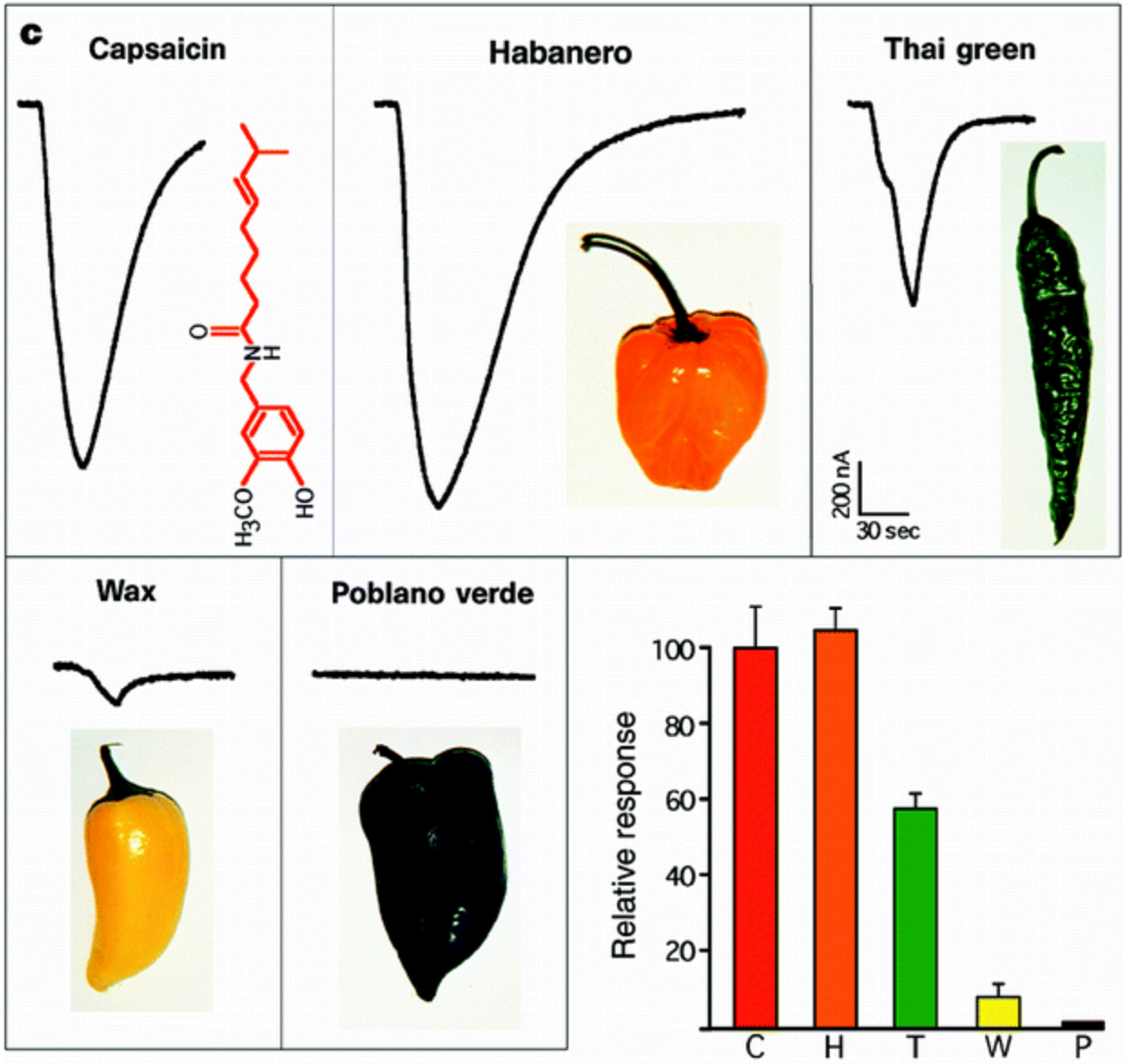




Can VR1 confer that “je ne sais ???” subtlety of chili experience?


PEPPERS
A COOKBOOK
BY ROBERT BERKLEY · PHOTOGRAPHS BY ERIC JACOBSON

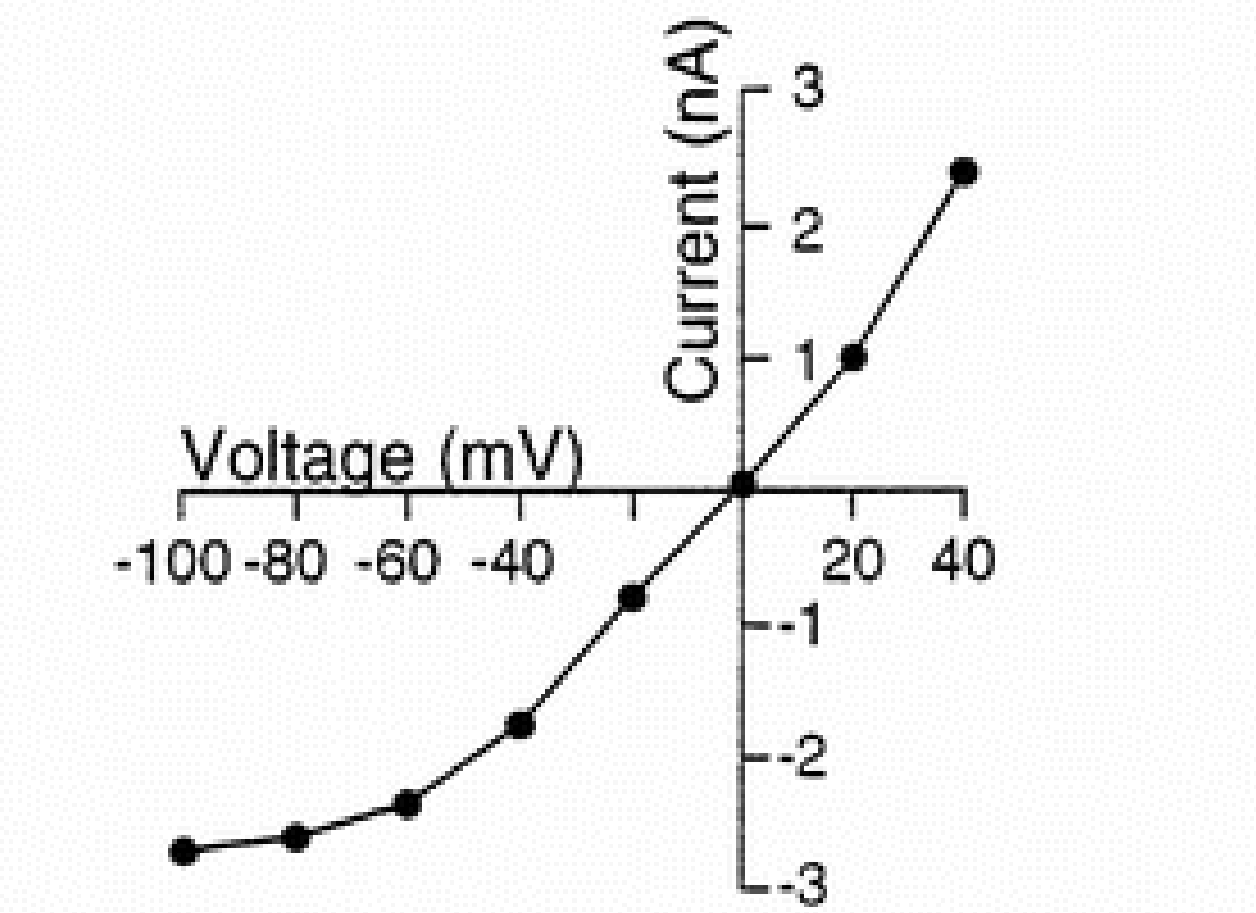
A More Rigorous Scale for Chilis



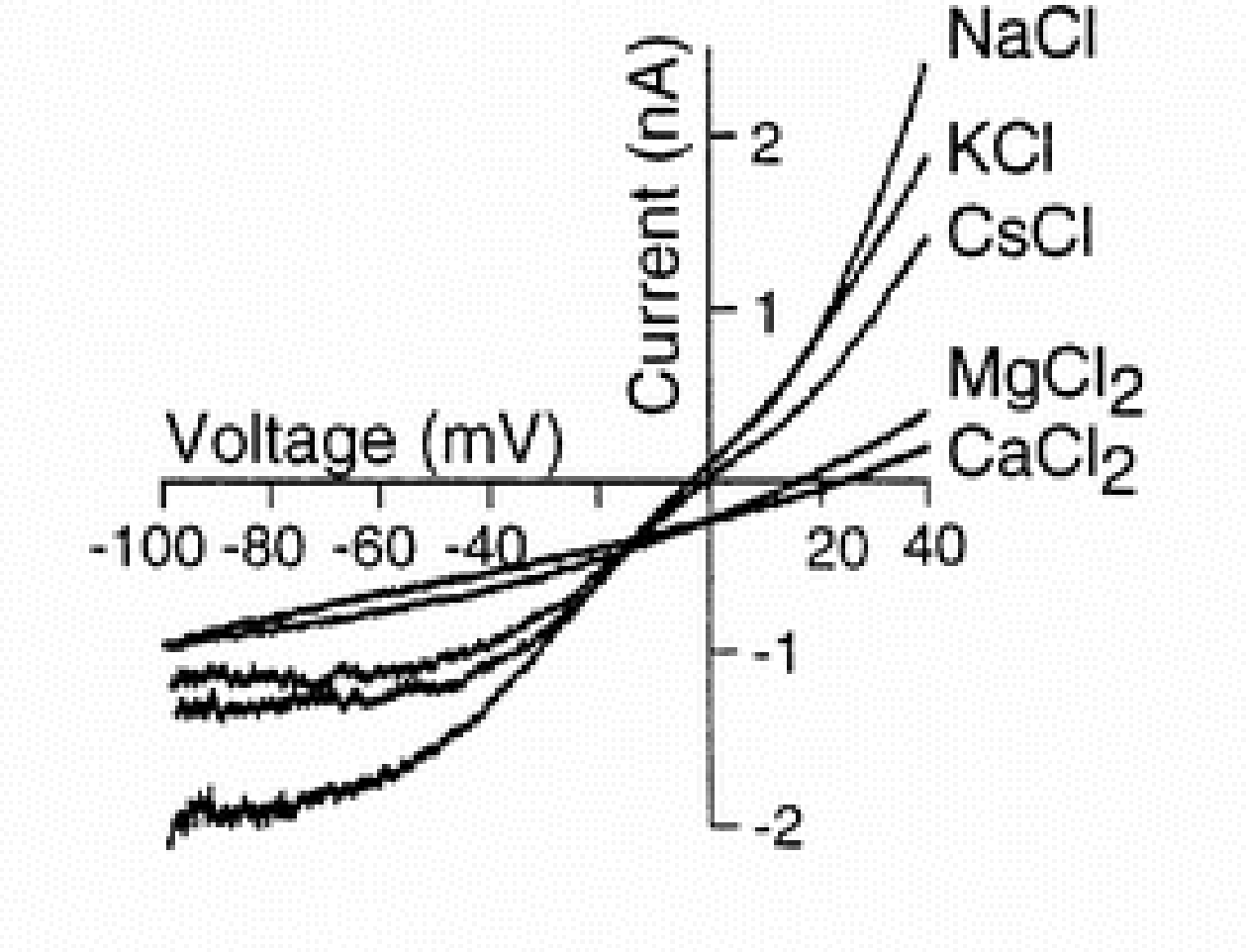
Control: is it specific for the function in question?

- Controls Tested:
  - ATP, serotonin, ACh, bradykinin, substance P, histamine, glutamate, hypertonic solution

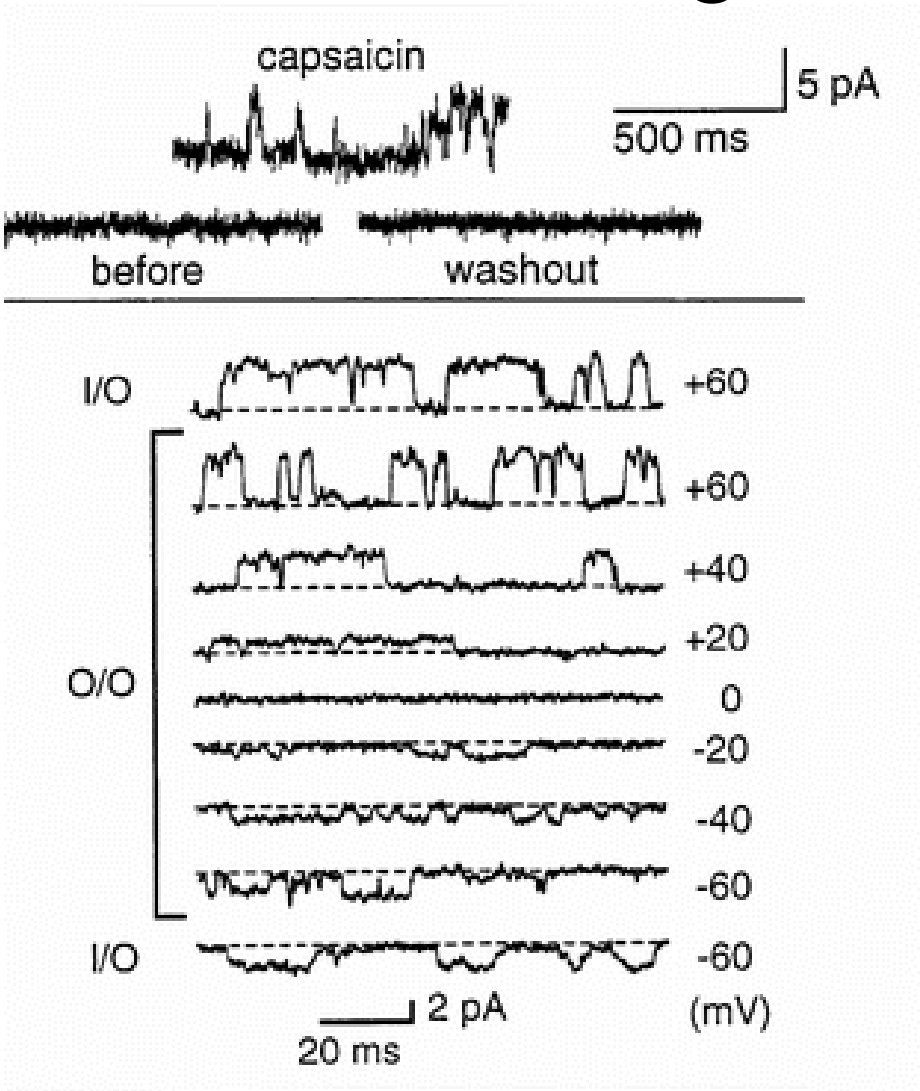
“I-V Curve” for capsaicin currents during different holding voltages

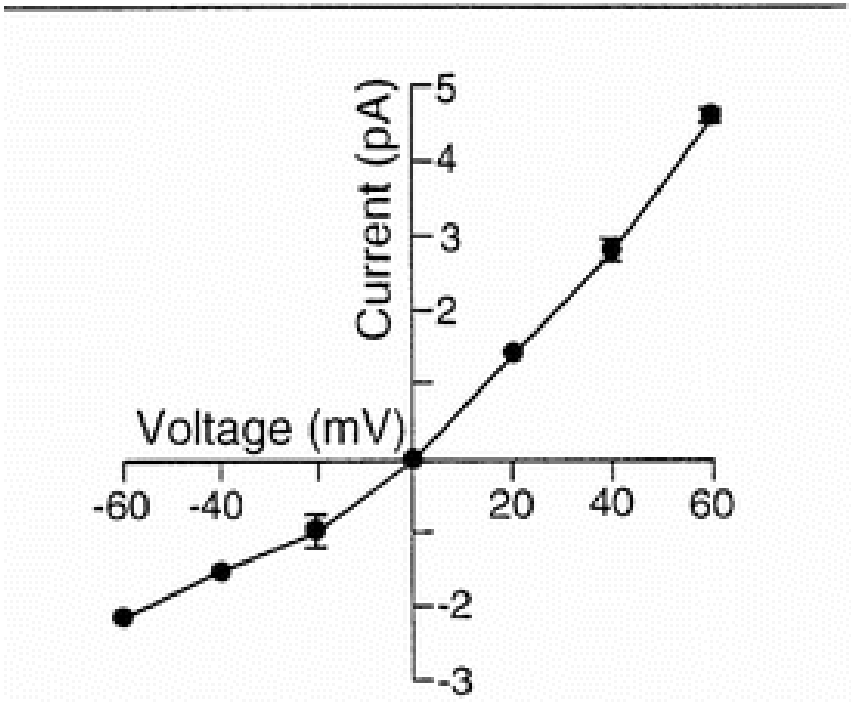


Which Ions Carry the Charge?

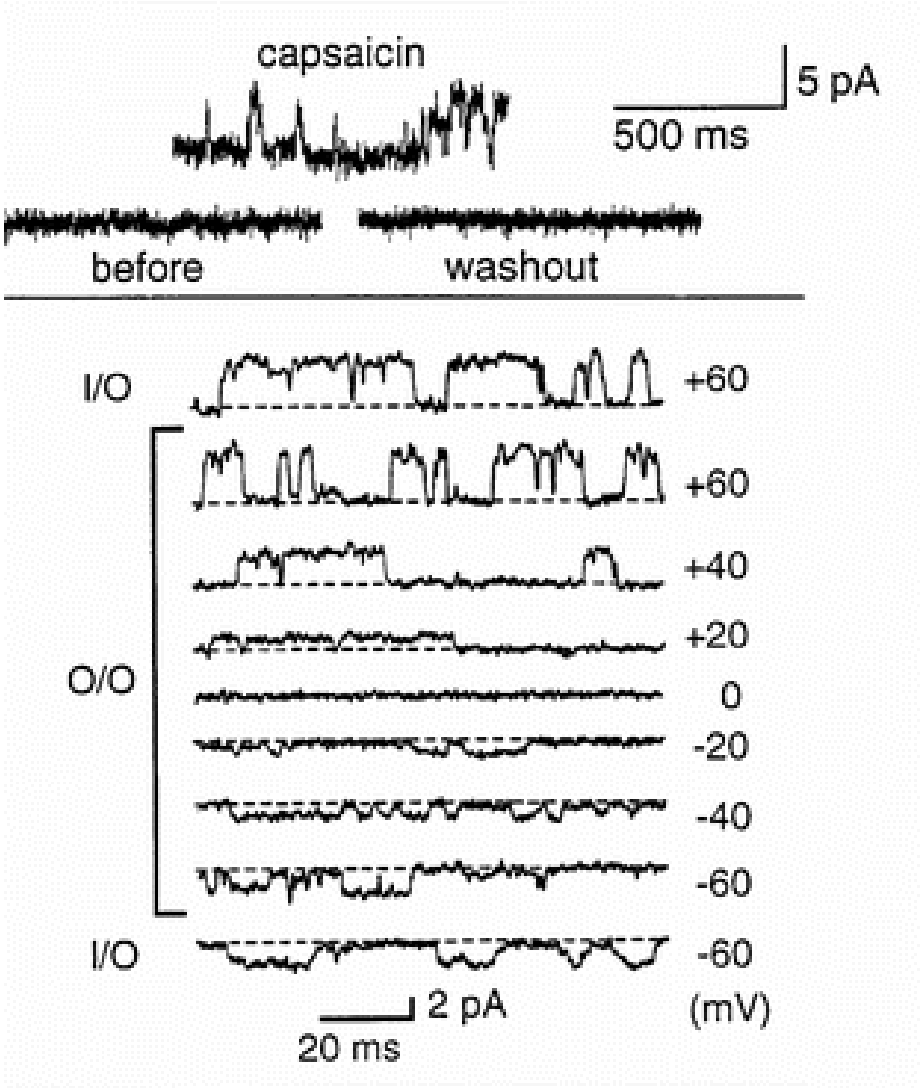


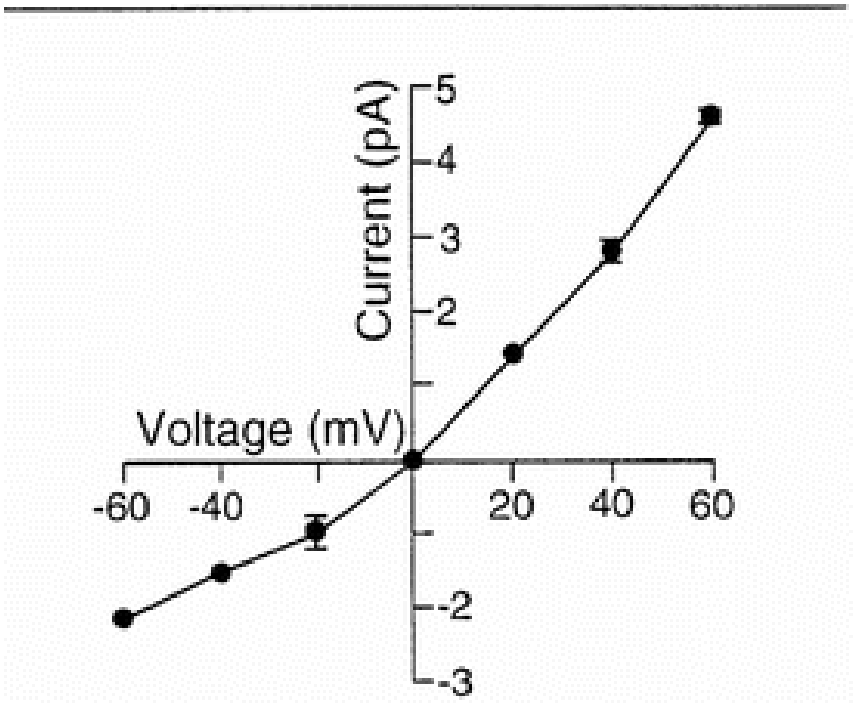
Monitoring activity of single channels



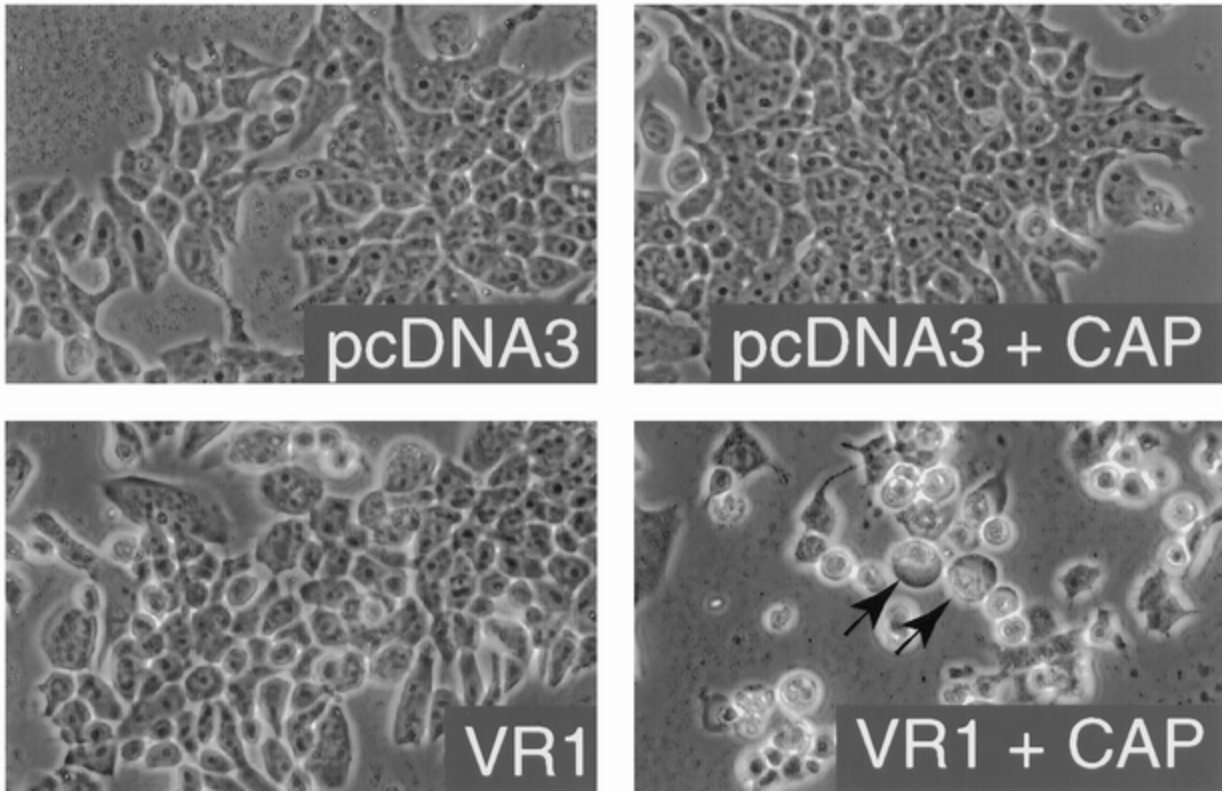


Capsaicin works on either side of the membrane

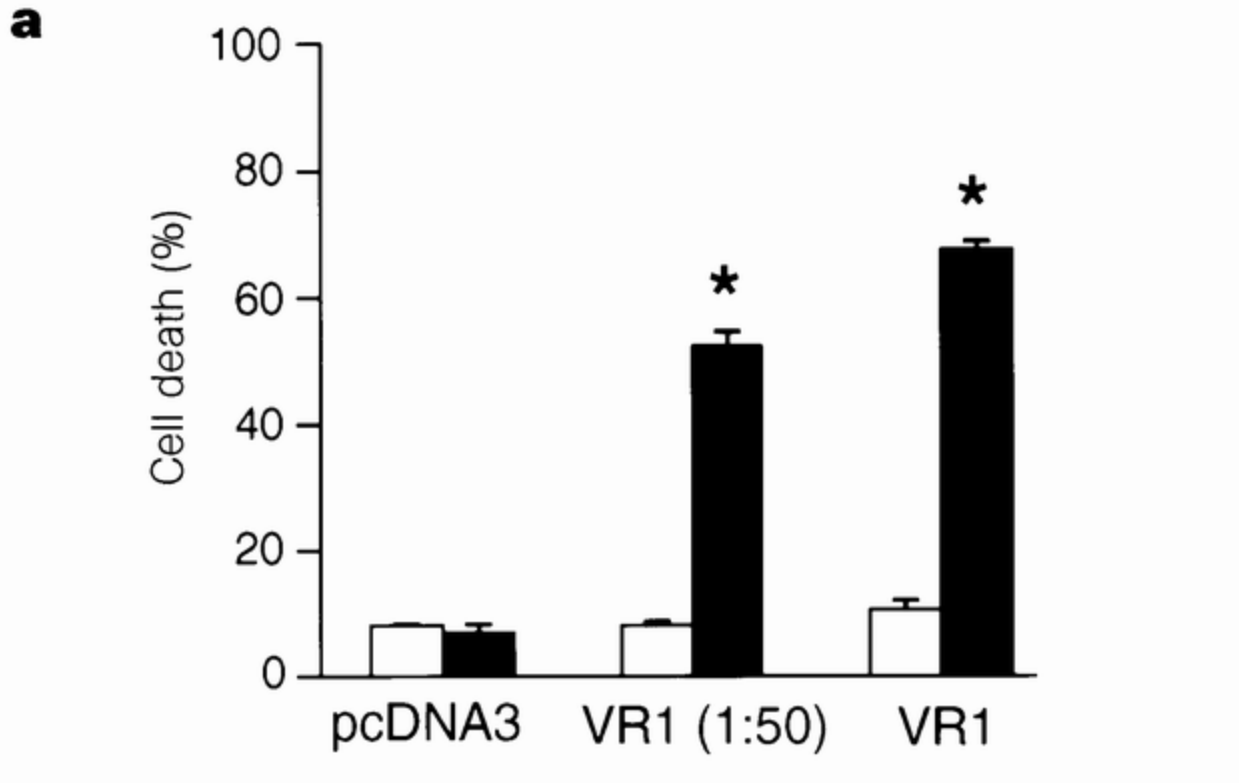




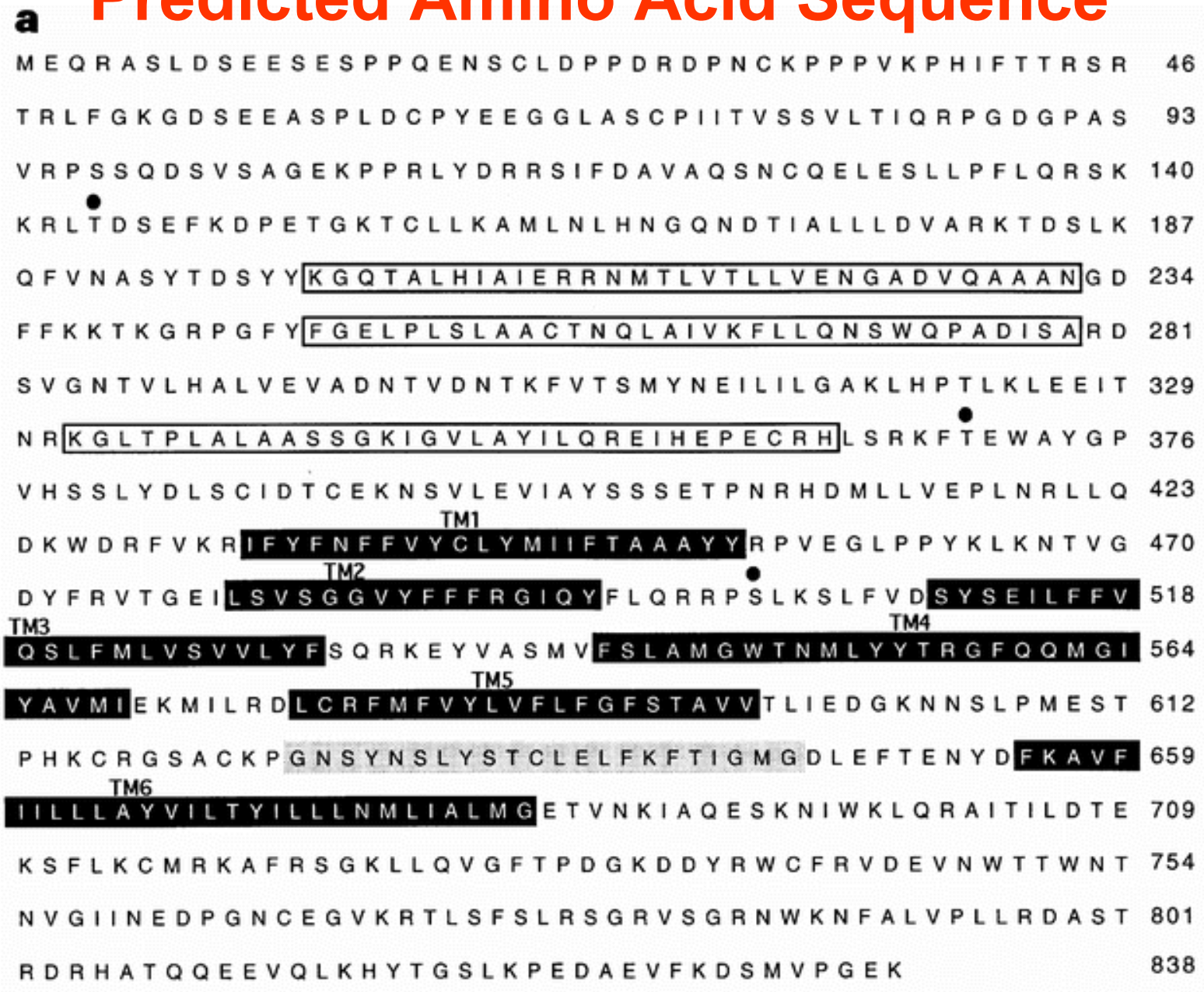
The receptor, with capsaicin, is an effectively lethal accomplice



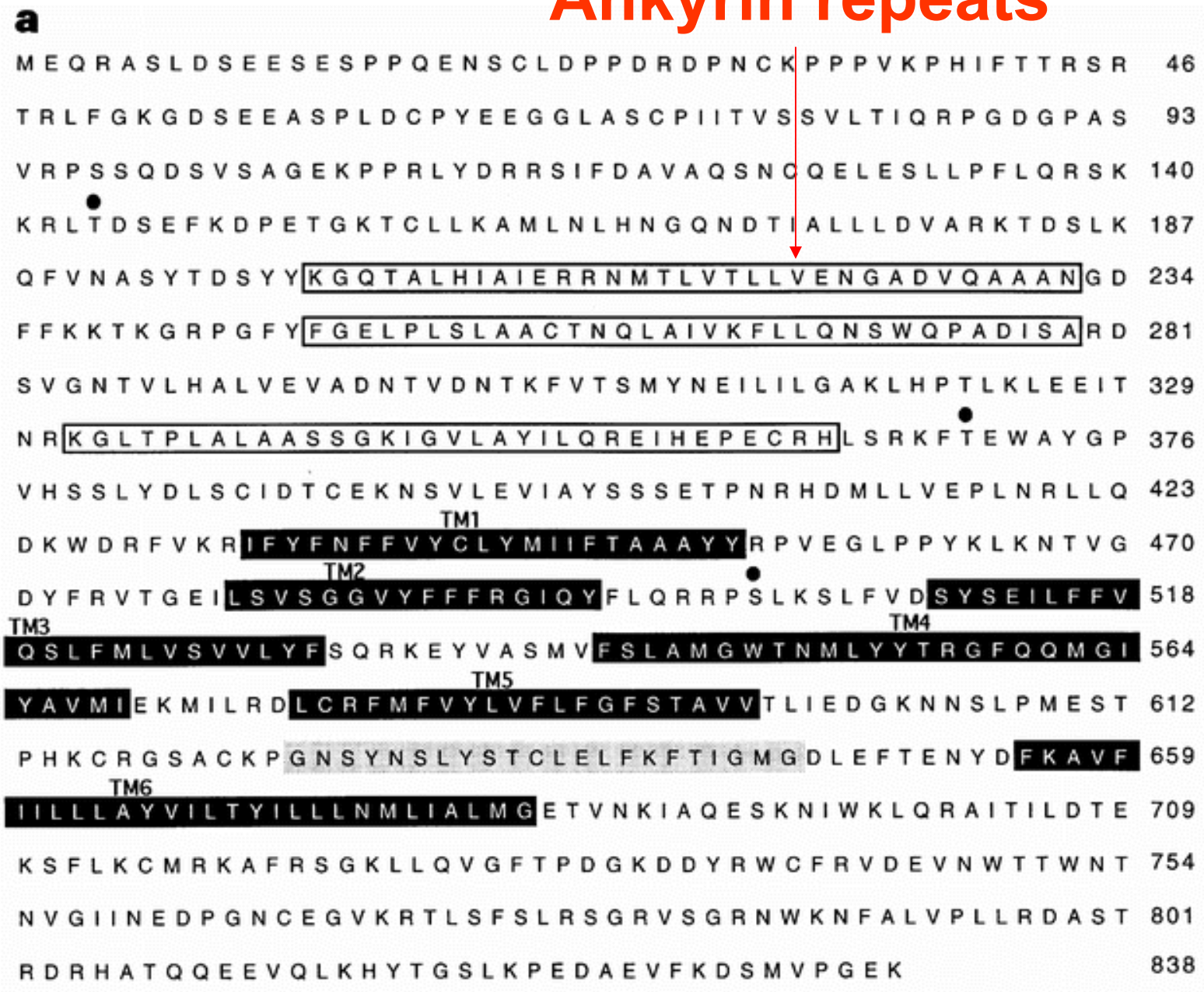
The receptor, with capsaicin, is an effectively lethal accomplice



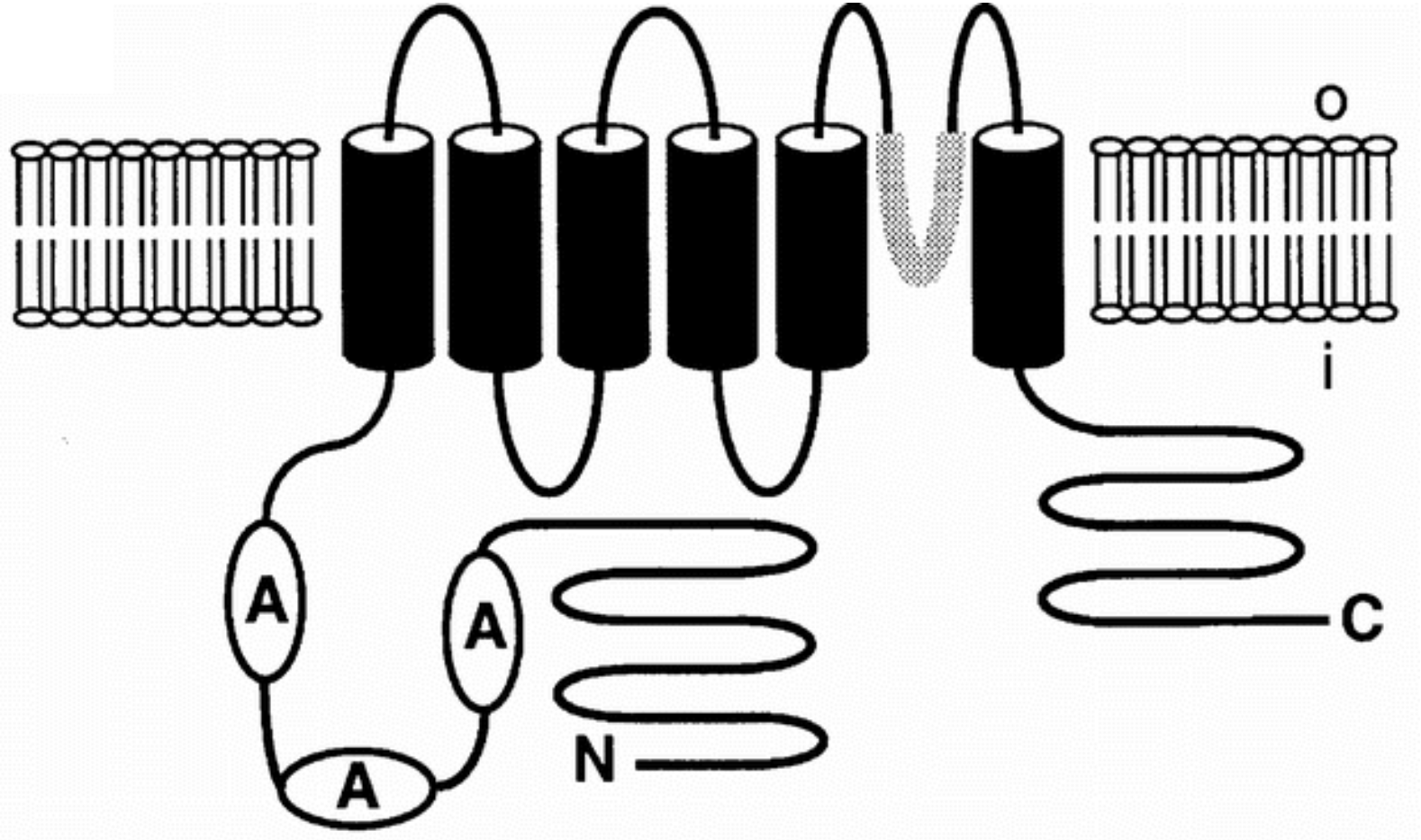
Predicted Amino Acid Sequence



Ankyrin repeats

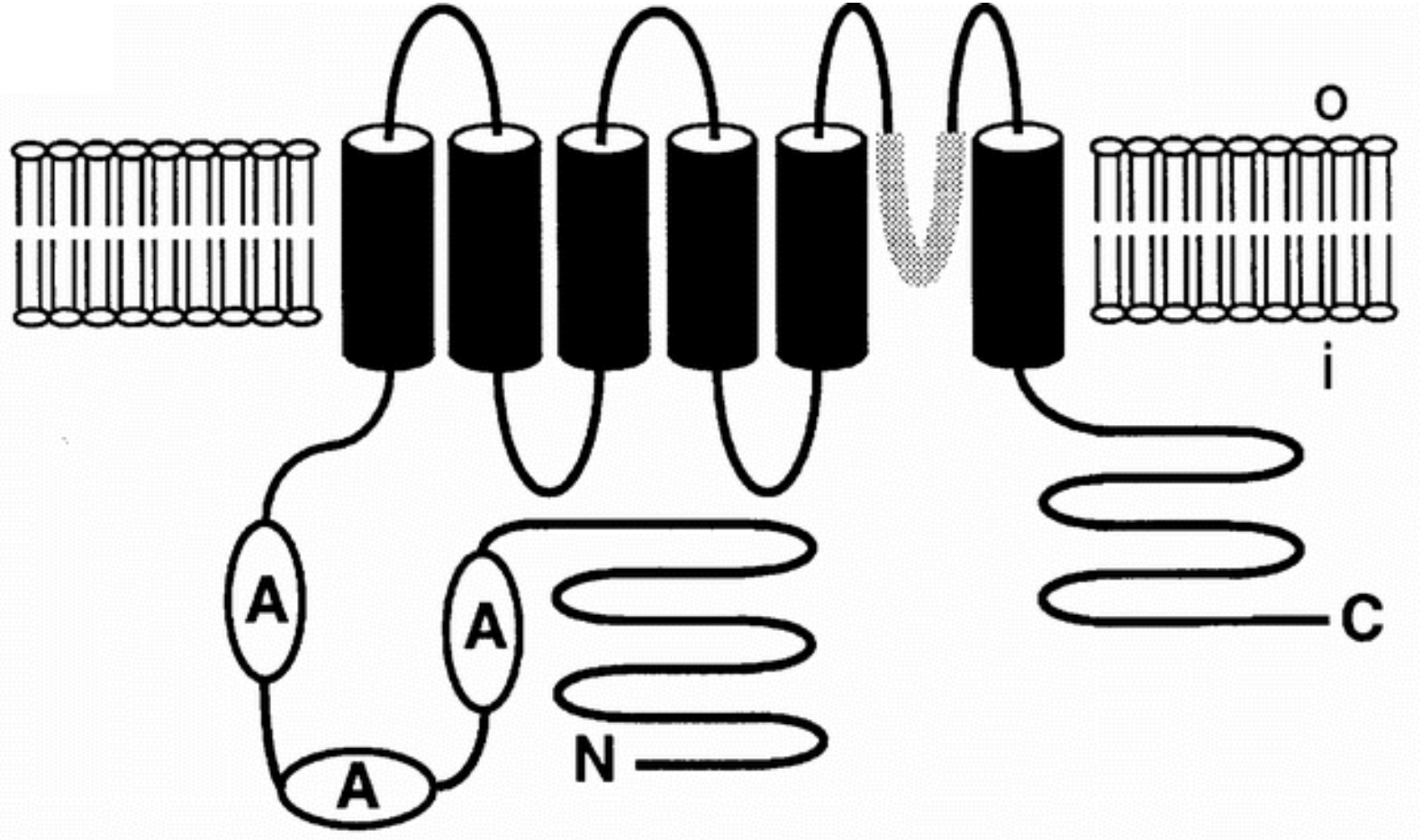


Predicted Structure



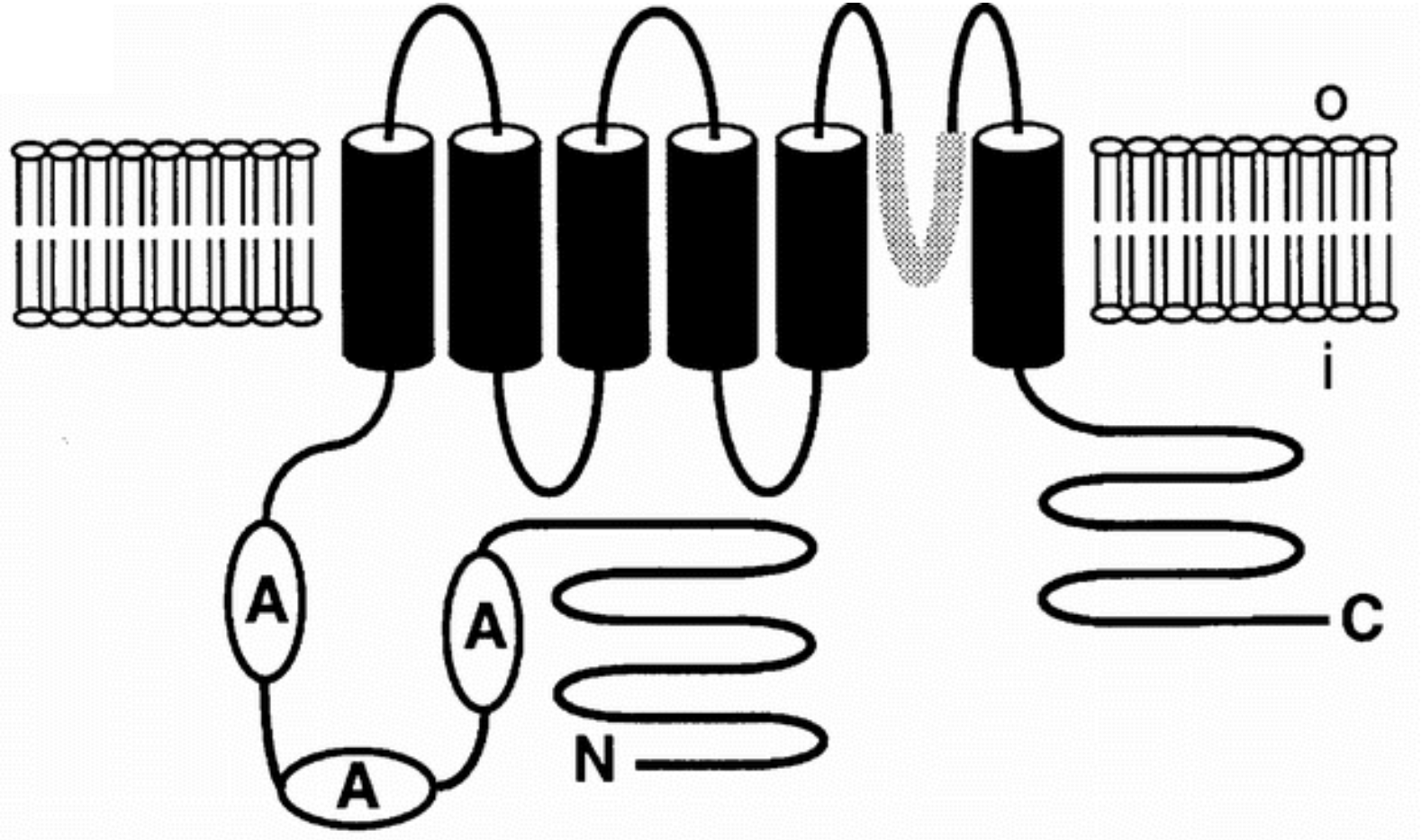
Predicted transmembrane domains

Predicted Structure



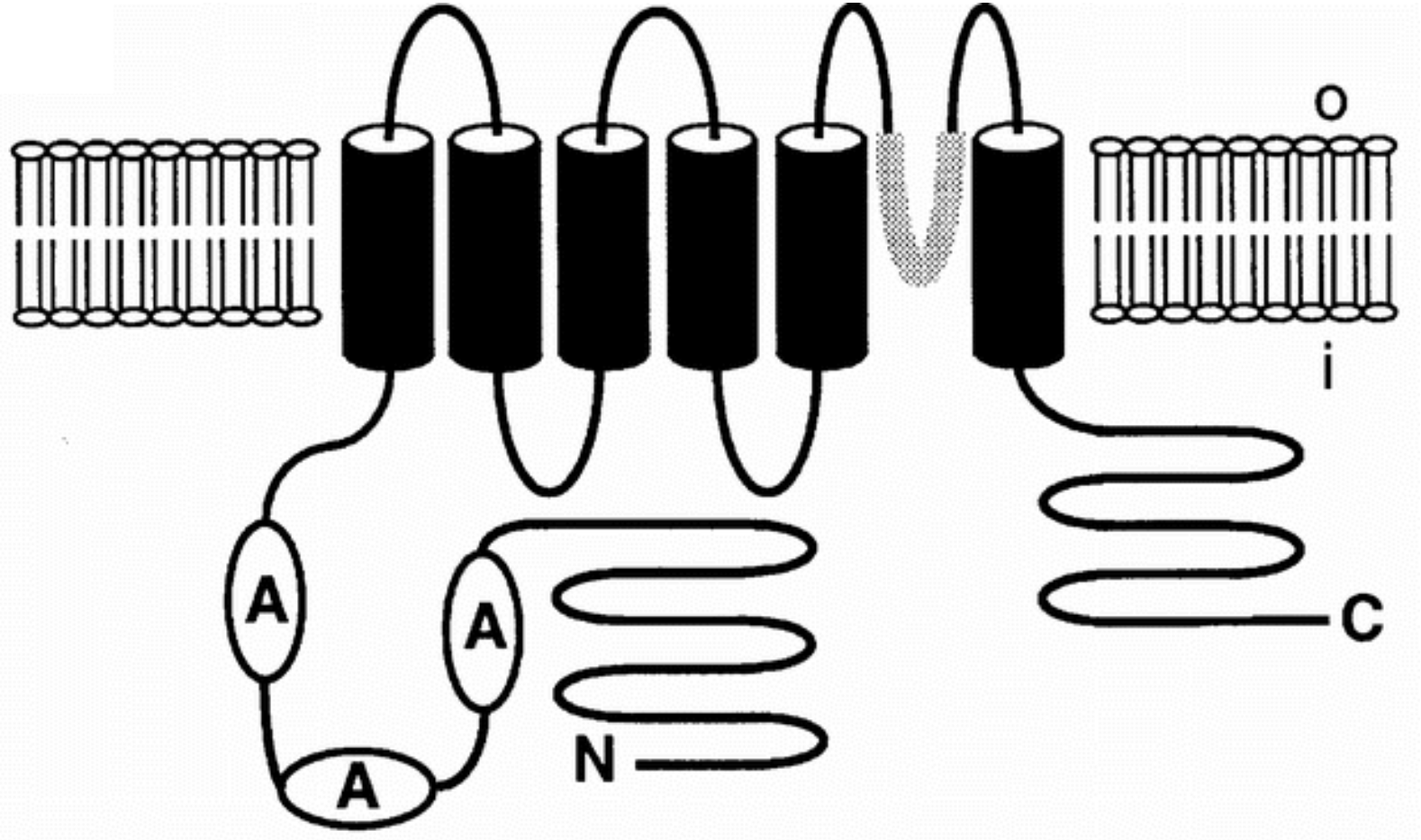
Predicted pore loop region

Predicted Structure

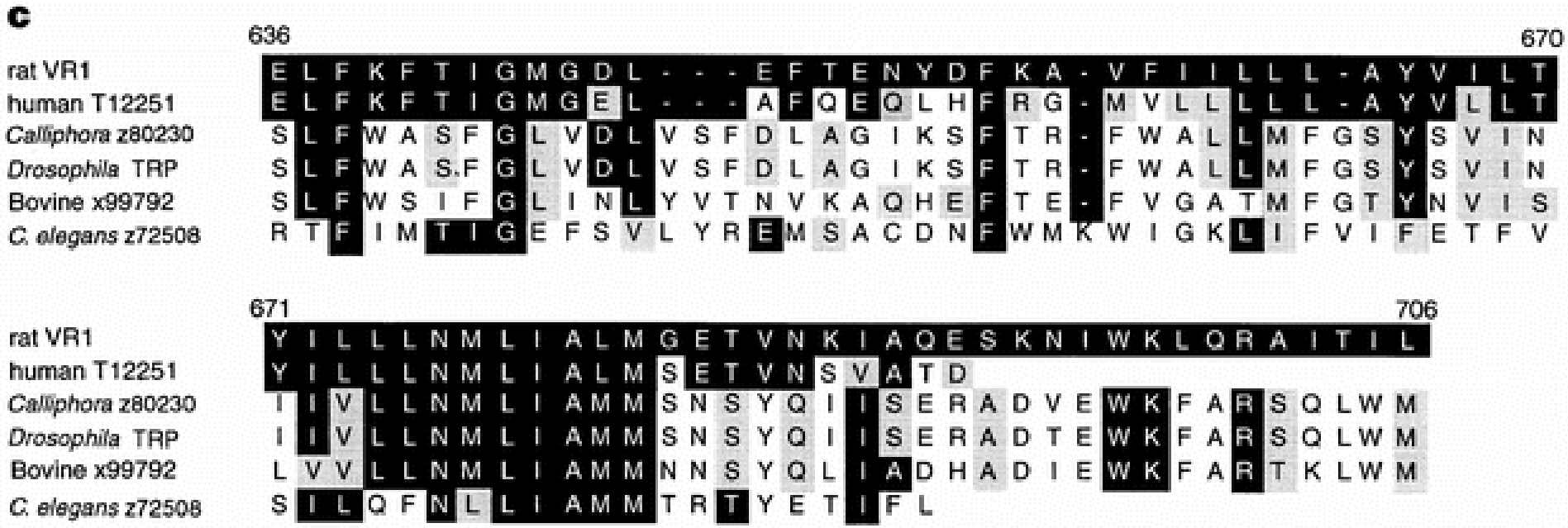


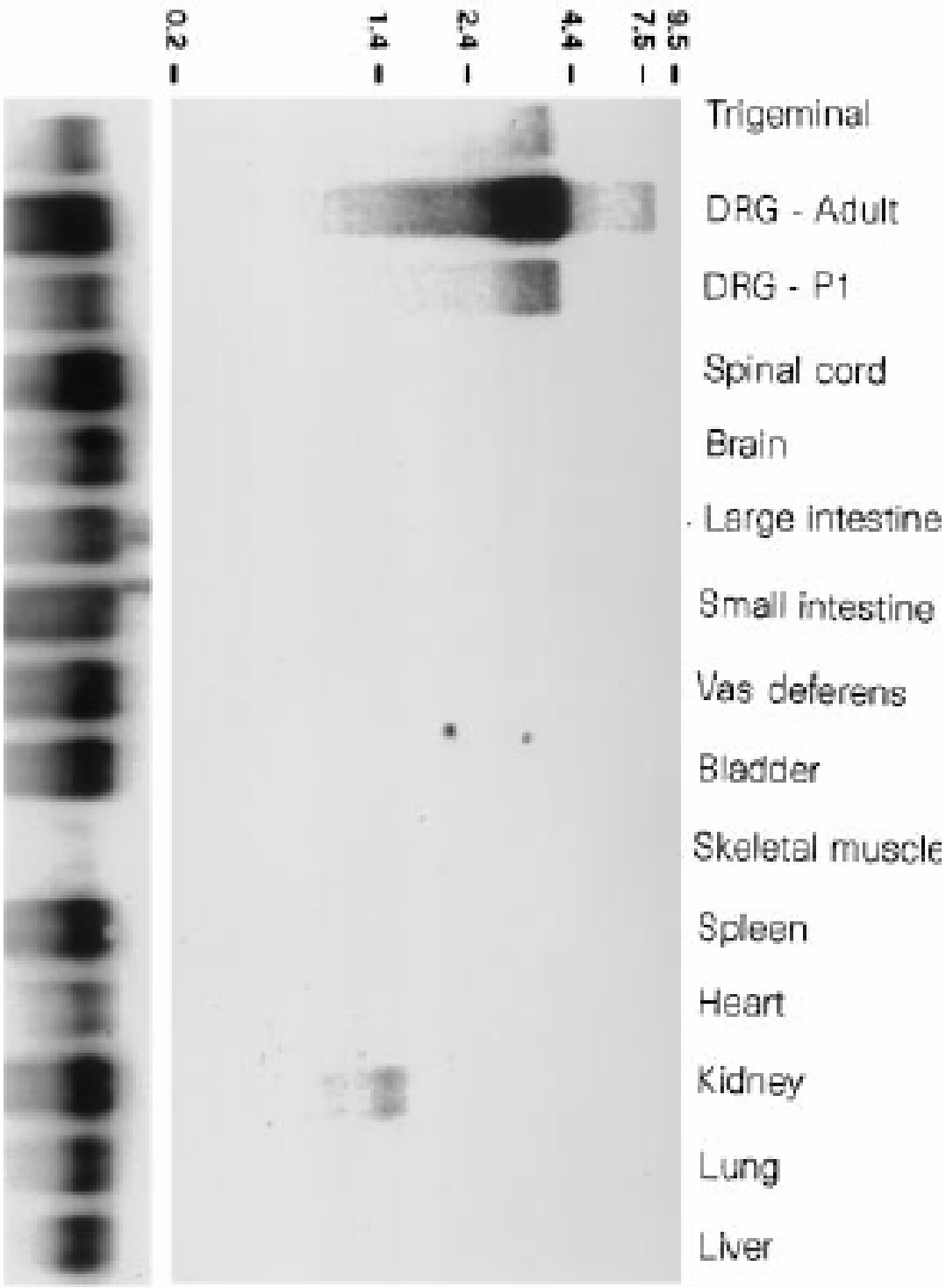
Predicted PKA binding sites

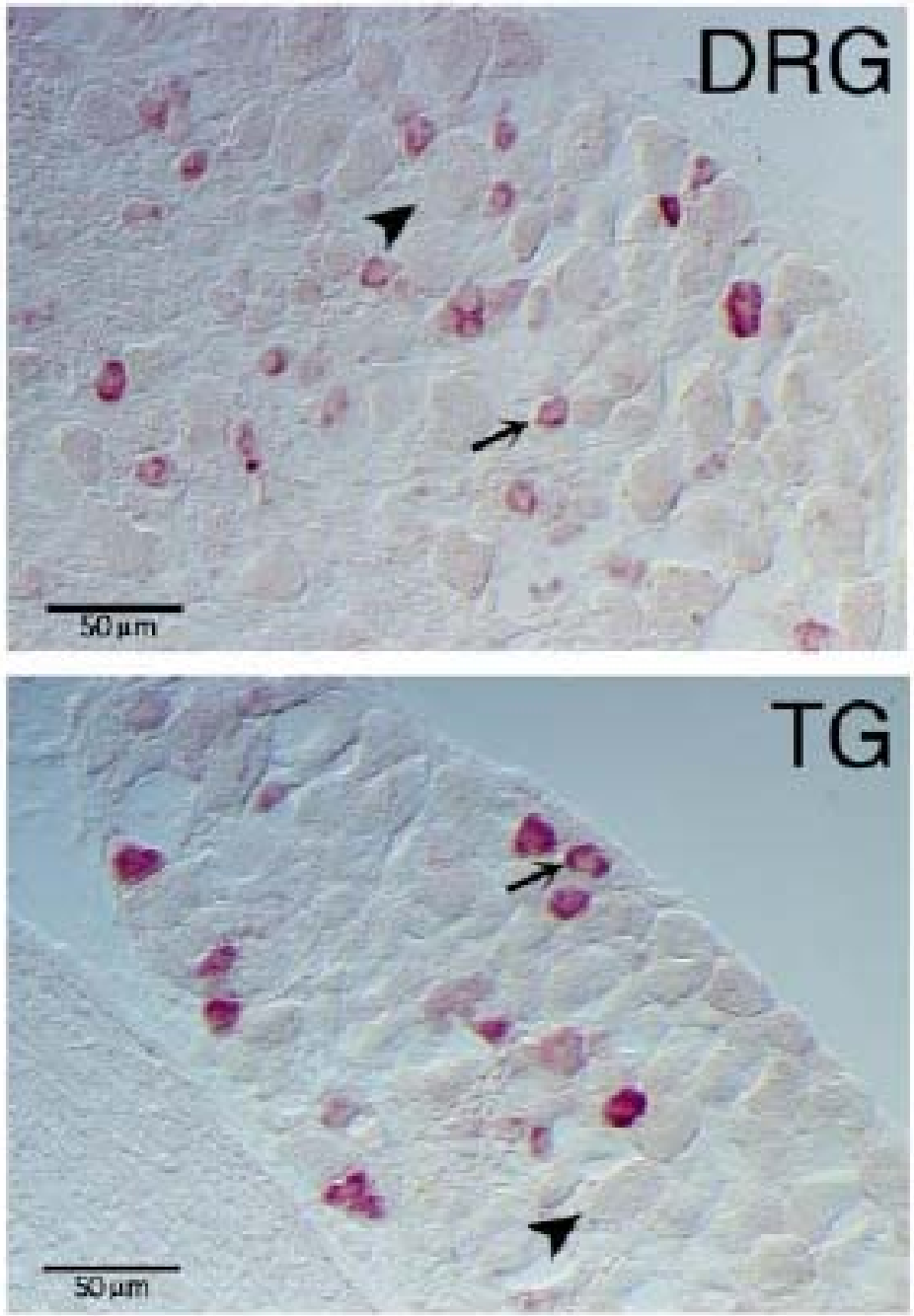
Predicted Structure



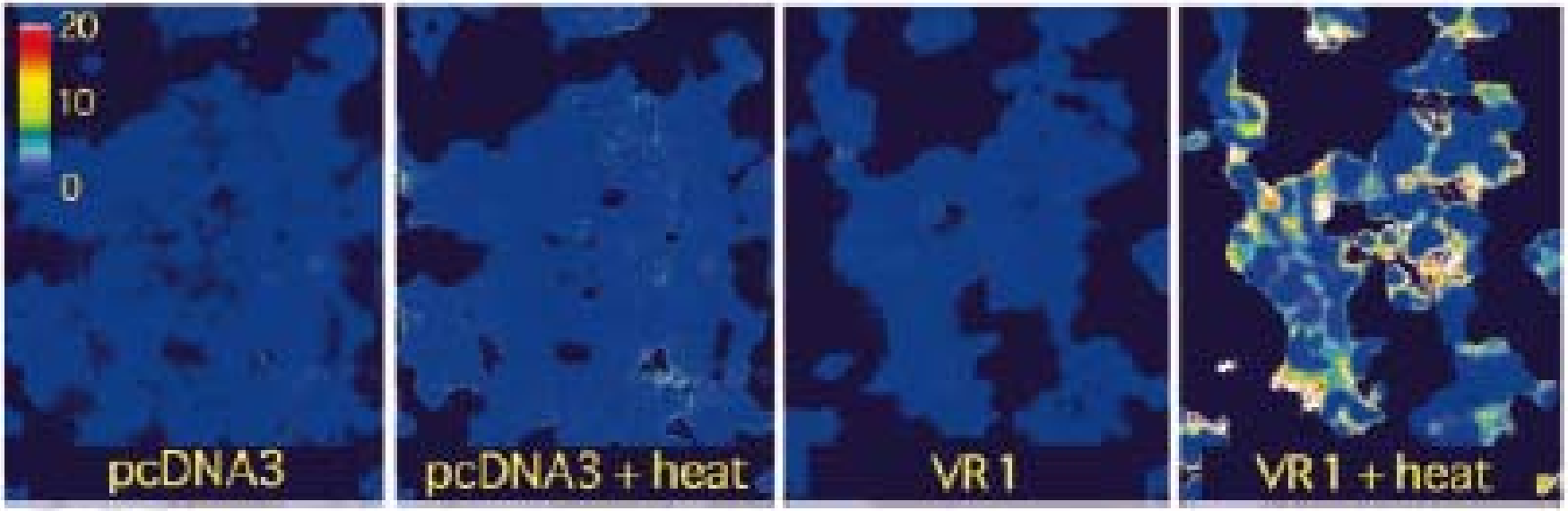
VR1 Resembles Store-Operated Channels



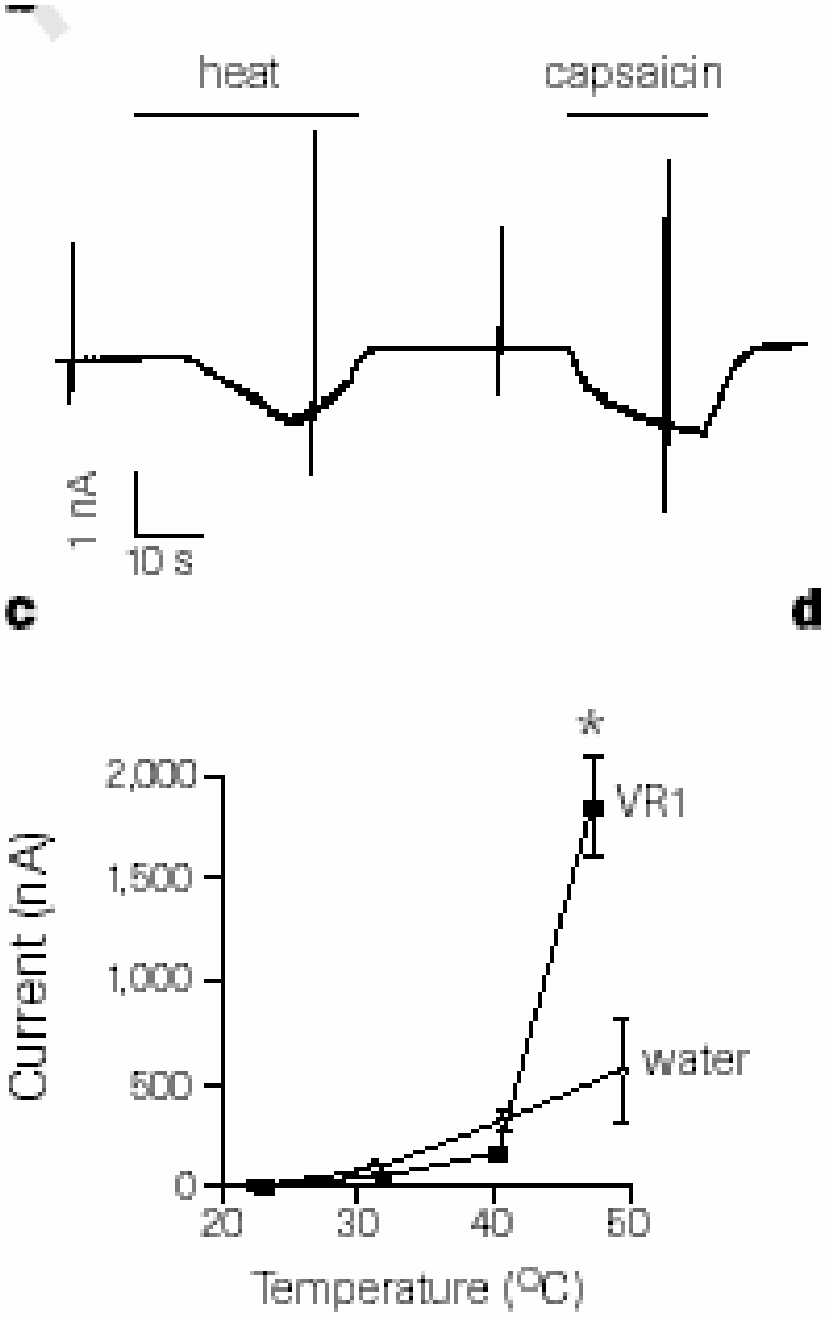

0.2
1.4
2.4
4.4
7.5
9.5
Trigeminal
DRG - Adult
DRG - P1
Spinal cord
Brain
Large intestine
Small intestine
Vas deferens
Bladder
Skeletal muscle
Spleen
Heart
Kidney
Lung
Liver

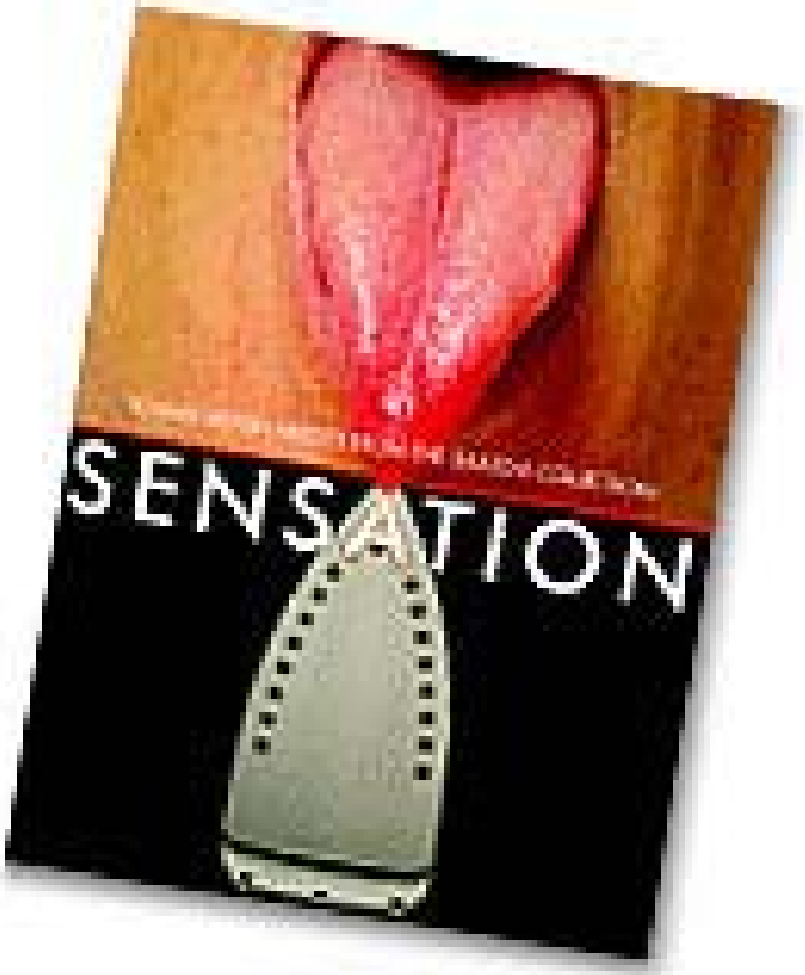

DRG
50 µm
TG
50 µm

Heat also activates our putative receptor!

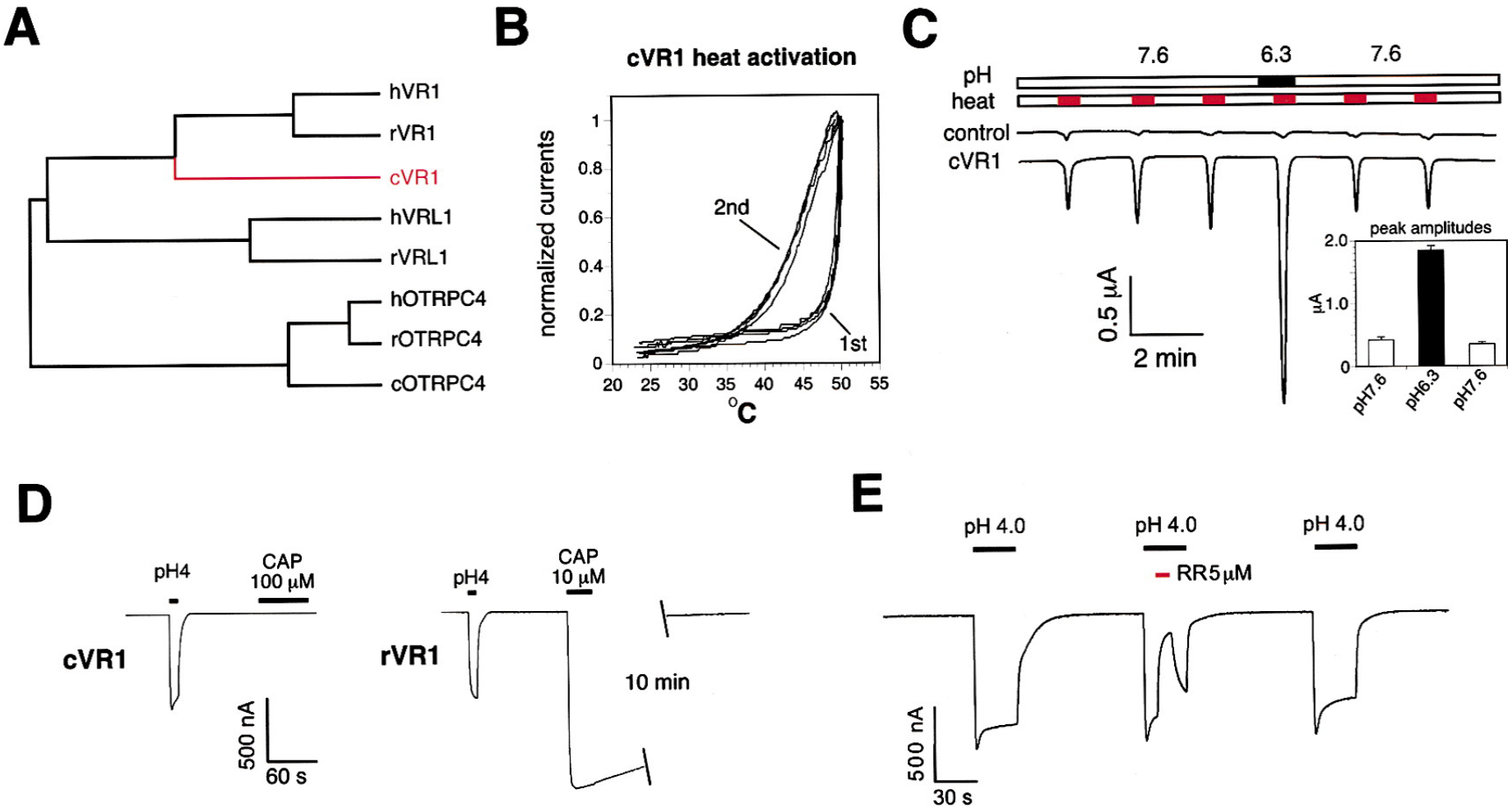


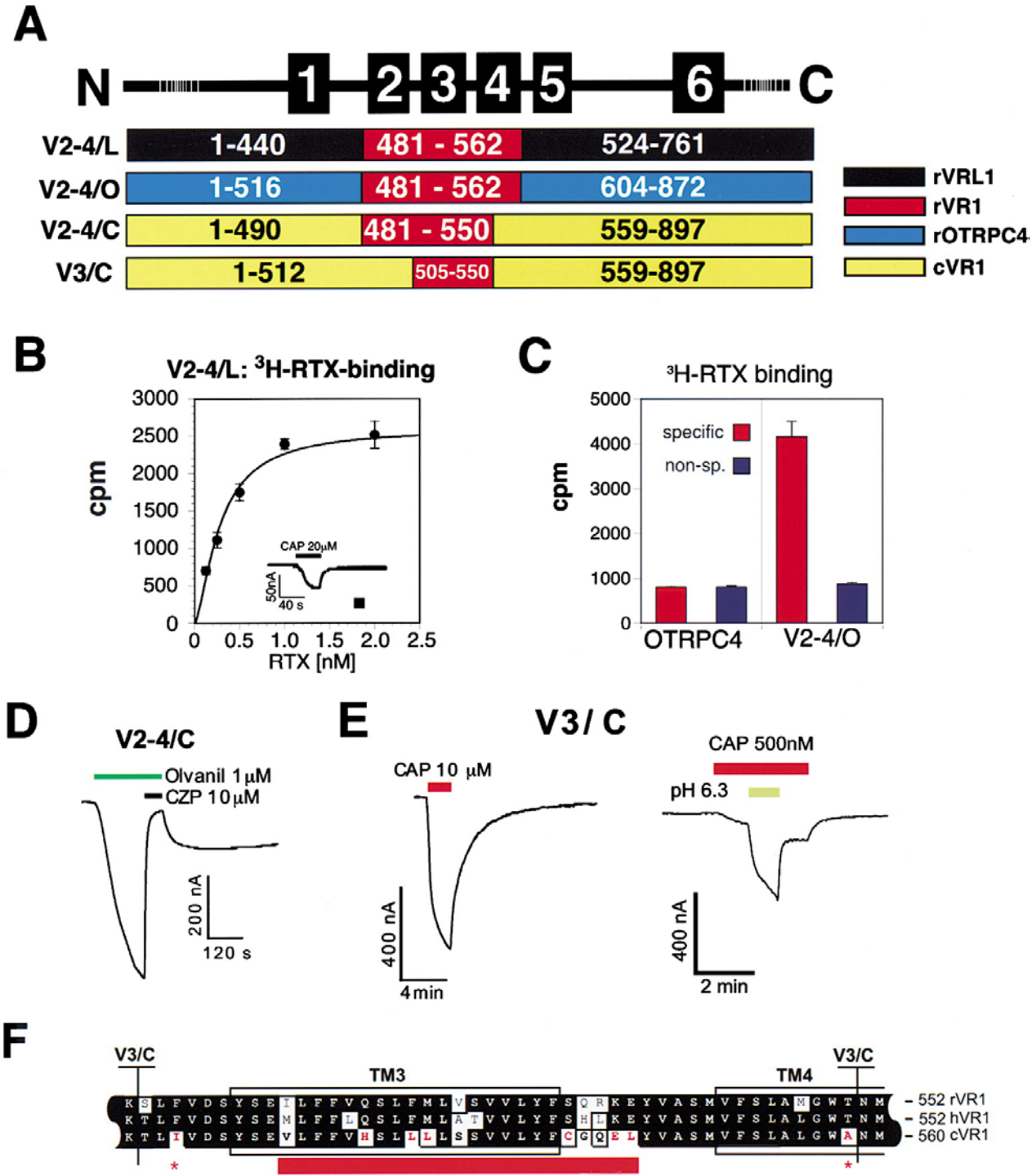
Heat and capsaicin are interchangeable for activating the VR1 receptor

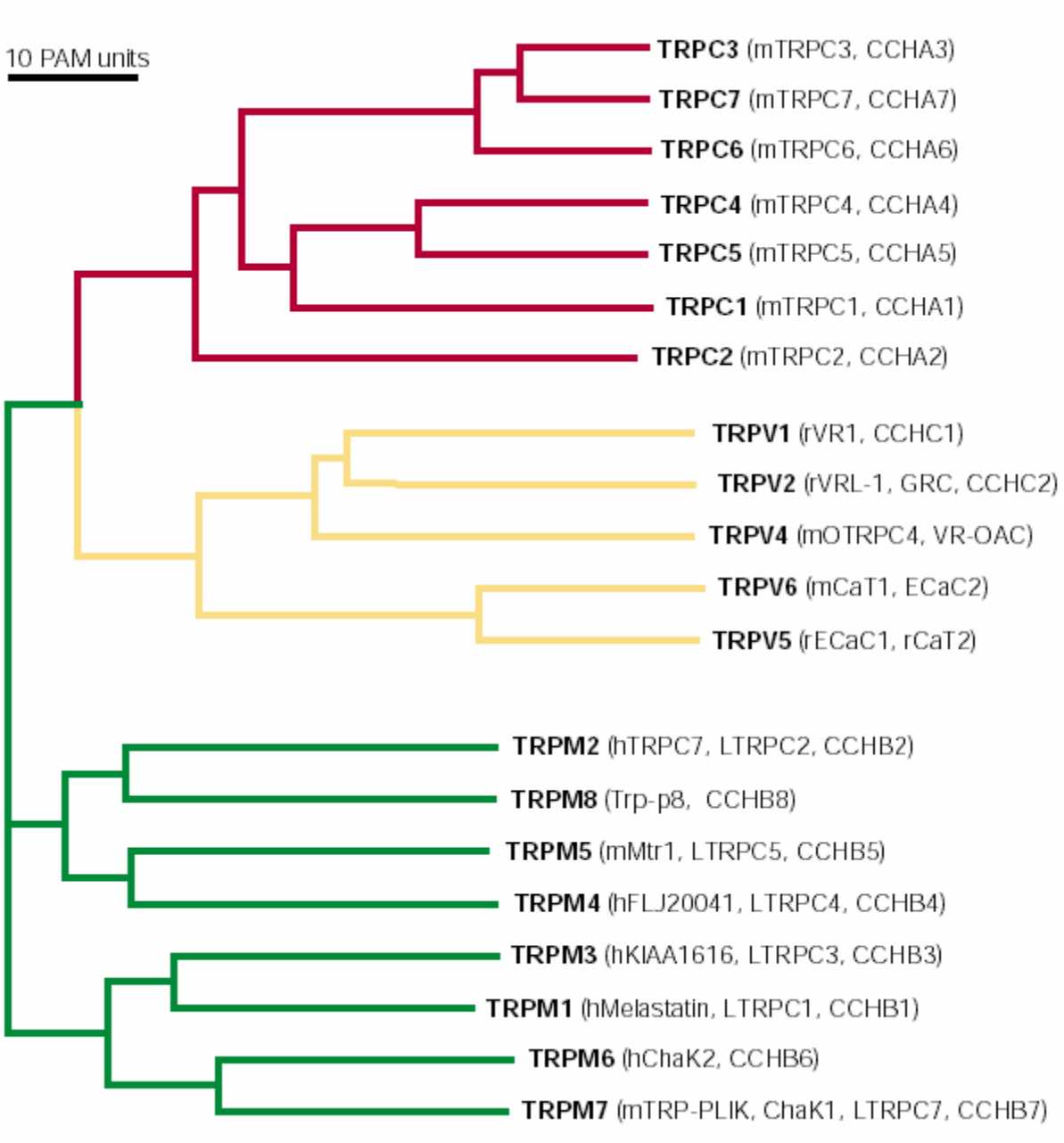



SENSATION

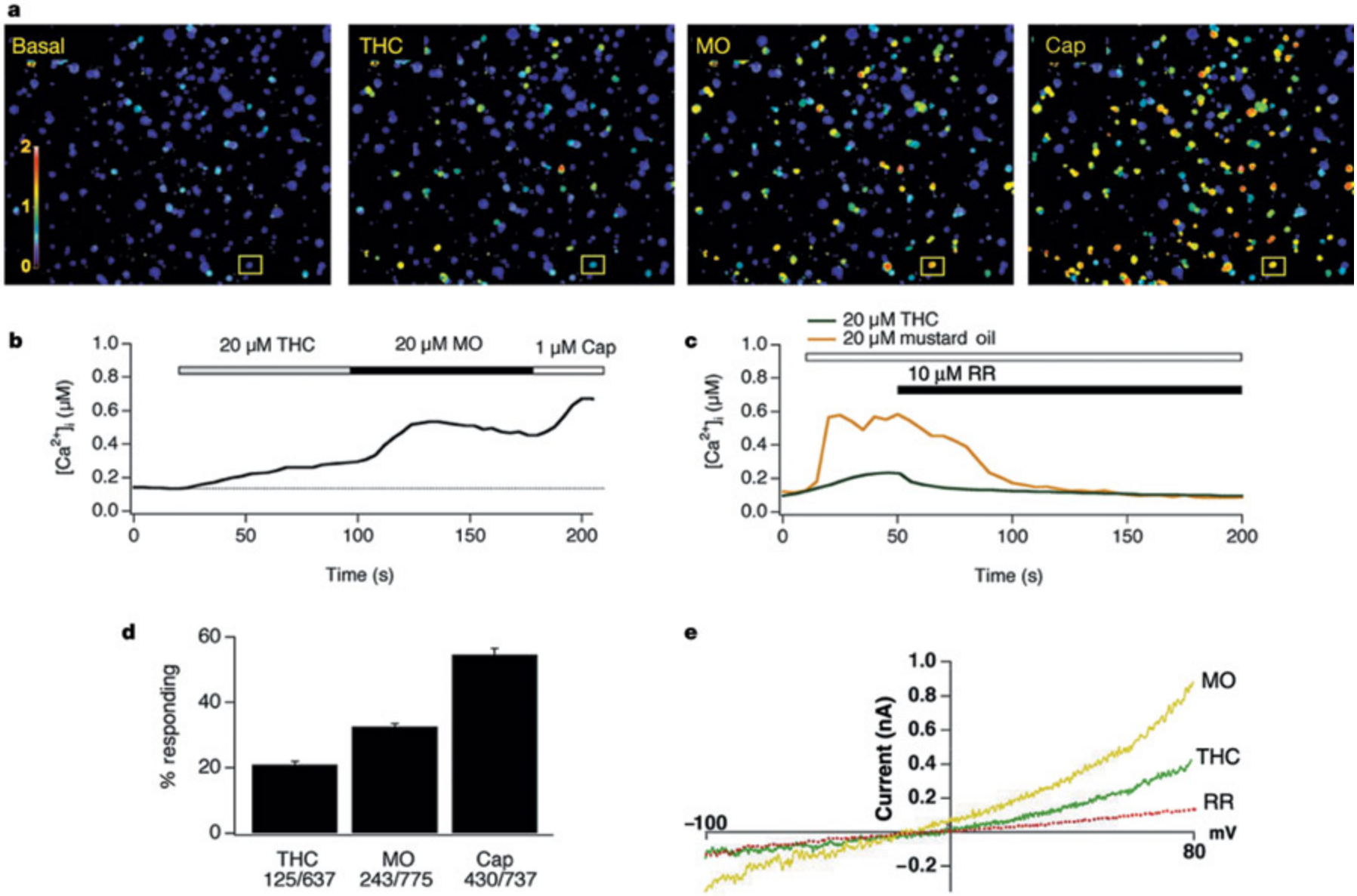

SQUIRRELS
PRIMITIVE MAMMALS
SHREWS
MOLES
OPOSSUM
RABBITS
BATS
GROUND SQUIRRELS
MICE
WOODCHUCK
BEAVER
PORCUPINE
RAT

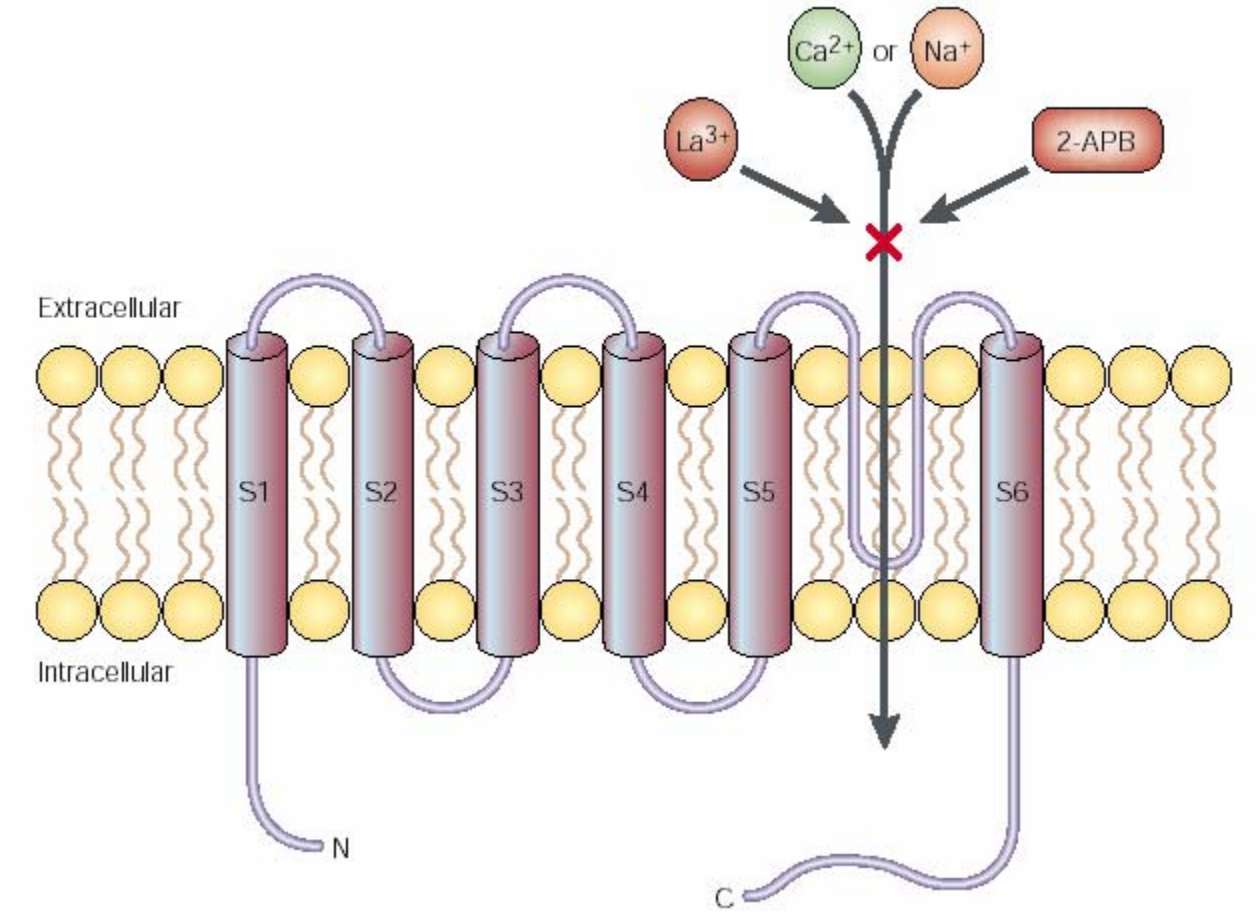

A
hVR1
rVR1
cVR1
hVRL1
rVRL1
hOTRPC4
rOTRPC4
cOTRPC4
B
cVR1 heat activation
normalized currents
2nd
1st
°C
C
7.6
6.3
7.6
pH
heat
control
cVR1
0.5 µA
2 min
peak amplitudes
µA
pH7.6
pH6.3
pH7.6
D
pH4
CAP
100 µM
cVR1
500 nA
60 s
pH4
CAP
10 µM
rVR1
10 min
E
pH 4.0
pH 4.0
pH 4.0
RR5µM
500 nA
30 s


A
N
1
2
3
4
5
6
C
V2-4/L
1-440
481 - 562
524-761
V2-4/O
1-516
481 - 562
604-872
V2-4/C
1-490
481 - 550
559-897
V3/C
1-512
505-550
559-897
rVRL1
rVR1
rOTRPC4
cVR1
B
V2-4/L: 3H-RTX-binding
cpm
RTX [nM]
CAP 20µM
50nA
40 s
C
3H-RTX binding
specific
non-sp.
OTRPC4
V2-4/O
D
V2-4/C
Olvanil 1µM
CZP 10µM
200 nA
120 s
E
V3/ C
CAP 10 µM
400 nA
4 min
CAP 500nM
pH 6.3
400 nA
2 min
F
V3/C
TM3
TM4
V3/C
552 rVR1
552 hVR1
560 cVR1

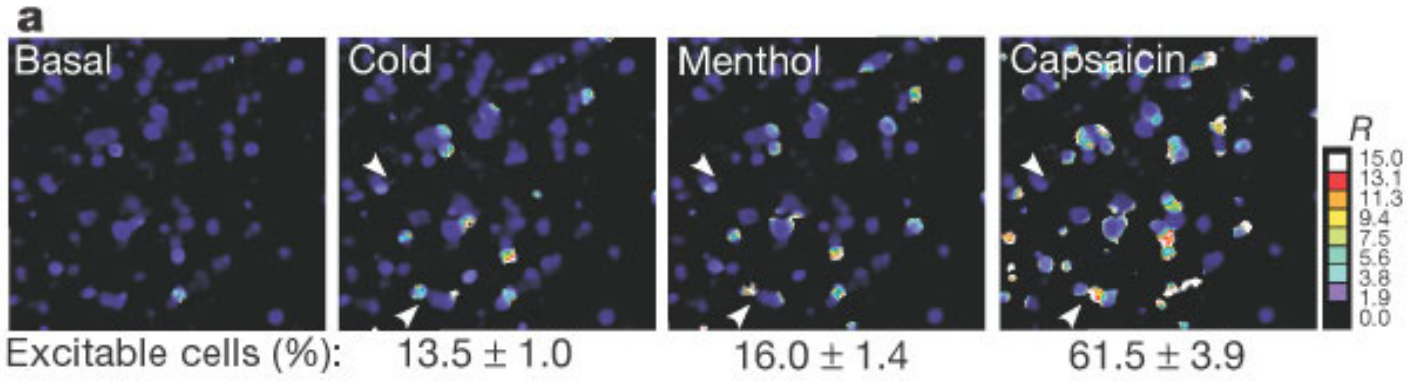

10 PAM units
TRPC3 (mTRPC3, CCHA3)
TRPC7 (mTRPC7, CCHA7)
TRPC6 (mTRPC6, CCHA6)
TRPC4 (mTRPC4, CCHA4)
TRPC5 (mTRPC5, CCHA5)
TRPC1 (mTRPC1, CCHA1)
TRPC2 (mTRPC2, CCHA2)
TRPV1 (rVR1, CCHC1)
TRPV2 (rVRL-1, GRC, CCHC2)
TRPV4 (mOTRPC4, VR-OAC)
TRPV6 (mCaT1, ECaC2)
TRPV5 (rECaC1, rCaT2)
TRPM2 (hTRPC7, LTRPC2, CCHB2)
TRPM8 (Trp-p8, CCHB8)
TRPM5 (mMtr1, LTRPC5, CCHB5)
TRPM4 (hFLJ20041, LTRPC4, CCHB4)
TRPM3 (hKIAA1616, LTRPC3, CCHB3)
TRPM1 (hMelastatin, LTRPC1, CCHB1)
TRPM6 (hChaK2, CCHB6)
TRPM7 (mTRP-PLIK, ChaK1, LTRPC7, CCHB7)

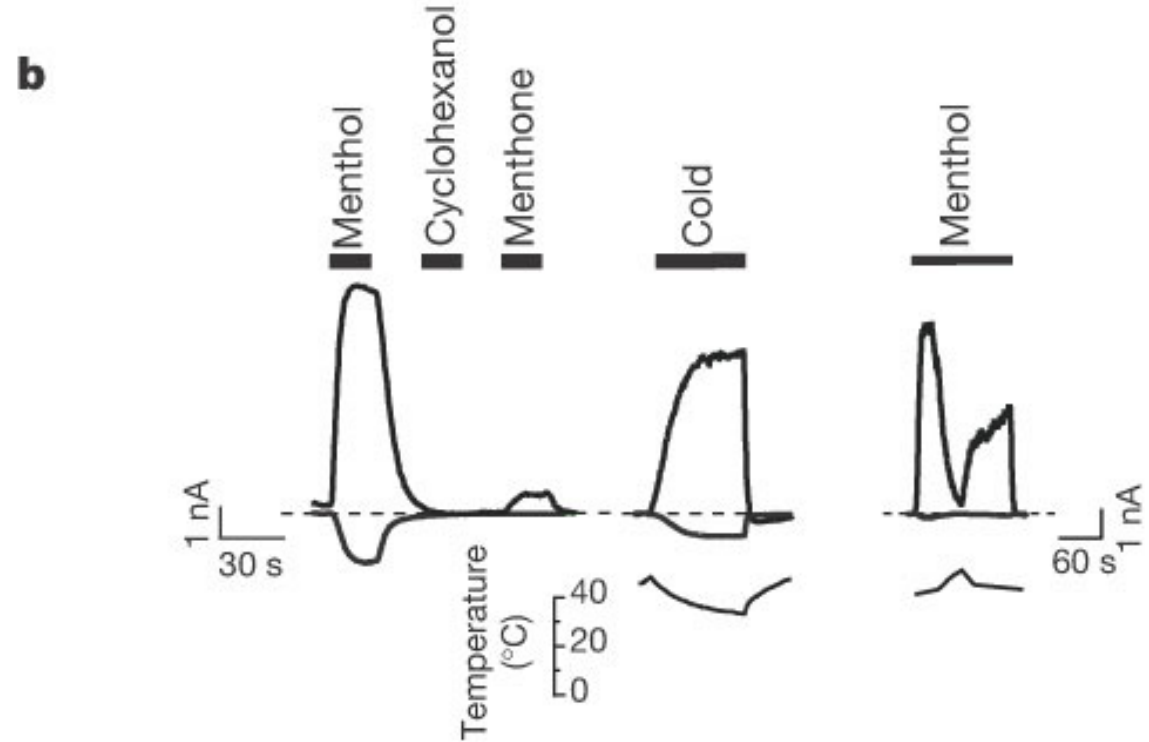
Trp => Mustard Oil, THC



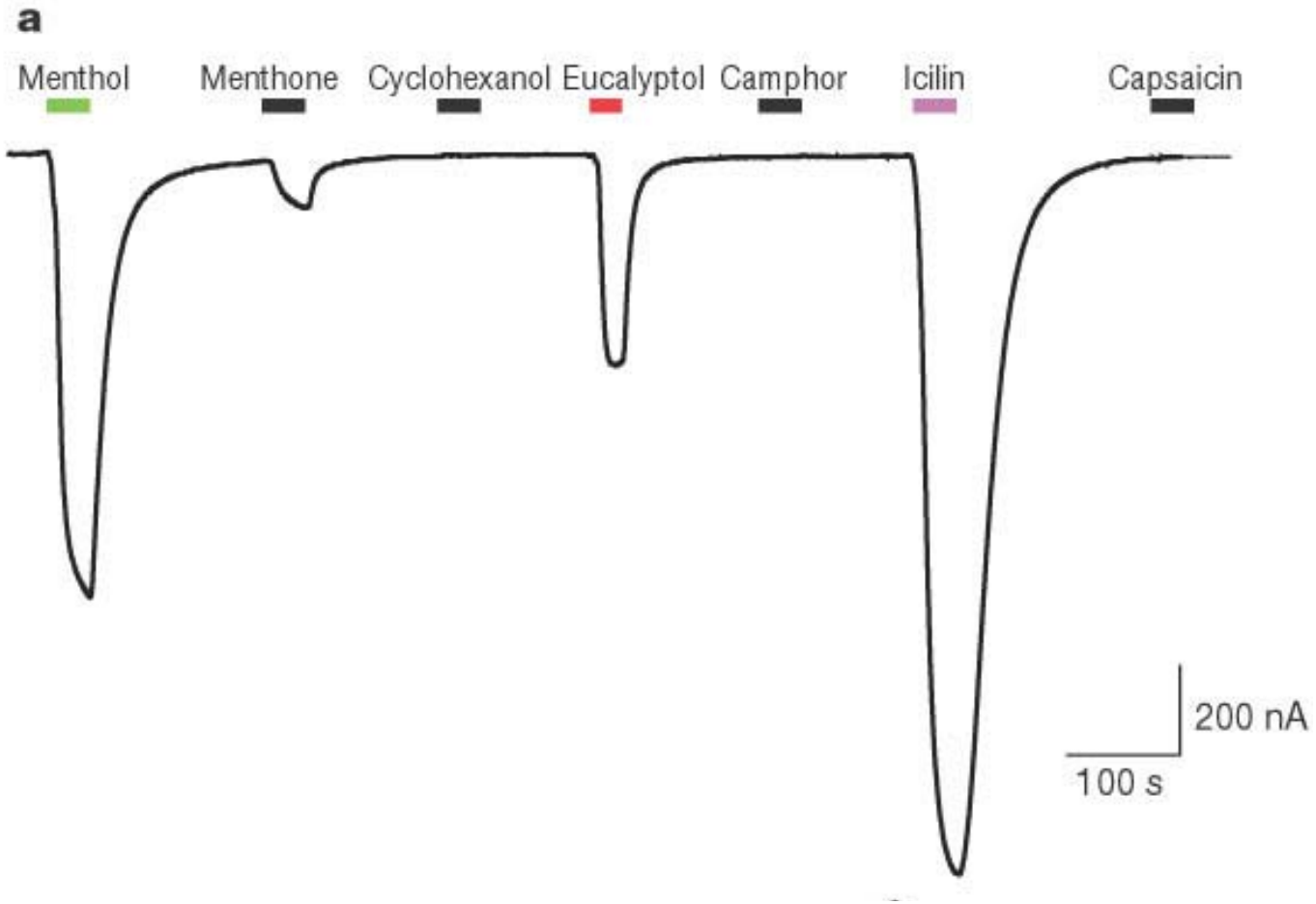

Ca^{2+}
or
Na^{+}
La^{3+}
2-APB
Extracellular
S1
S2
S3
S4
S5
S6
Intracellular
N
C

1. Calcium Imaging / Functional Cloning

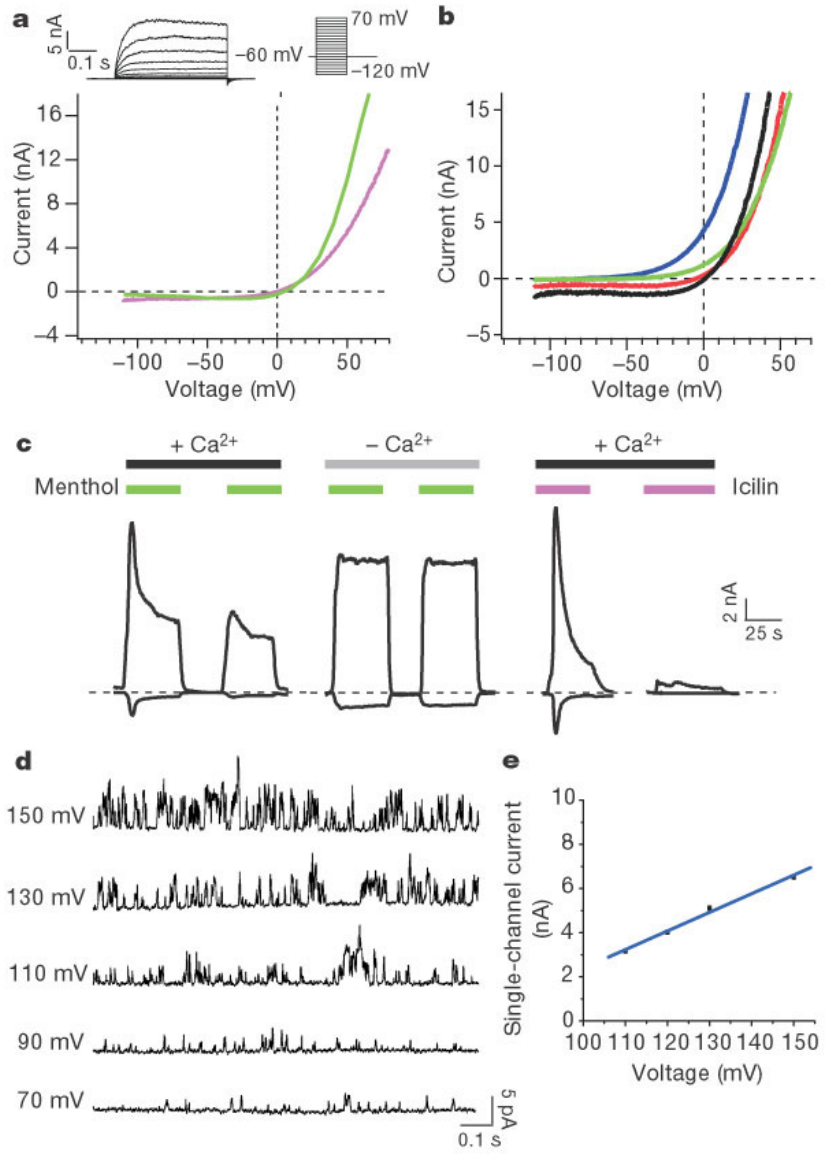




2. Recapitulate electrophys. sensitivity



3. Electrophys: Currents Same



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DAFGDIQFETLGKKGKYLRLSCDTDSETLYELLTQHWHLKTPNLVISVTG 150
GAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNT 200
ISRNSEENIVAIGIAAWGMVSNRDTLIRNCDDEGHFSAQYIMDDFMRDPL 250
YILDNNHTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTSQDSNYGGKIP 300
IVCFAQGGGRETLKAINTSVKSKIPCVVVEGSGQIADVIASLVEVEDVLT 350
SSMVKEKLVRFLPRTVSRLPEEEIESWIKWLKEILESPHLLTVIKMEEAG 400
DEVVSSAISYALYKAFSTNEQDKDNWNGQLKLLLEWNQLDLASDEIFTHD 450
RRWESADLQEVMFTALIKDRPKFVRLFLENGLNLQKFLTNEVLTELFSTH 500
FSTLVYRNLQIAKNSYNDALLTFVWKLVANFRRSFWKEDRSSREDLDVEL 550
HDASLTTRHPLQALFIWAILQNKKELSKVIWEQTKGCTLAALGASKLLKT 600
LAKVKNDINAAGESEELANEYETRAVELFTECYSSDEDLAEQLLVYSCEA 650

                                                  S1
WGGSNCLELAVEATDQHFIAQPGVQNFLSKQWYGEISRDTKNWKIILCLF 700
                                        S2
IIPLVGCGLVSFRKKPIDKHKKLLWYYVAFFTSPFVVFSWNVVFYIAFLL 750

LFAYVLLMDFHSVPHTPELILYALVFVLFCDEVRQWYMNGVNYFTDLWNV 800
          S3                              S4
MDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIFTLRLIHIFTVS 850
                        S5
RNLGPKIIMLQRMLIDVFFFLFLFAVWMVAFGVARQGILRQNEQRWRWIF 900

RSVIYEPYLAMFGQVPSDVDSTTYDFSHCTFSGNESKPLCVELDEYNLPR 950
          S6
FPEWITIPLVCIYMLSTNILLVNLLVAMFGYTVGIVQENNDQVWKFQRYF 1,000

LVQEYCNRLNIPFPFVVFAYFYMVVKKCFKCCCKEKNTESSACCFRNEDN 1,050
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NKIK                                                1,104
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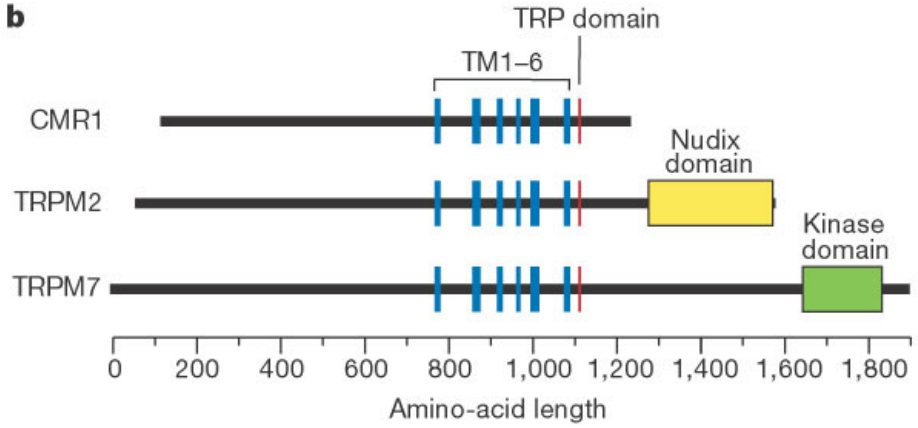
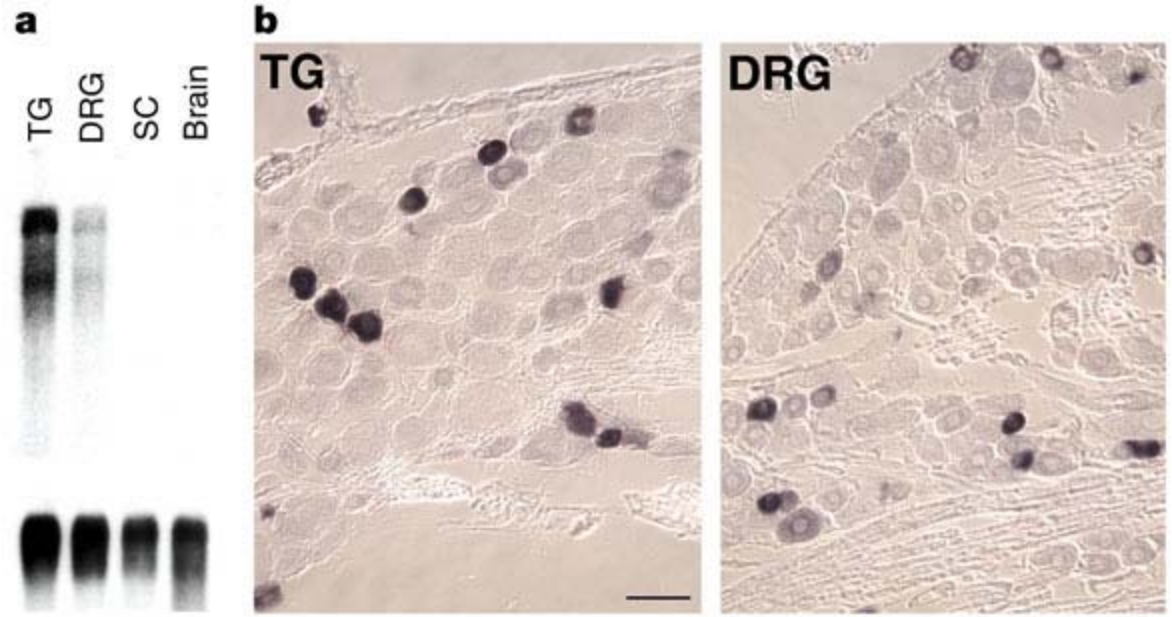
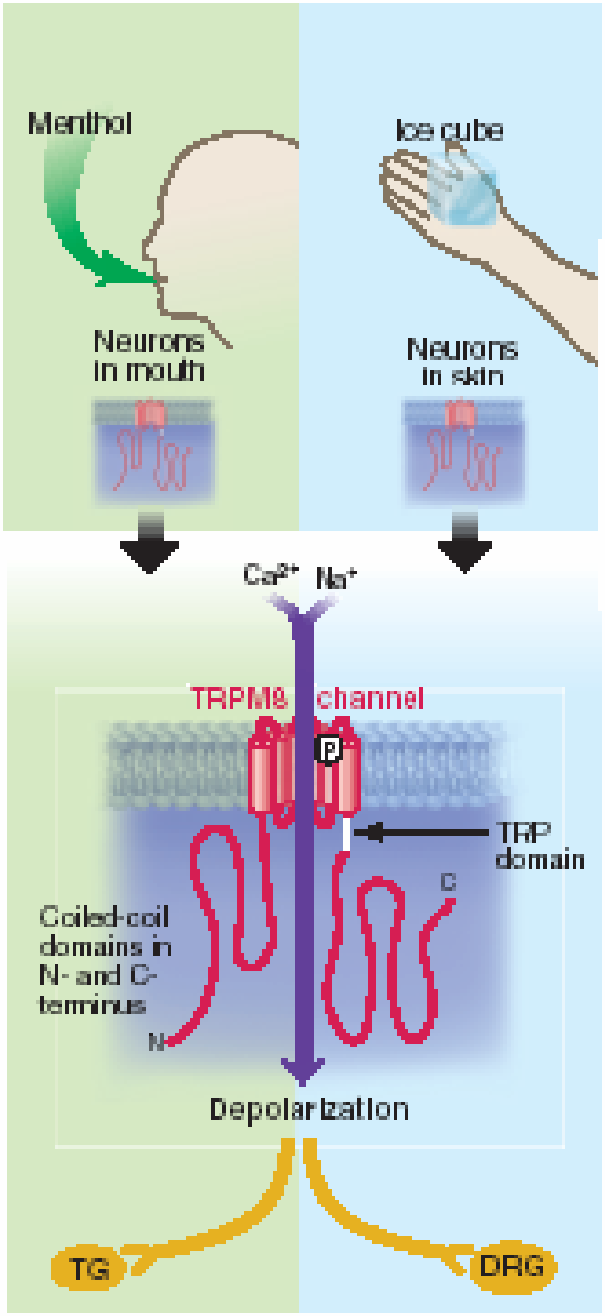
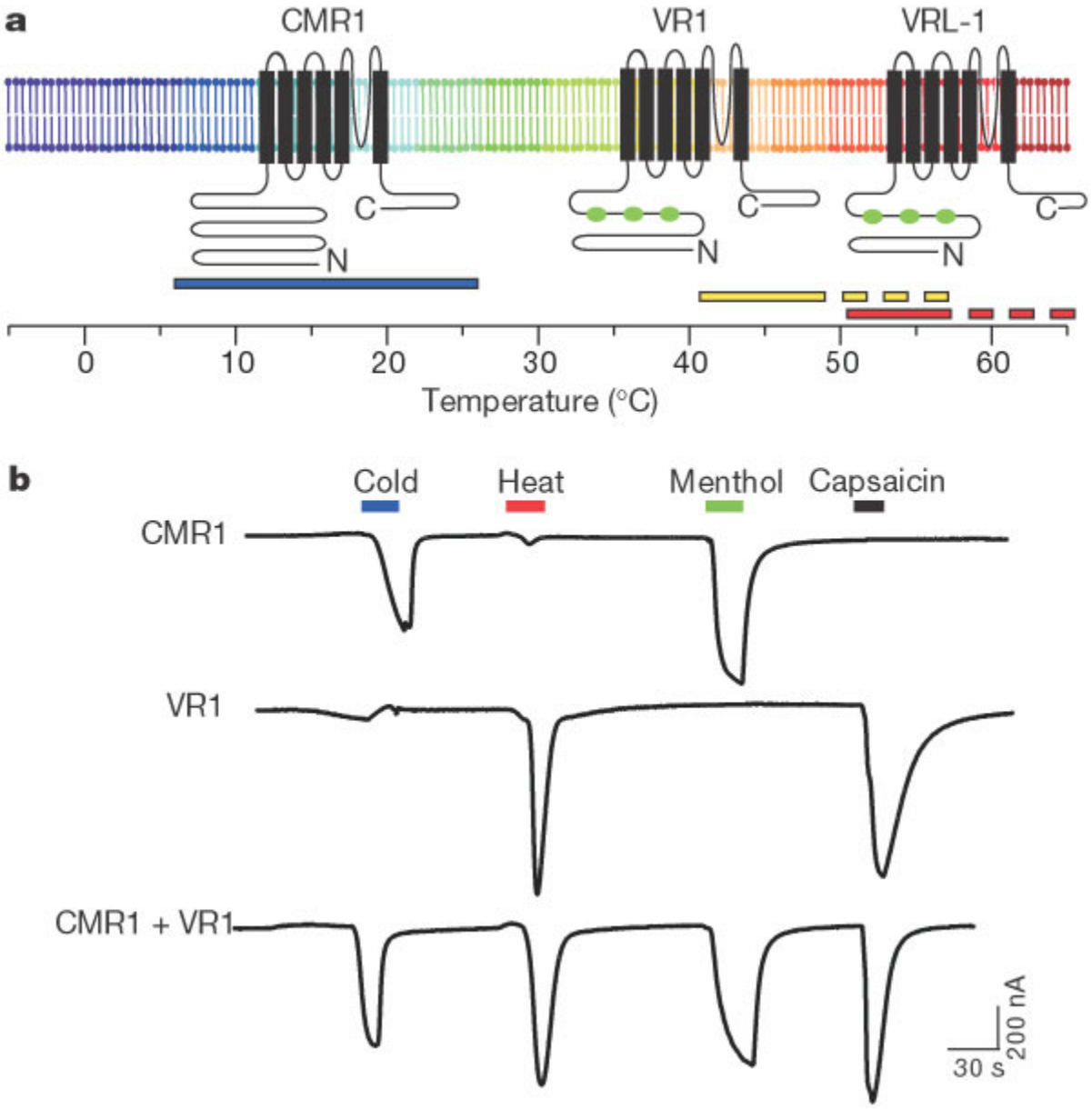
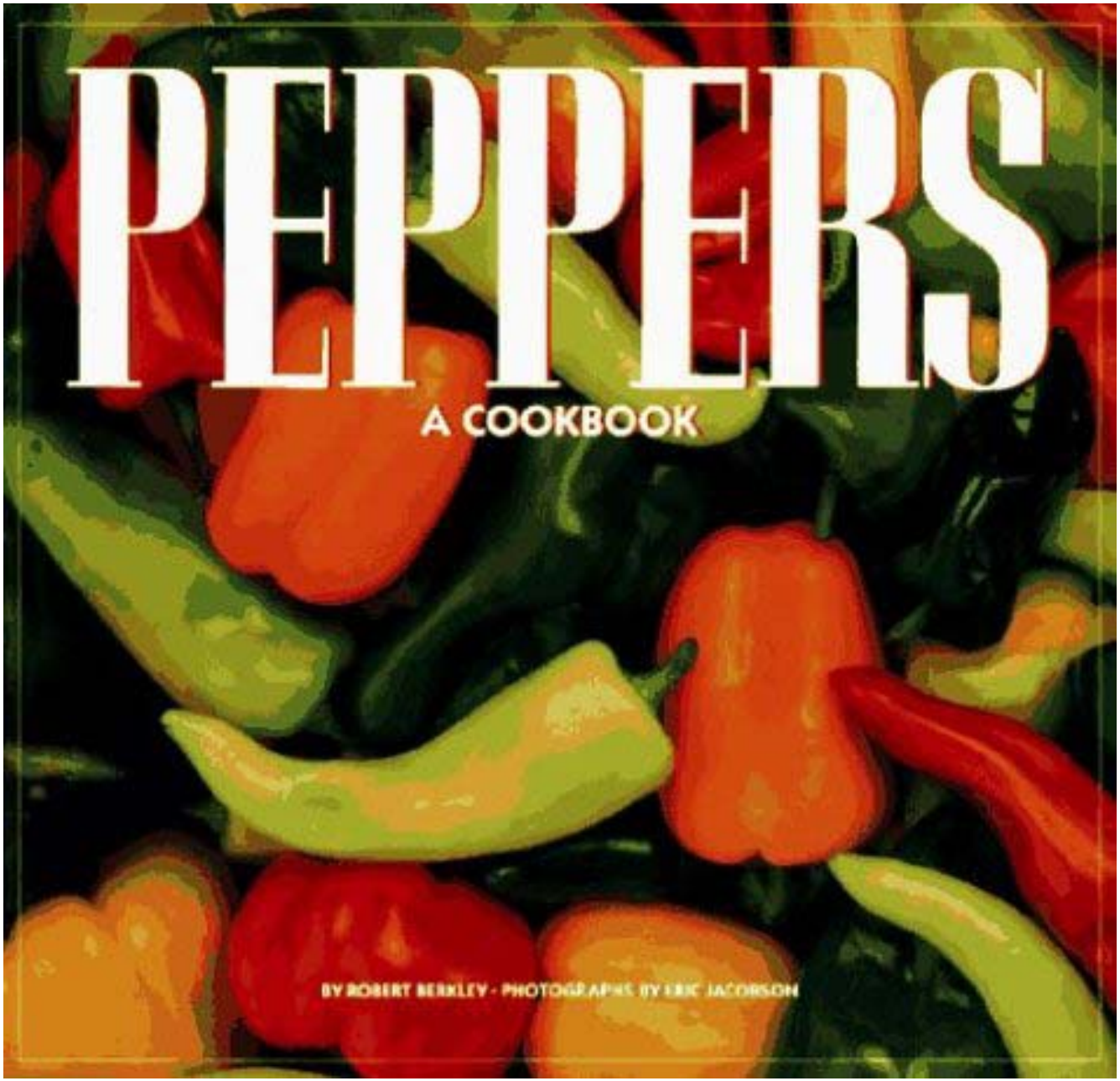


Right Place, Makes Sense




Menthol
Ice cube
Neurons in mouth
Neurons in skin
Ca^{2+} Na^{+}
TRPM8 channel
P
TRP domain
C
Coiled-coil domains in N- and C-terminus
N
Depolarization
TG
DRG


a
CMR1
VR1
VRL-1
C
N
C
N
C
N
0
10
20
30
40
50
60
Temperature (°C)
b
Cold
Heat
Menthol
Capsaicin
CMR1
VR1
CMR1 + VR1
30 s
200 nA


PEPPERS
A COOKBOOK
BY ROBERT BERKLEY · PHOTOGRAPHS BY ERIC JACOBSON