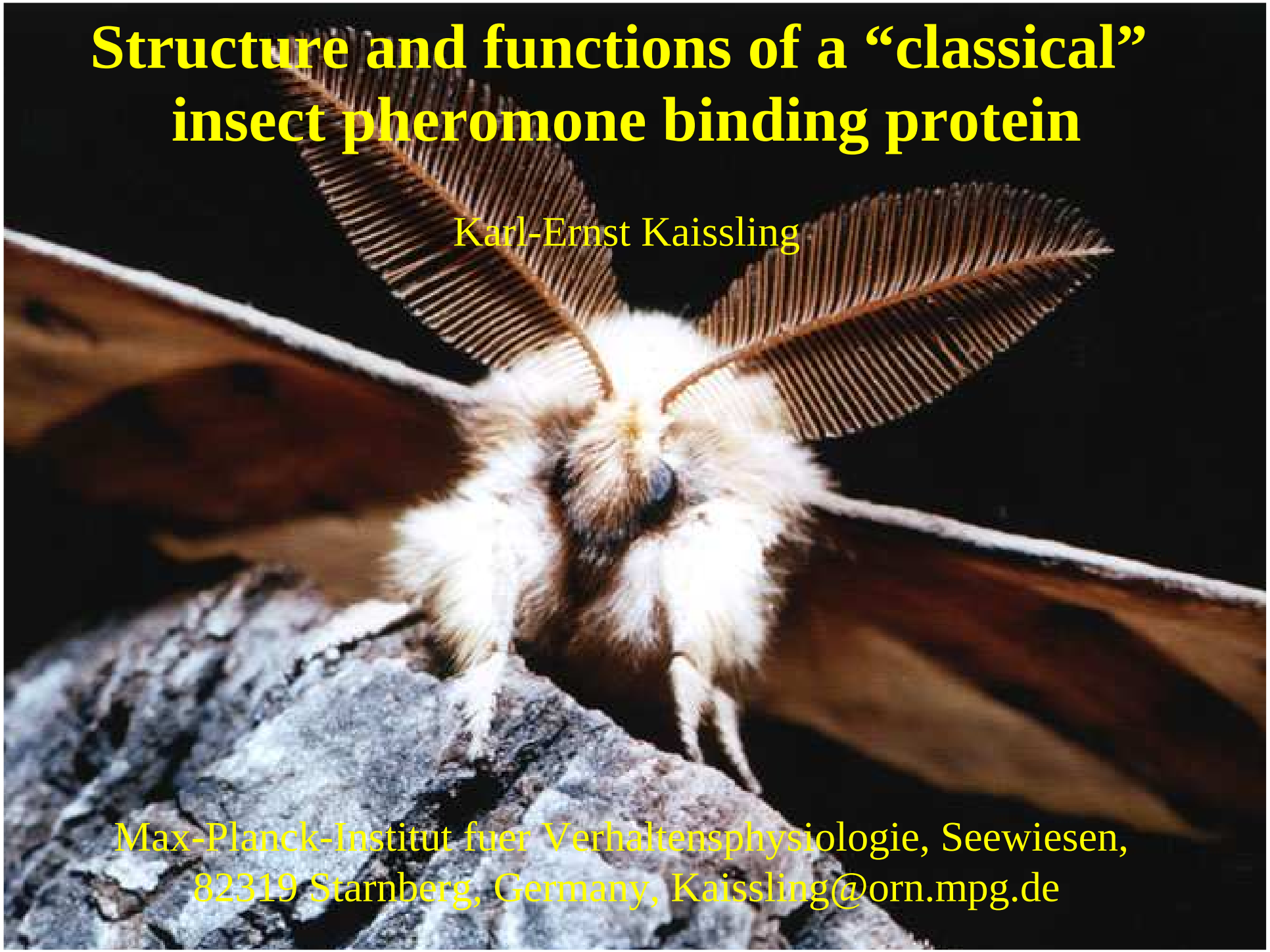
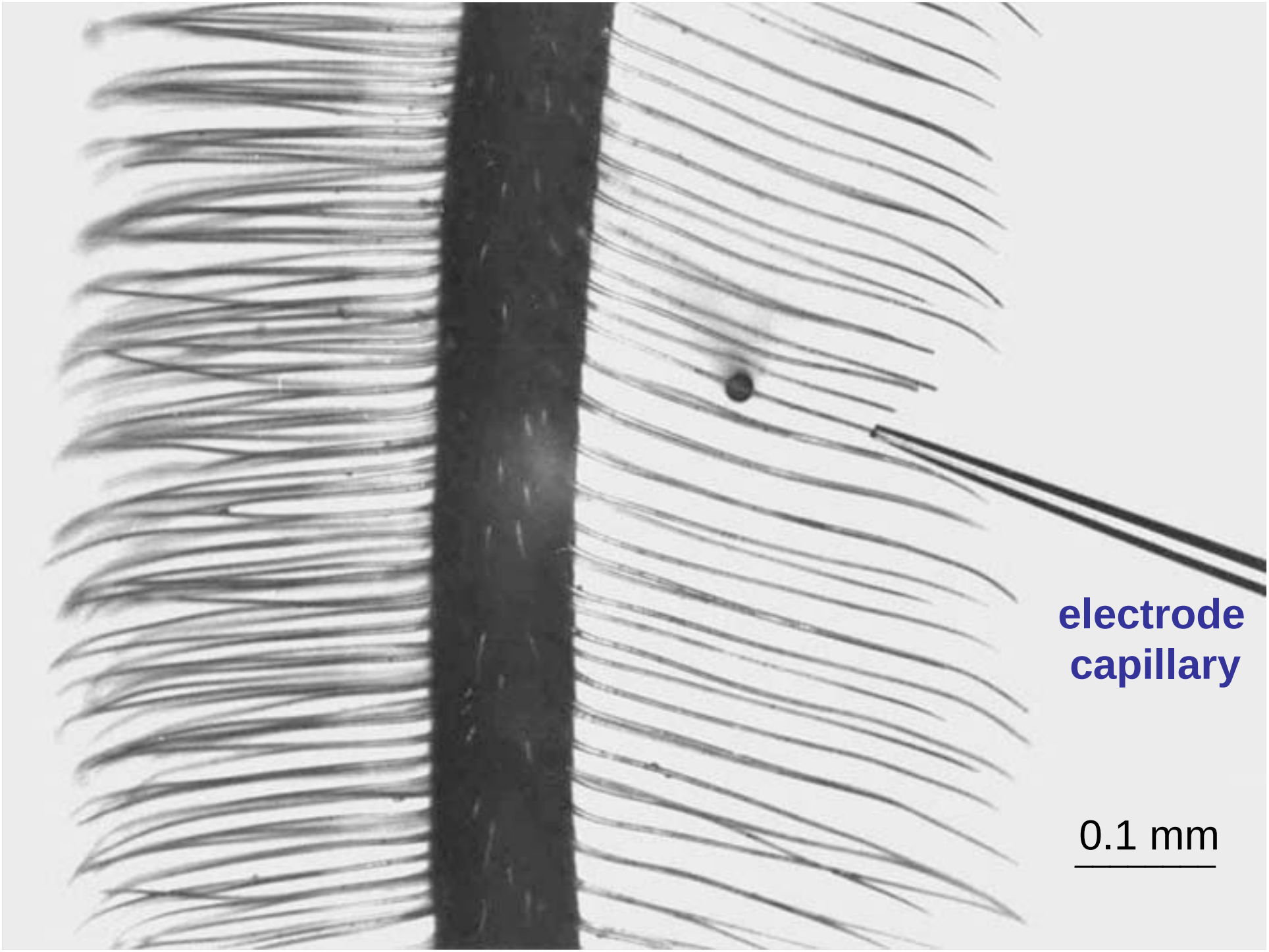
Structure and functions of a “classical” insect pheromone binding protein



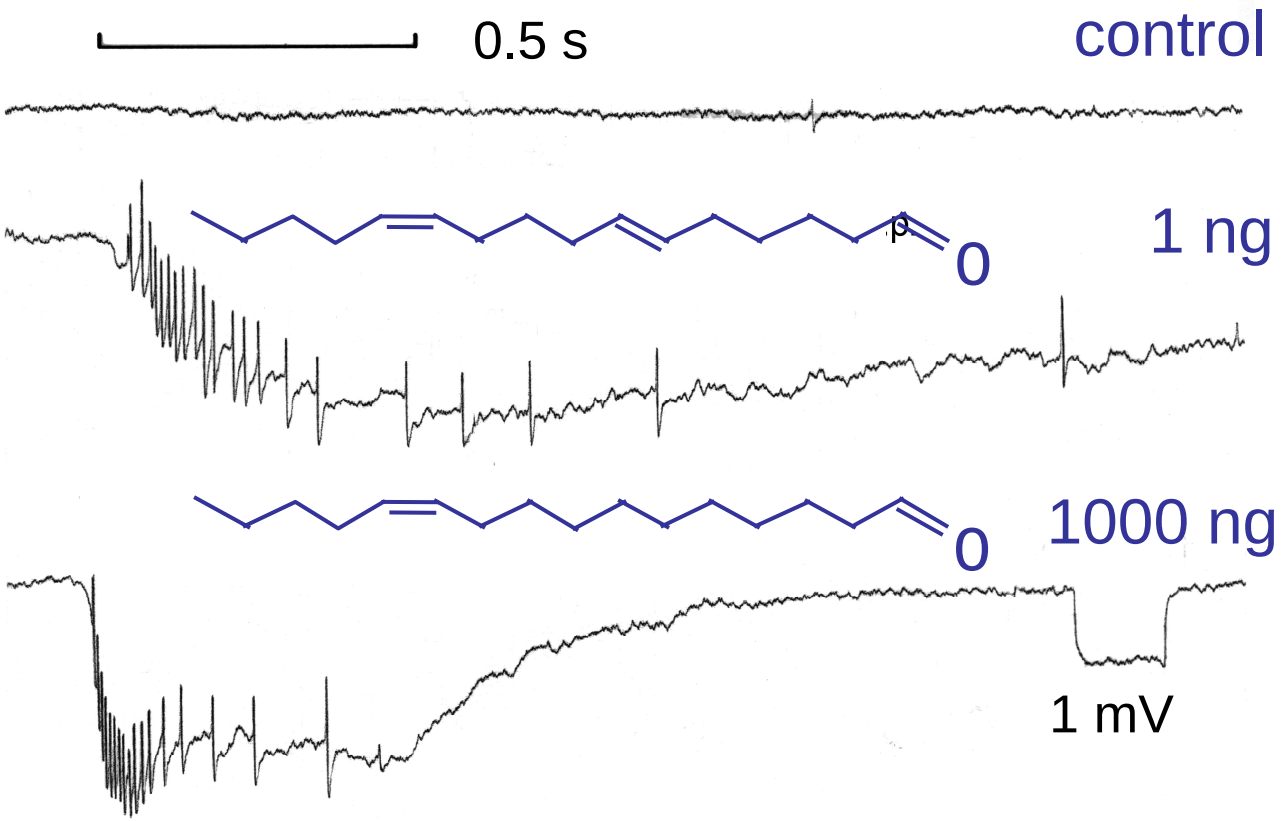
Karl-Ernst Kaissling

Max-Planck-Institut fuer Verhaltensphysiologie, Seewiesen, 82319 Starnberg, Germany, Kaissling@orn.mpg.de

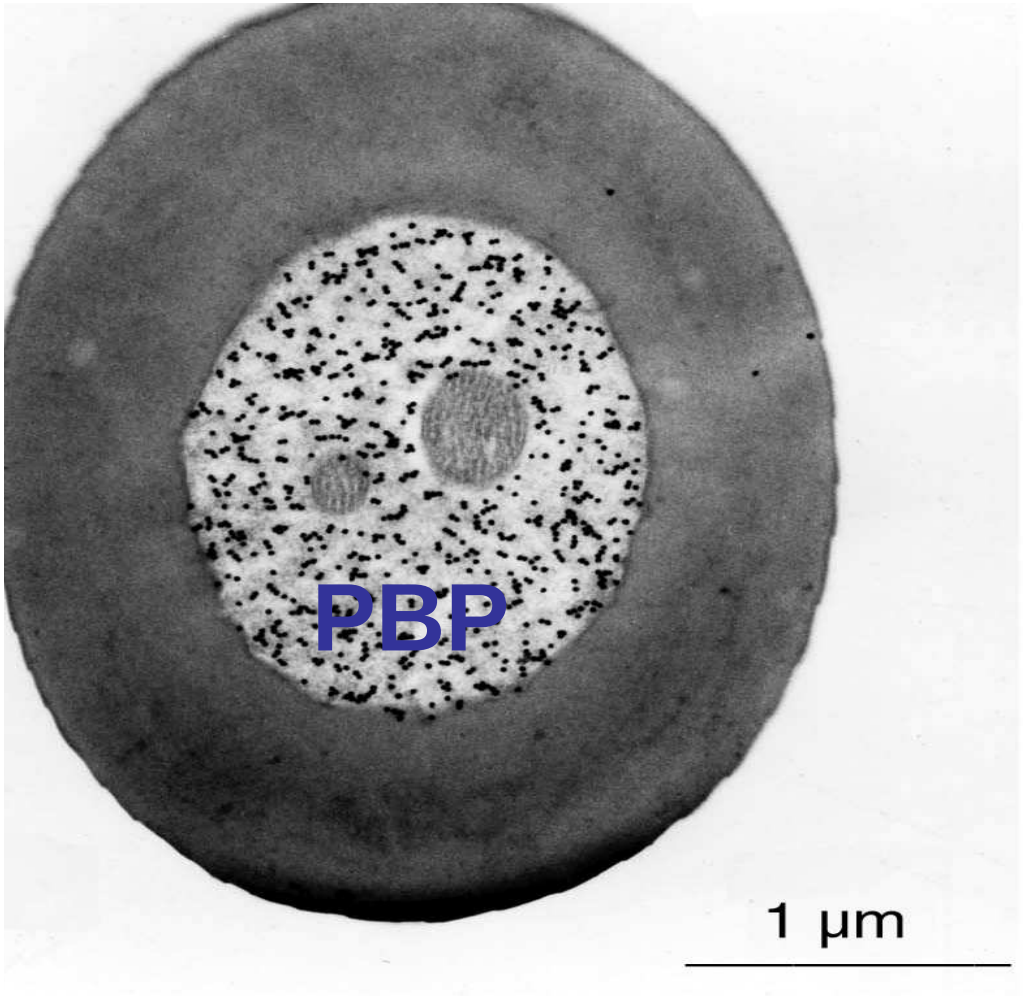

E. Priesner

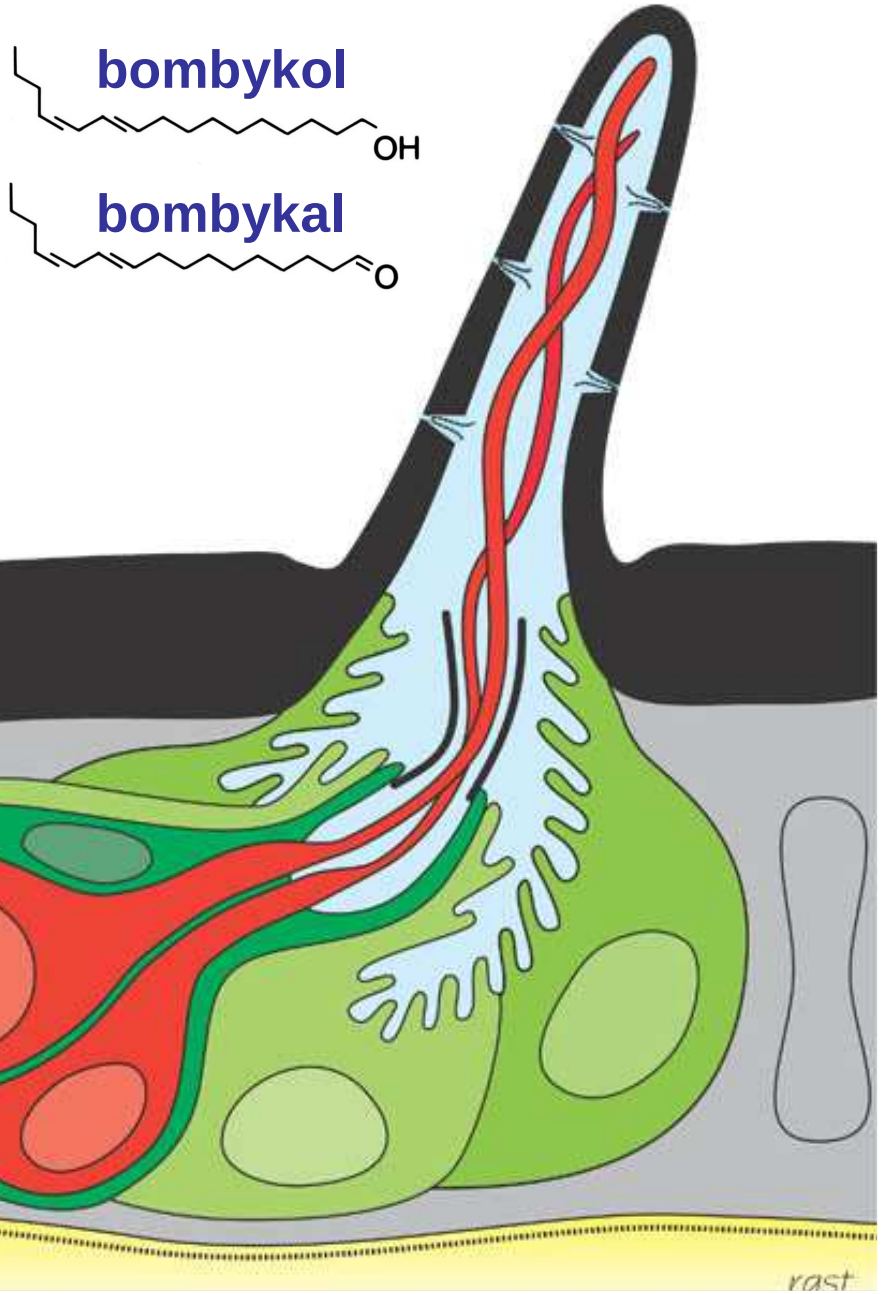

electrode
capillary
0.1 mm

Antheraea pernyi



Kaissling 1977





Steinbrecht et al., 1995

Possible functions of the
pheromone binding protein (PBP)

1 **solubilizes** the pheromone (Van den Berg, Ziegelberger)

a **transports** it through the sensillum lymph (**carrier**)

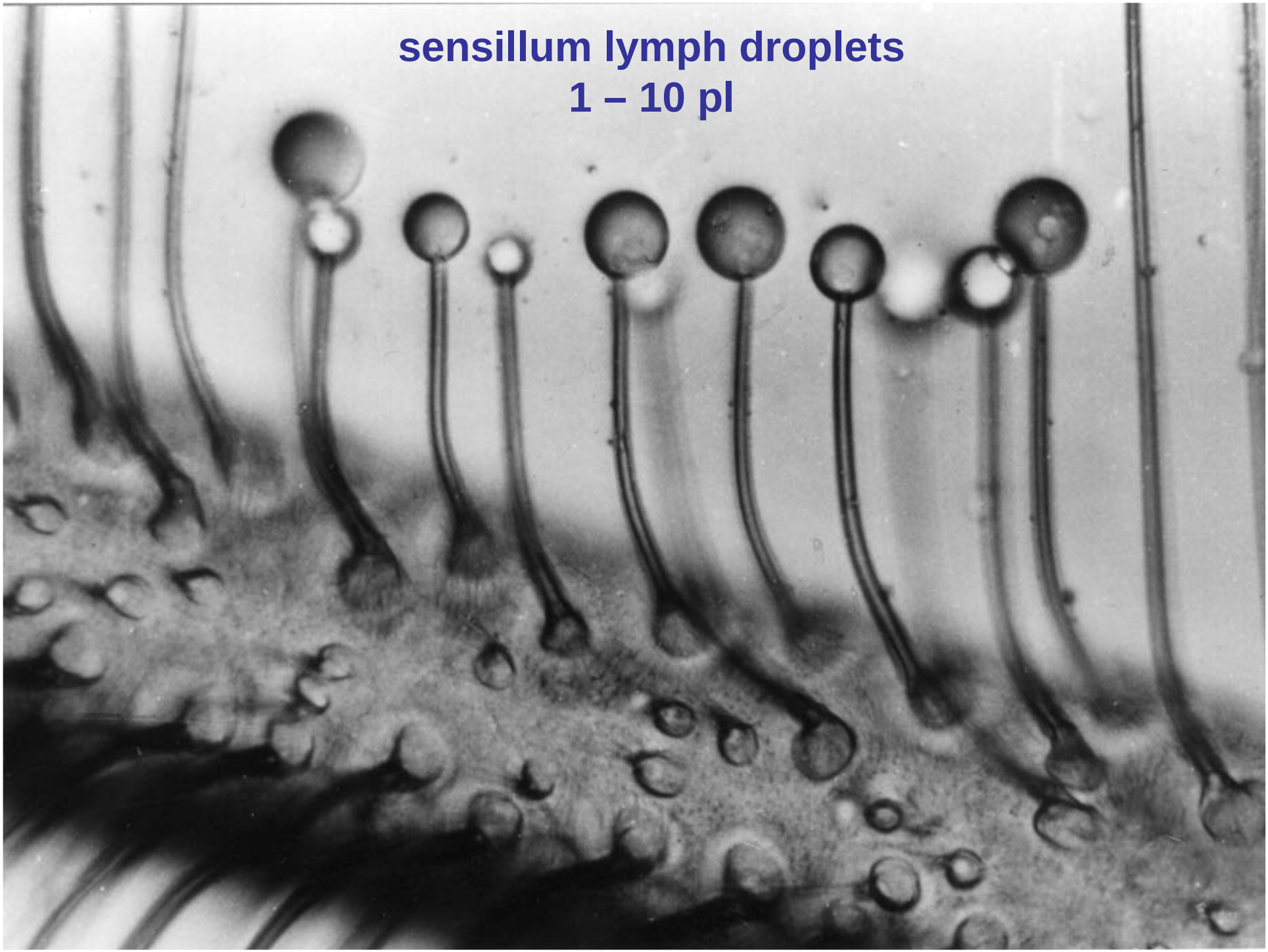
b **prevents** it from entering the cell membrane (unpubl.)

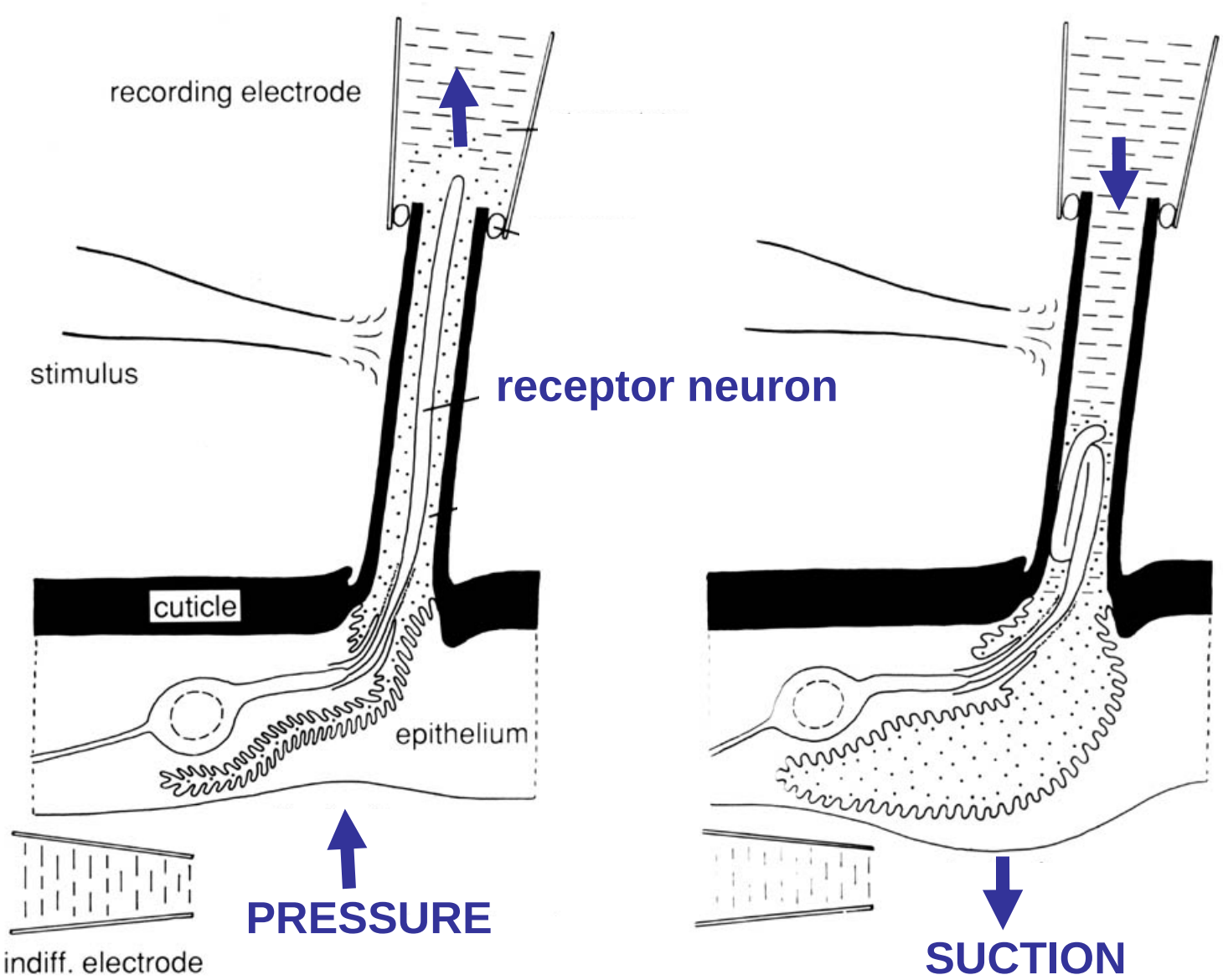
2

3

4

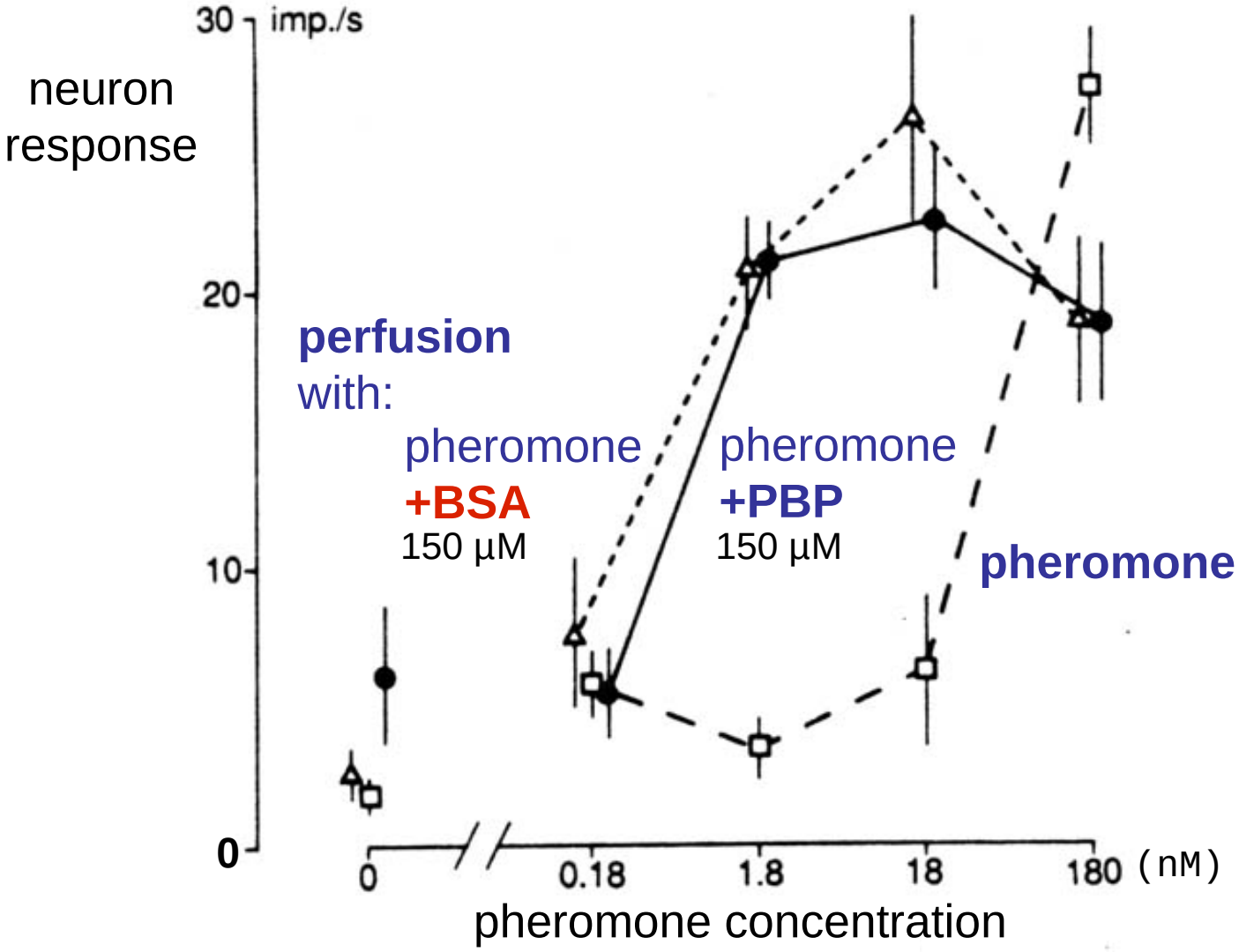
5


sensillum lymph droplets
1 – 10 pl

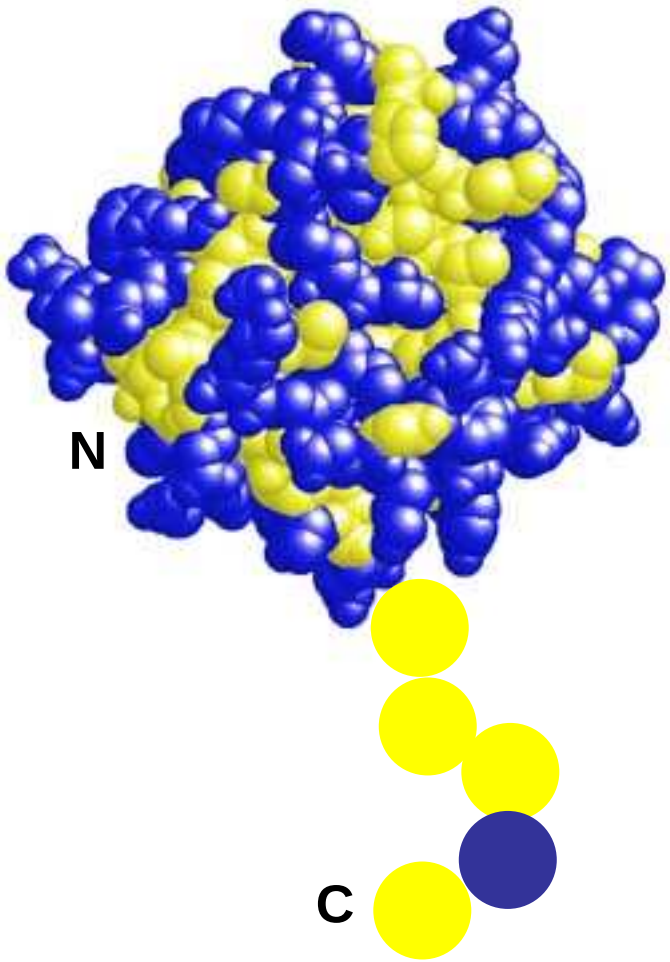


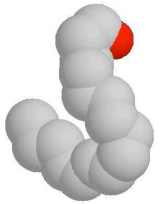
Kaissling, Keil, Williams 1991

Antheraea polyphemus



Van den Berg & Ziegelberger, 1991



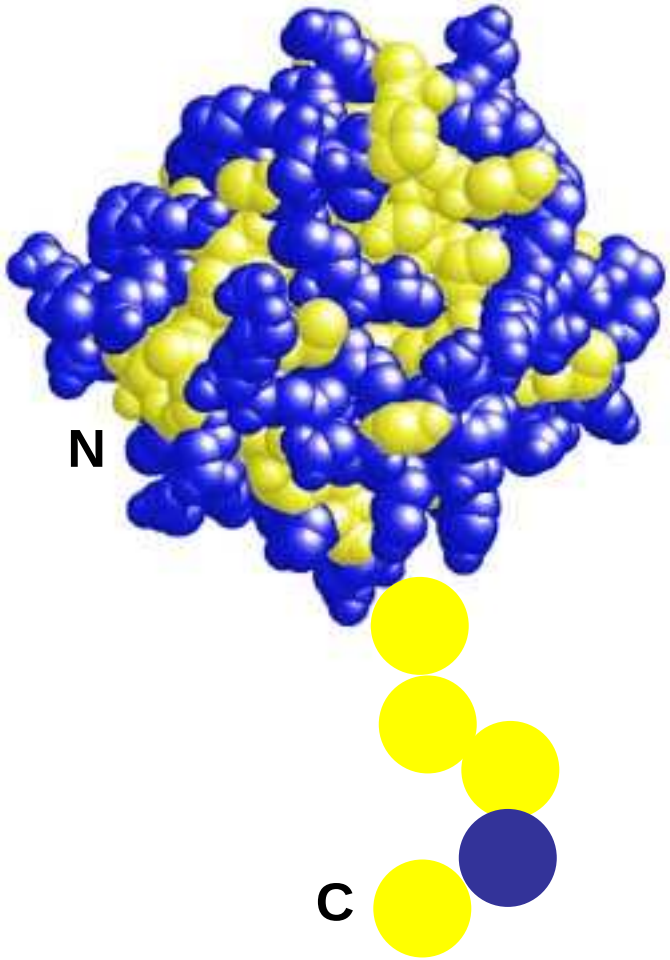


bombykol

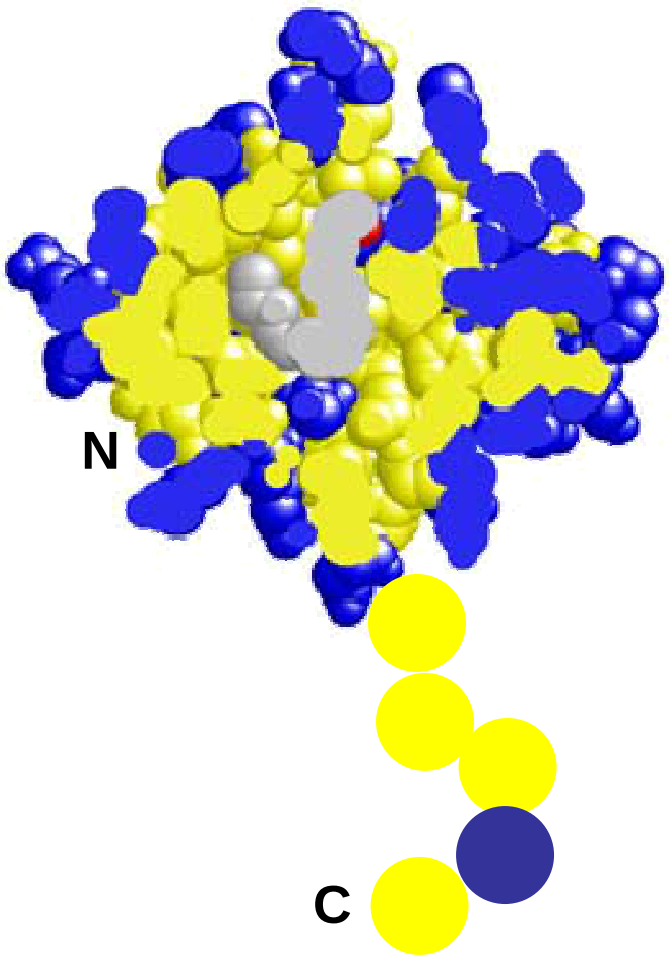
hydrophilic
hydrophobic

3 nm

Sandler et al., Chemistry & Biology **7**, 143 (2000)



X-ray



hydrophilic
hydrophobic

3 nm

Sandler et al., Chemistry & Biology **7**, 143 (2000)

Possible functions of the pheromone binding protein (PBP)

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2 **protects** the pheromone from enzymatic degradation (Vogt)

3

4

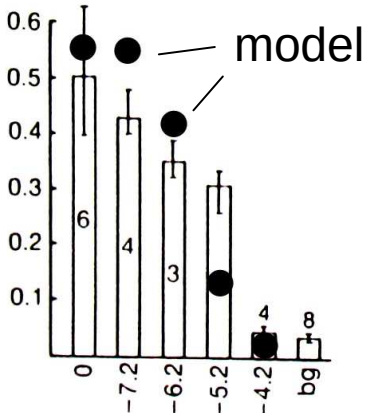
5

pheromone degradation

in vitro

velocity of degradation

(metabolite/pher. after 10 min)



log M [**PBP**]

PBP

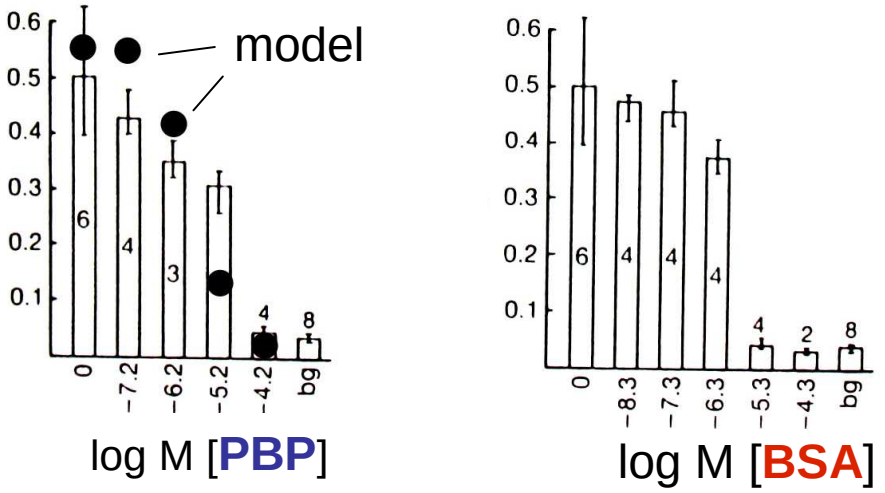
+ enzyme + pheromone

Vogt & Riddiford, 1986

pheromone degradation
in vitro

velocity of degradation

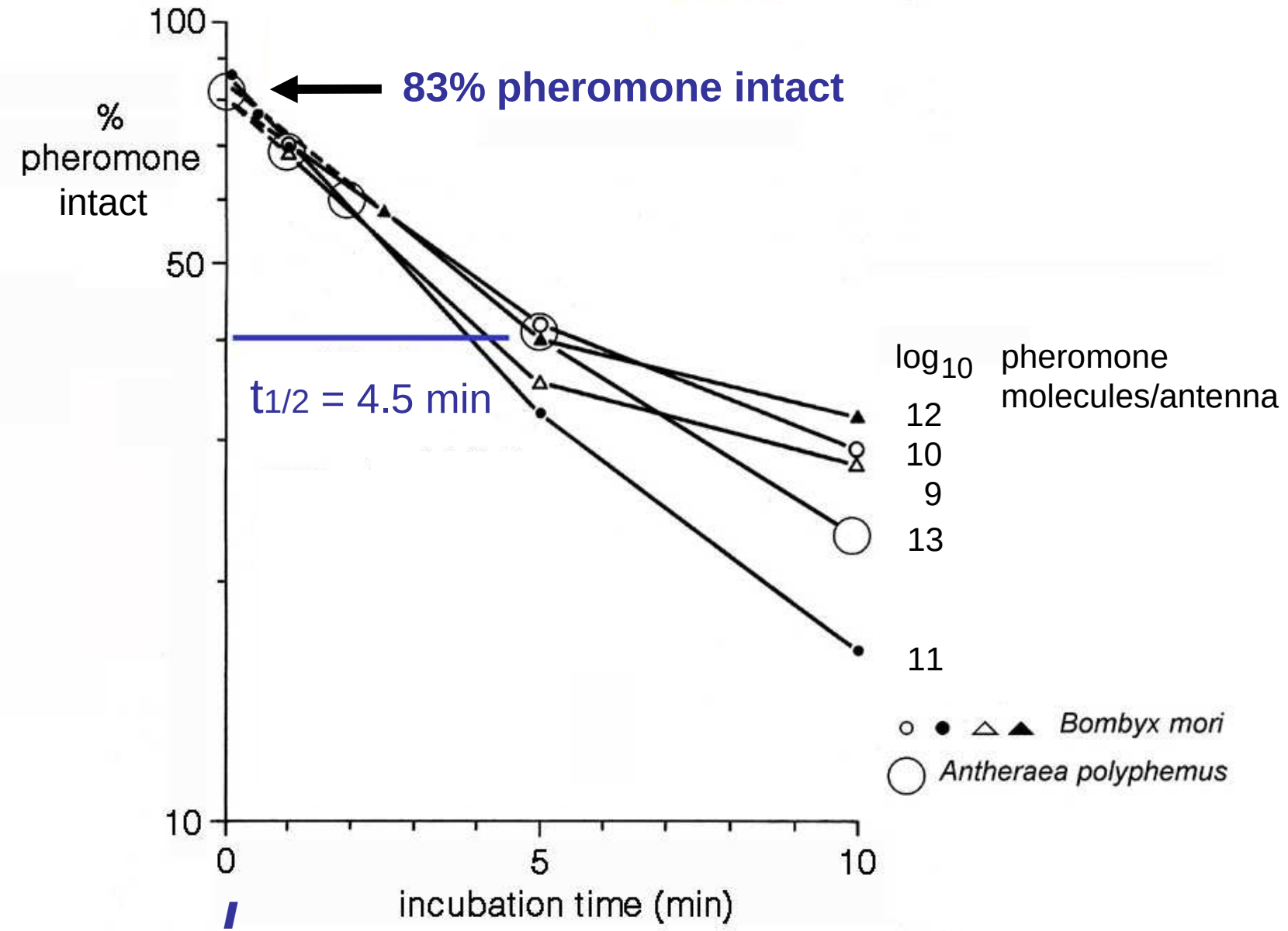
(metabolite/pher. after 10 min)



PBP or **BSA**
+ enzyme + pheromone

Vogt & Riddiford, 1986

pheromone degradation *in vivo*



10 s pheromone stimulus

Kasang et al. 1971 - 1989

Kasang

Vogt

$$\frac{\text{pher. degraded}}{\text{pher. protected}} = \frac{17\,\%}{83\,\%} = \frac{\frac{k10 \cdot Etot}{Km9,10}}{k2 \cdot A}$$

(bound to PBP)

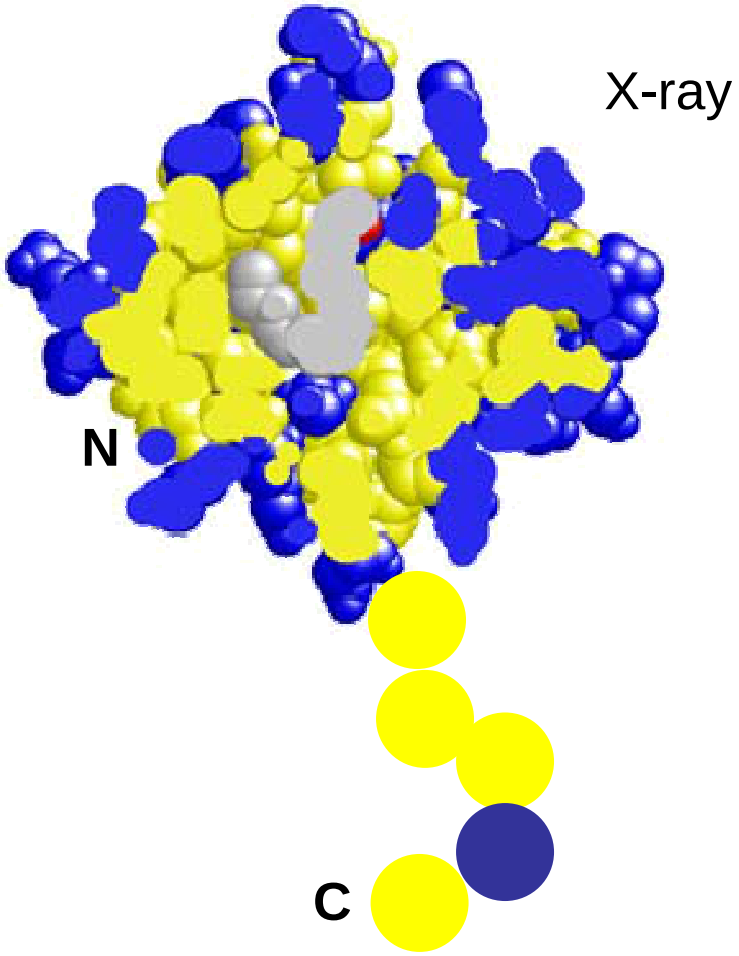
Leal

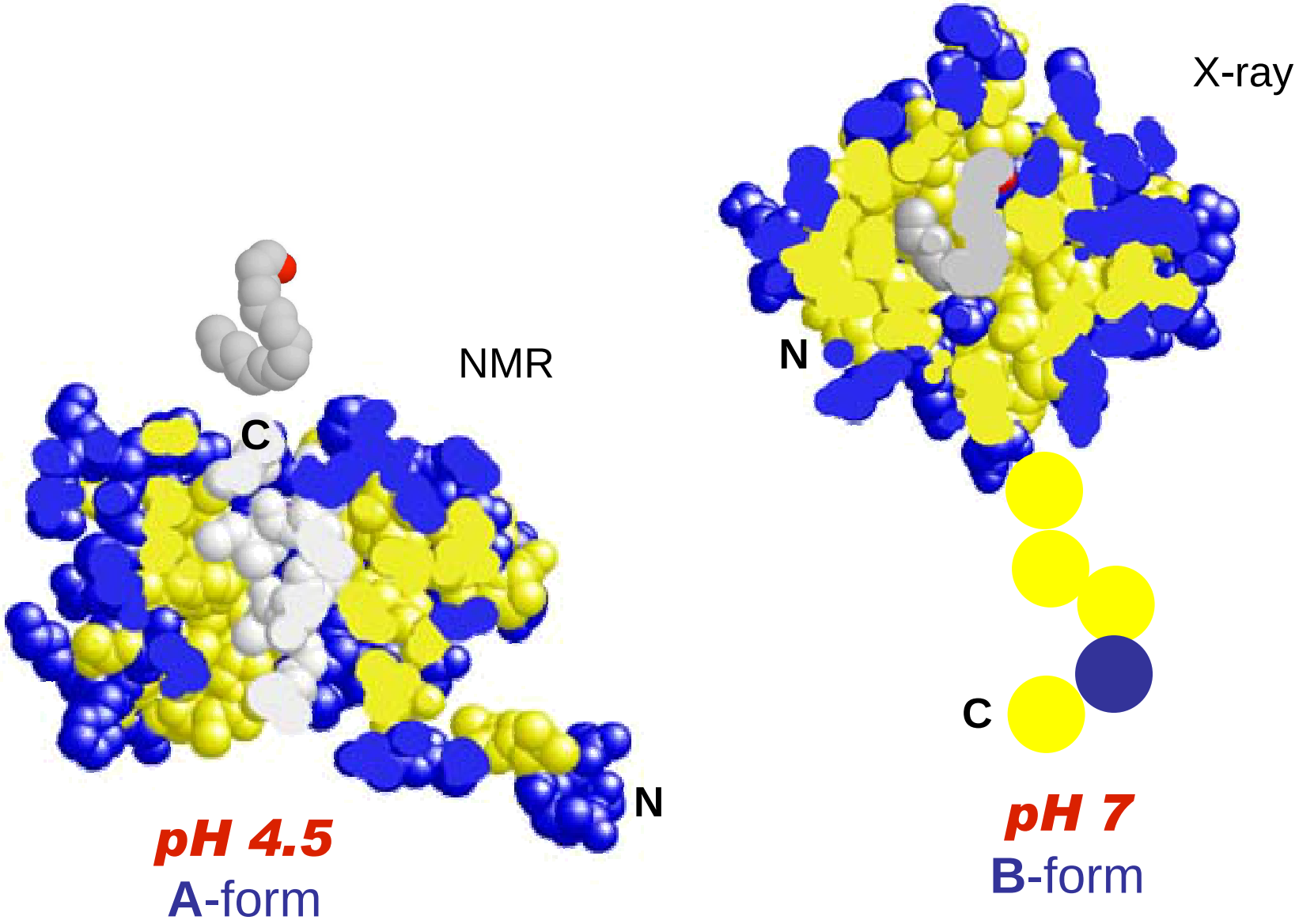
$$\frac{\text{PBP}}{Enzyme} = \frac{A}{Etot} = \frac{\frac{k10}{Km9,10} \cdot 83\,\%}{k2 \cdot 17\,\%} = \frac{7600}{1}$$

$A = 5 - 10\ mM$

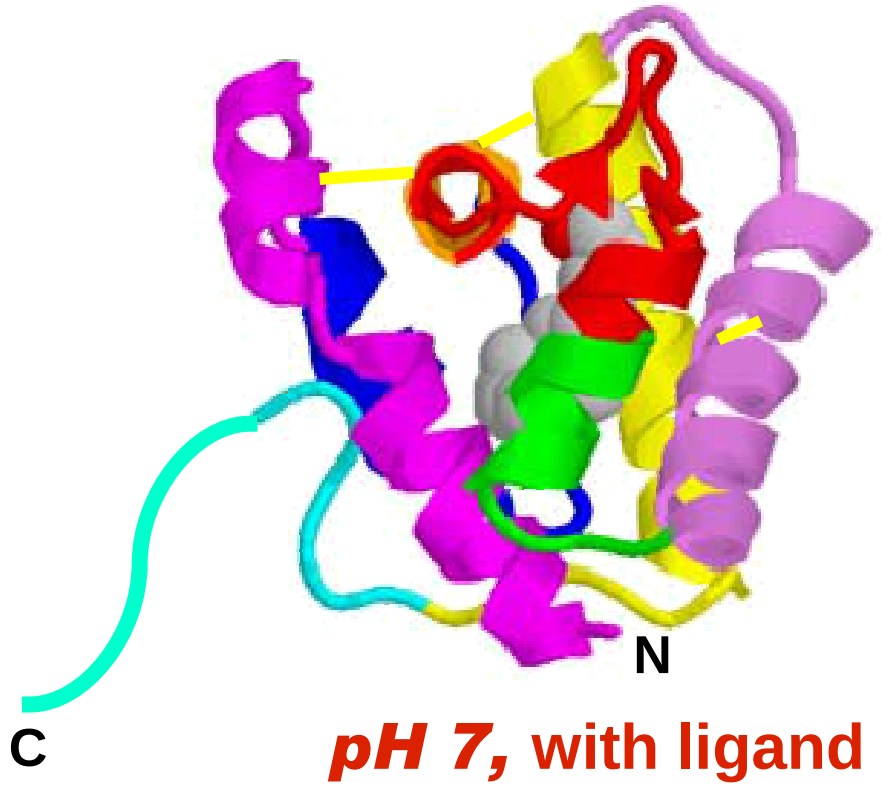
Vogt et al. 1985
Klein 1987

$Etot$ *about* $1\ \mu M$


X-ray
N
C
pH 7
B-form



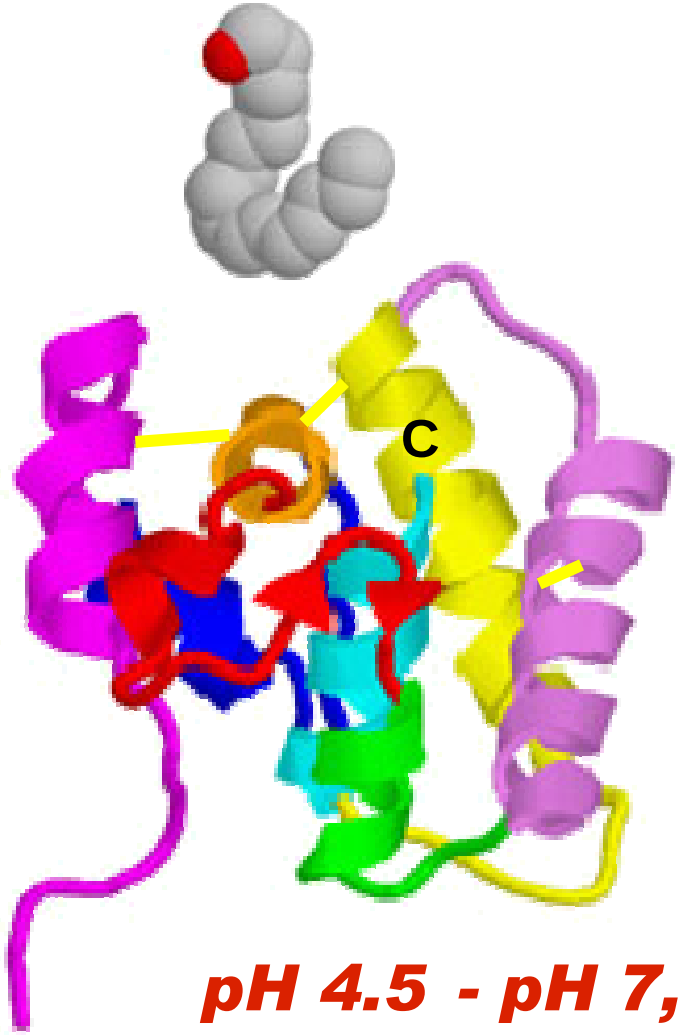
Horst et al., PNAS USA 98, 14374 (2001)

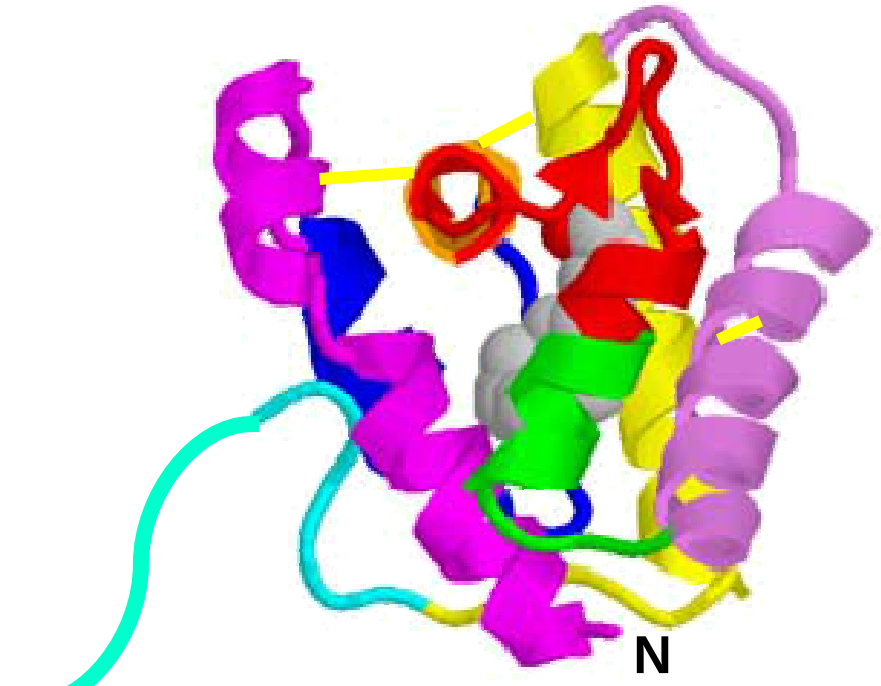


***pH 7,* with ligand**

B-form

Sandler et al. (2001)





***pH 7,* with ligand**

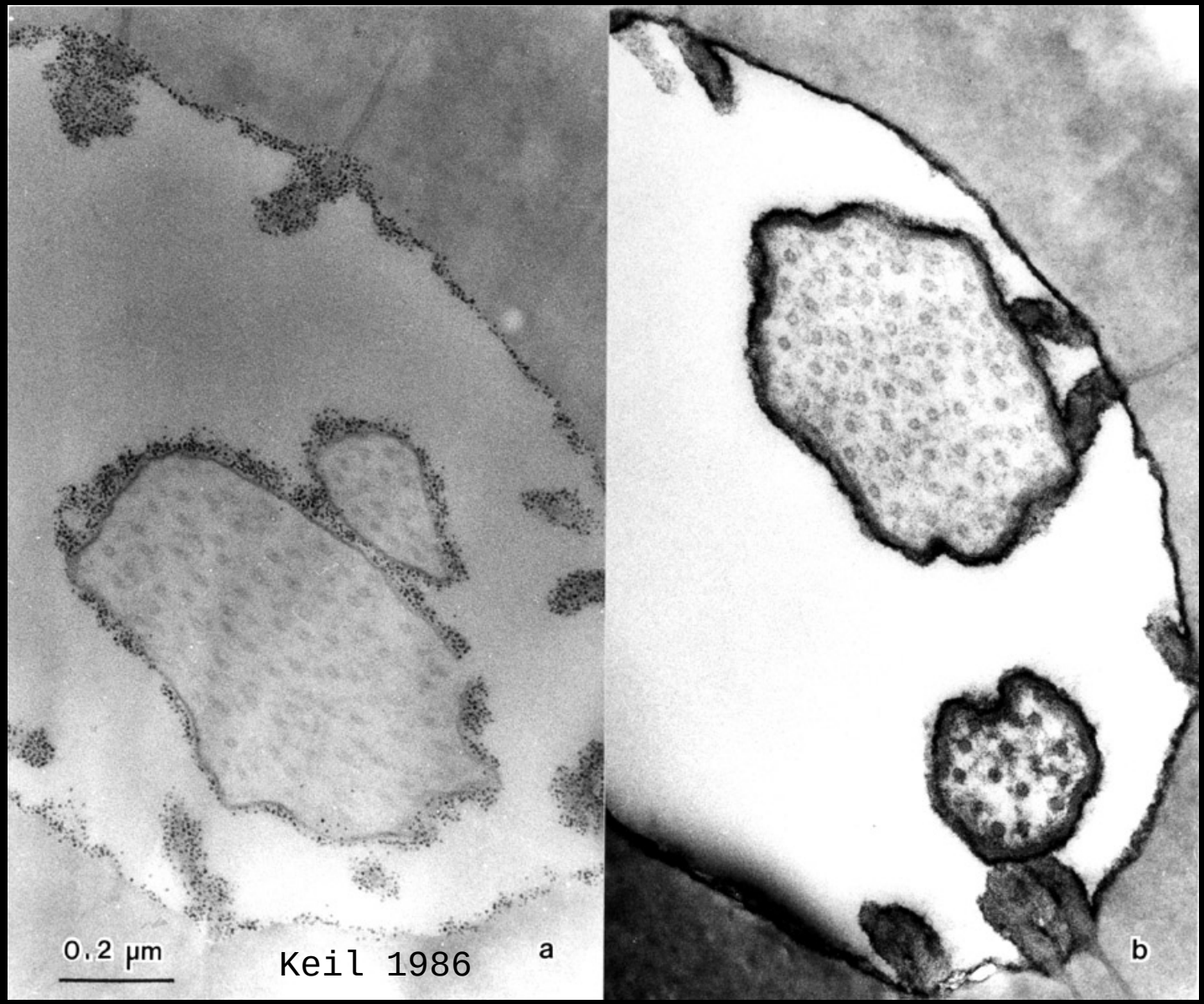
B-form

***pH 4.5 - pH 7,* without ligand**

A-form

Horst et al. (2001)
Lautenschlager et al. (2005)

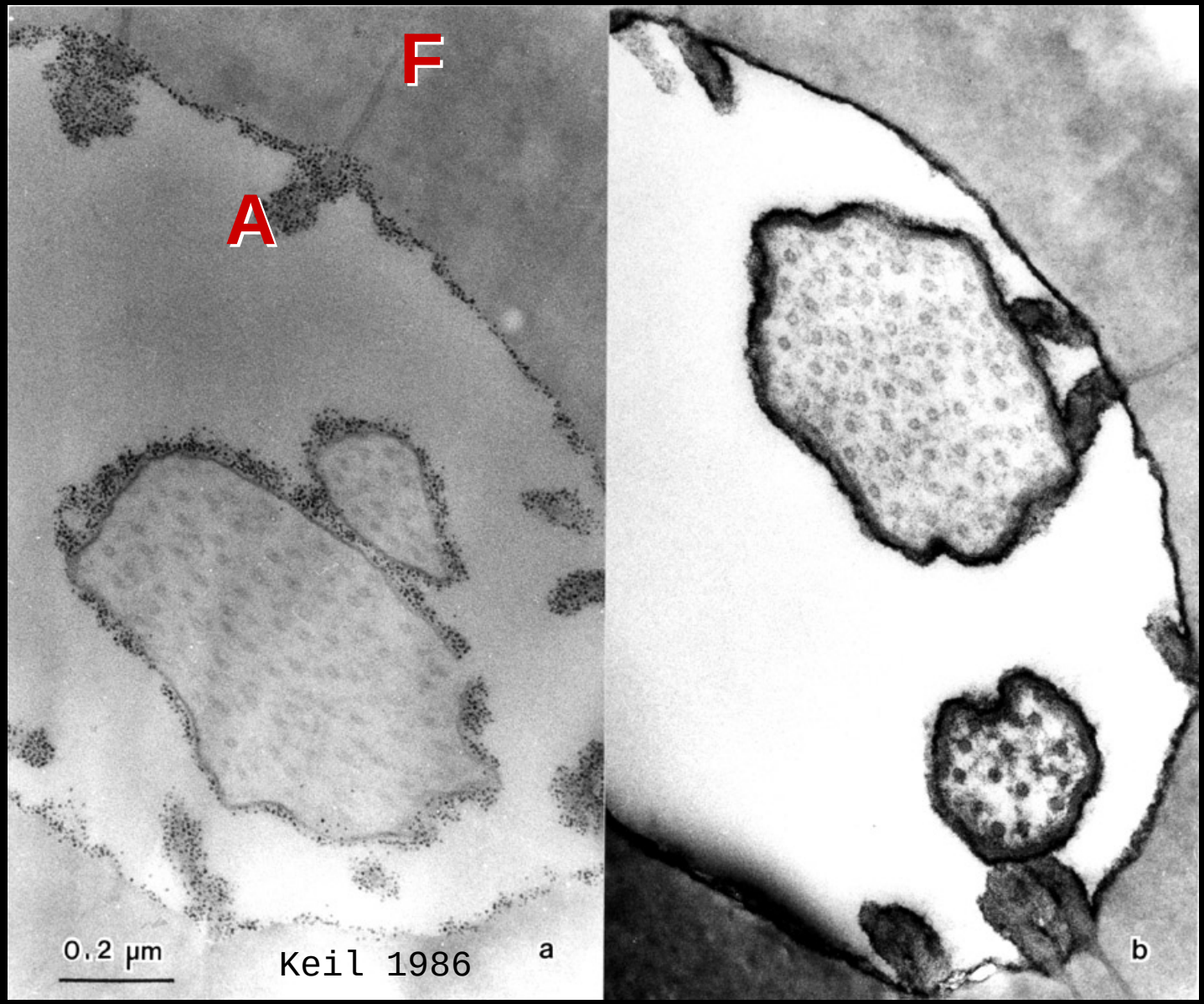
Marker for fixed negative charges



cationised Ferritin

Ruthenium Red

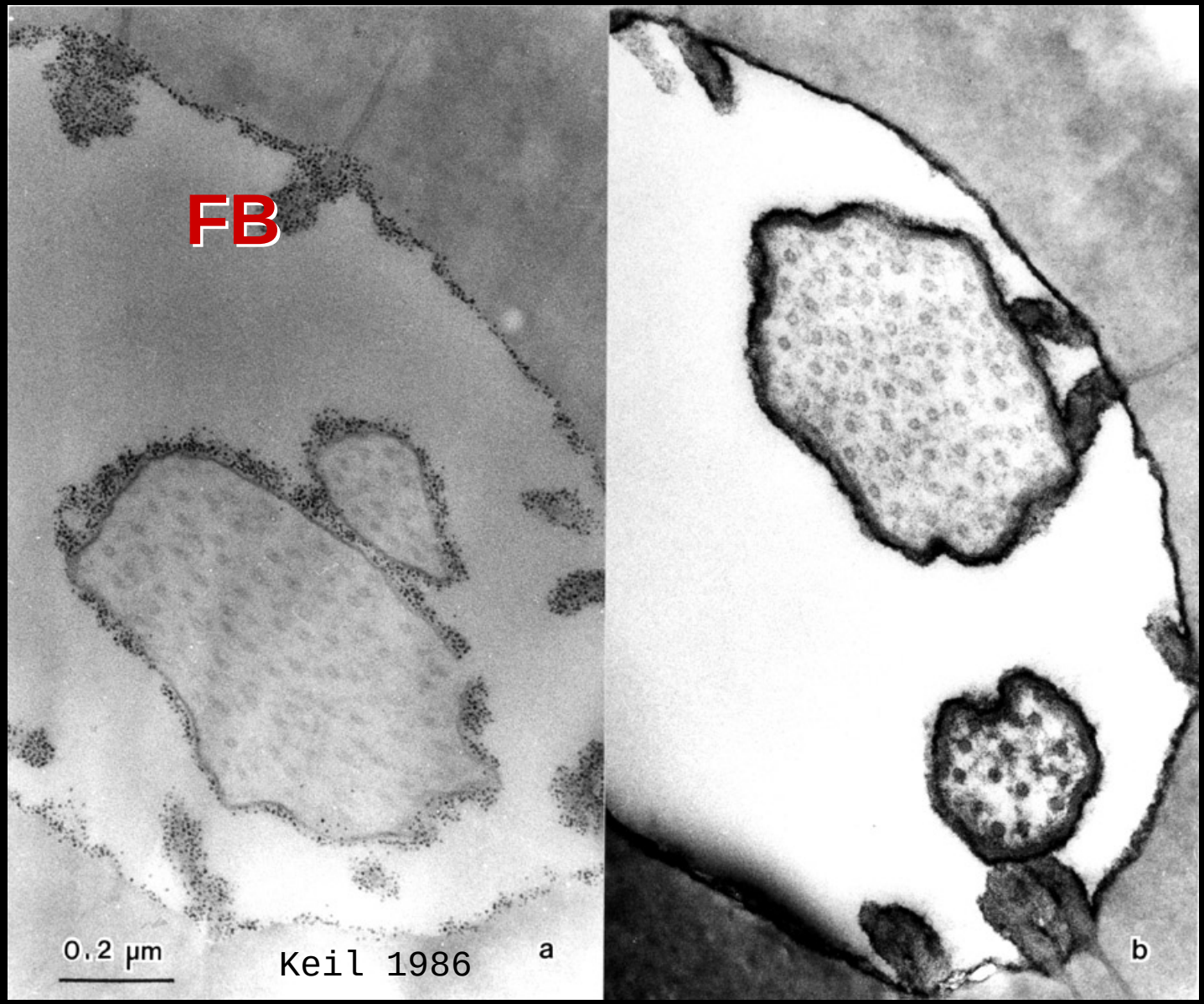
Marker for fixed negative charges



cationised Ferritin

Ruthenium Red

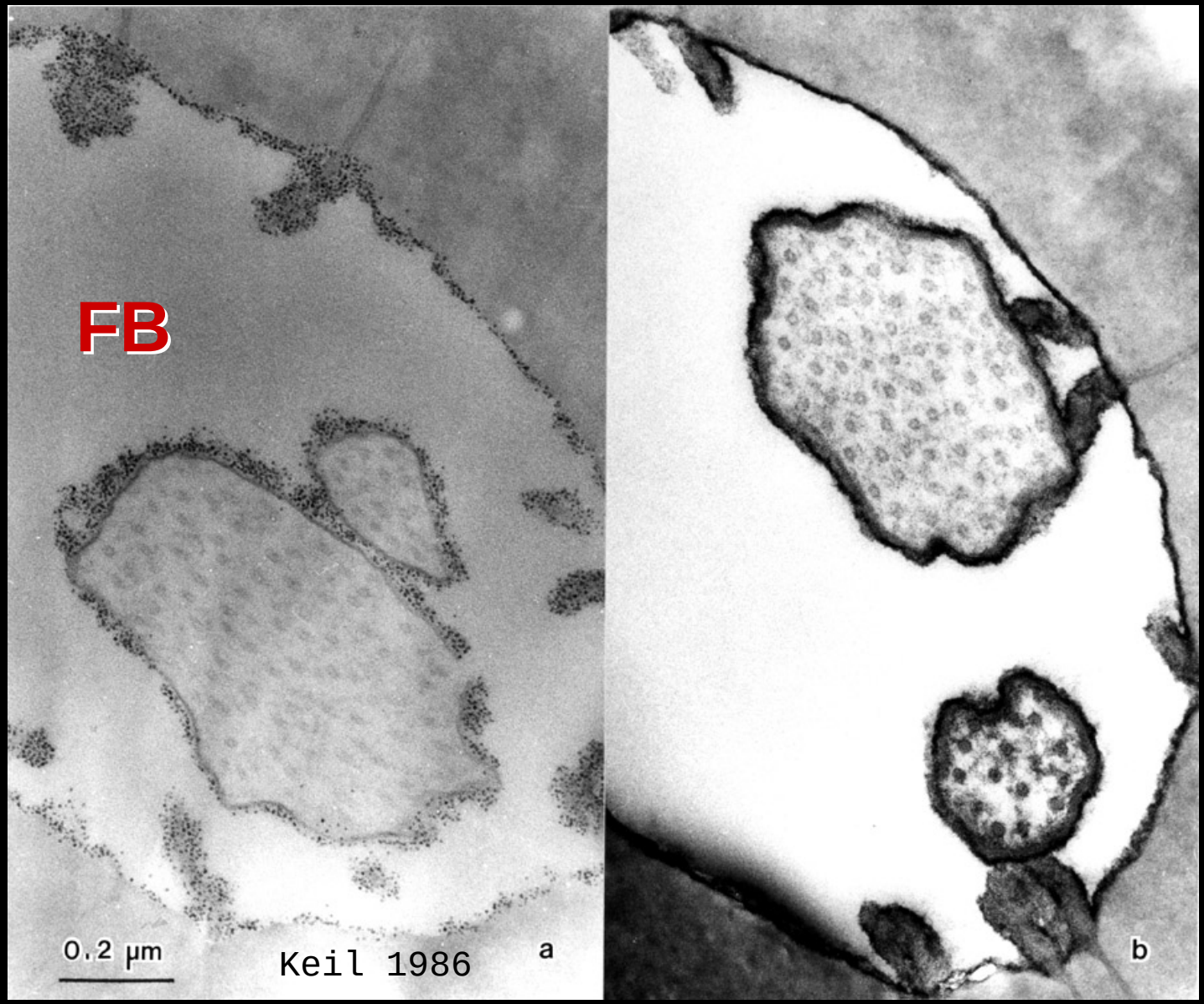
Marker for fixed negative charges



cationised Ferritin

Ruthenium Red

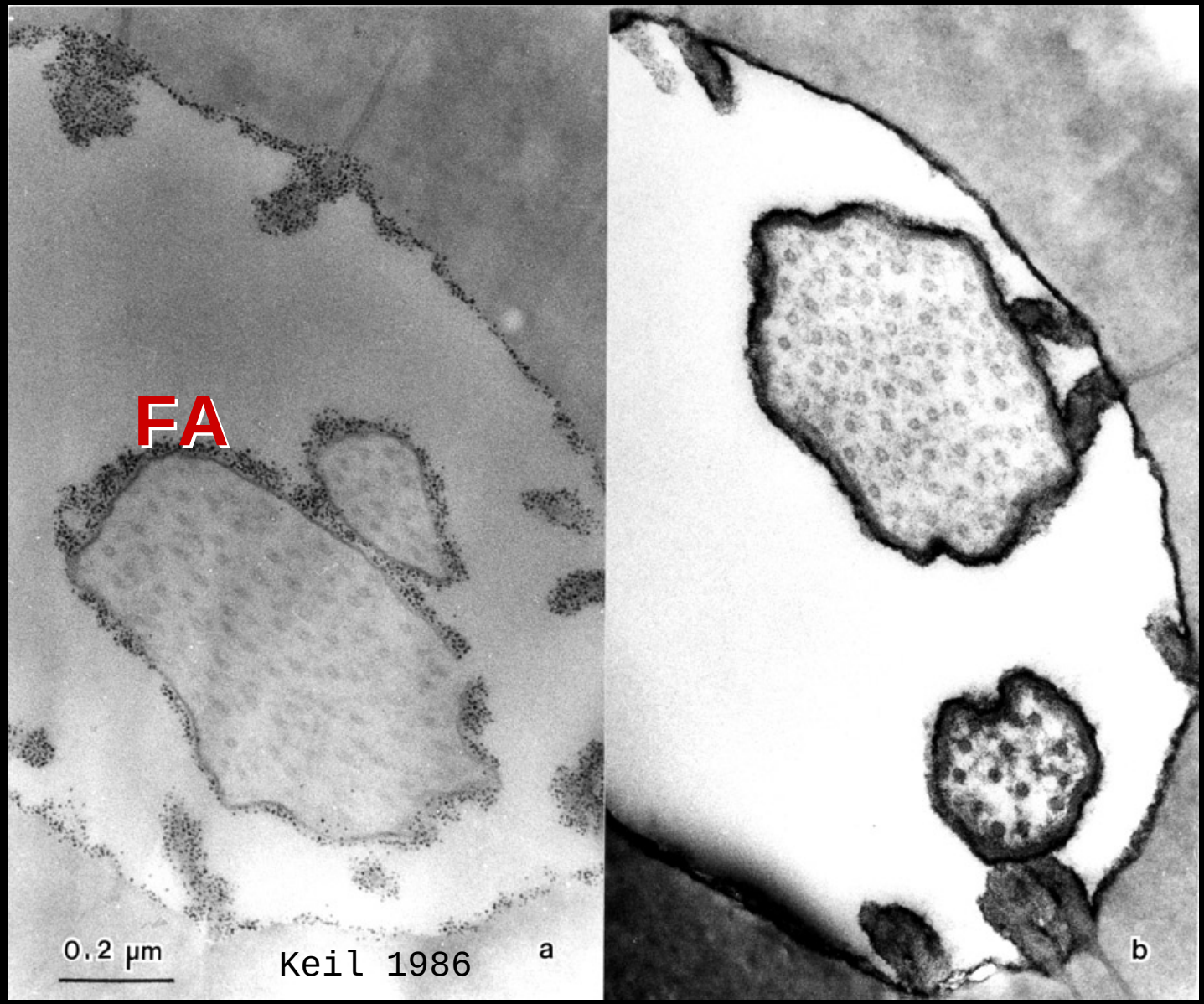
Marker for fixed negative charges



cationised Ferritin

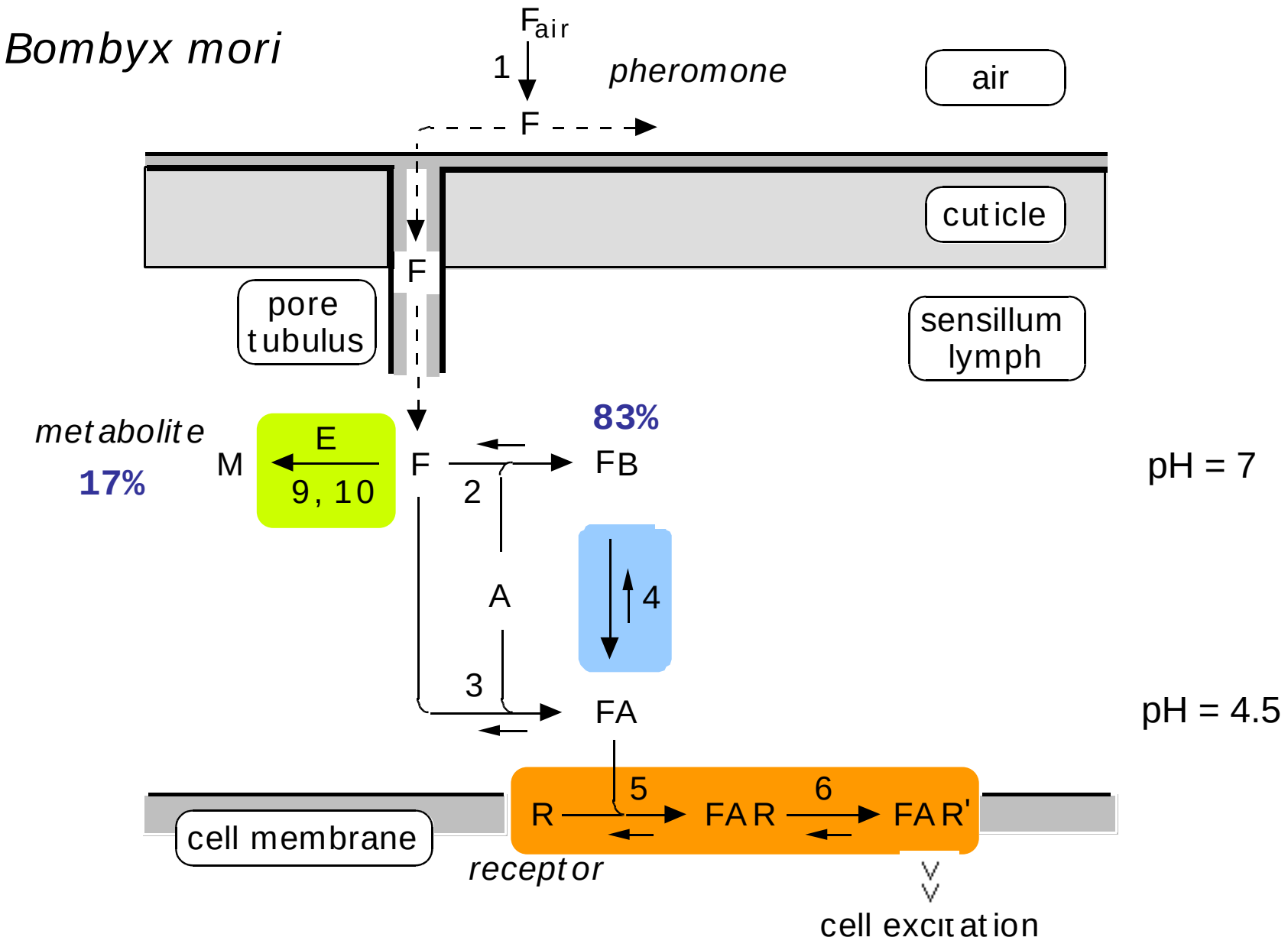
Ruthenium Red

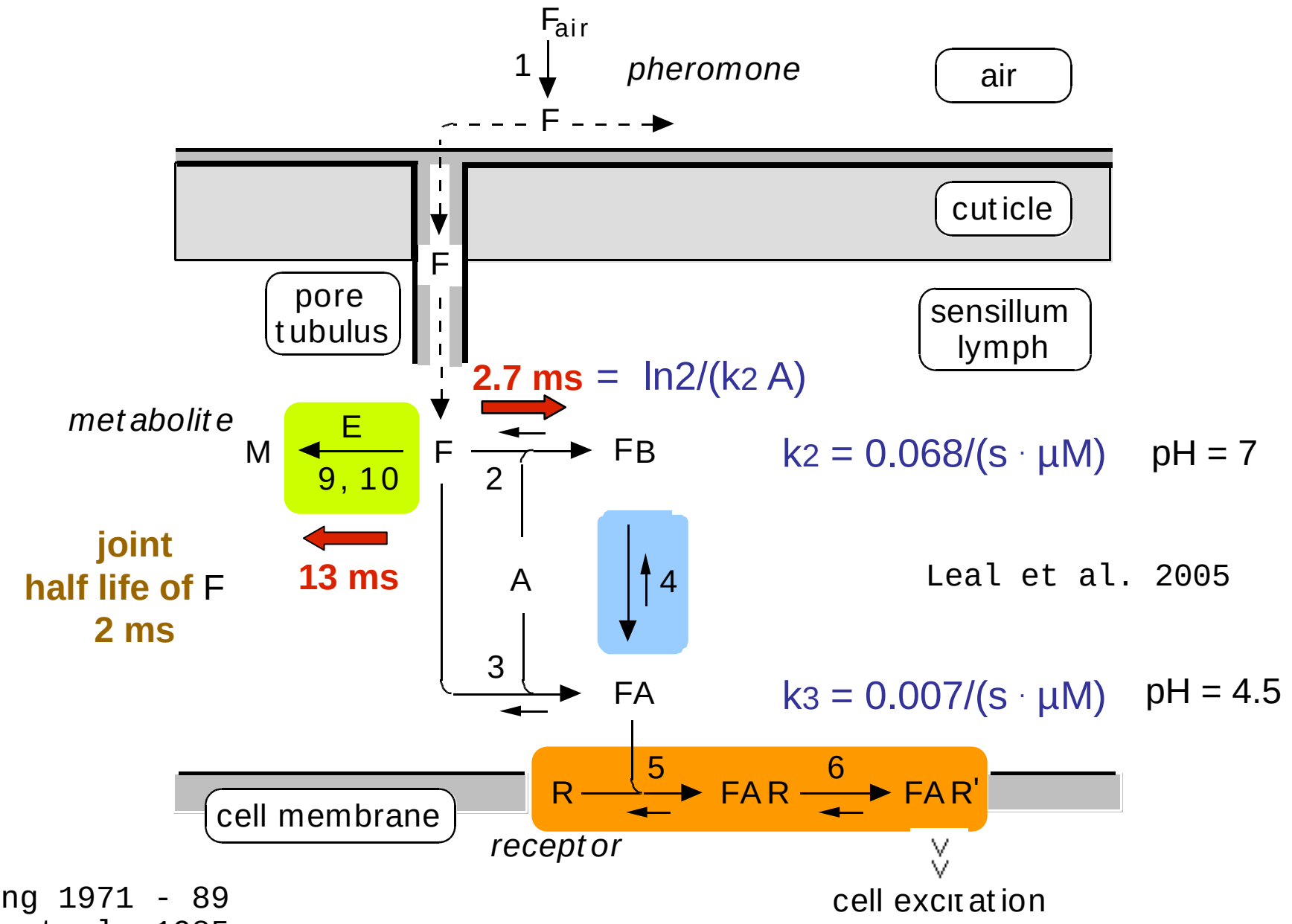
Marker for fixed negative charges



cationised Ferritin

Ruthenium Red


Bombyx mori
F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
metabolite
17%
M
E
9, 10
F
83%
FB
2
pH = 7
A
4
3
FA
pH = 4.5
5
6
R
FAR
FAR'
cell membrane
receptor
cell excitation



Kasang 1971 - 89
Vogt et al. 1985
Ishida & Leal 2005

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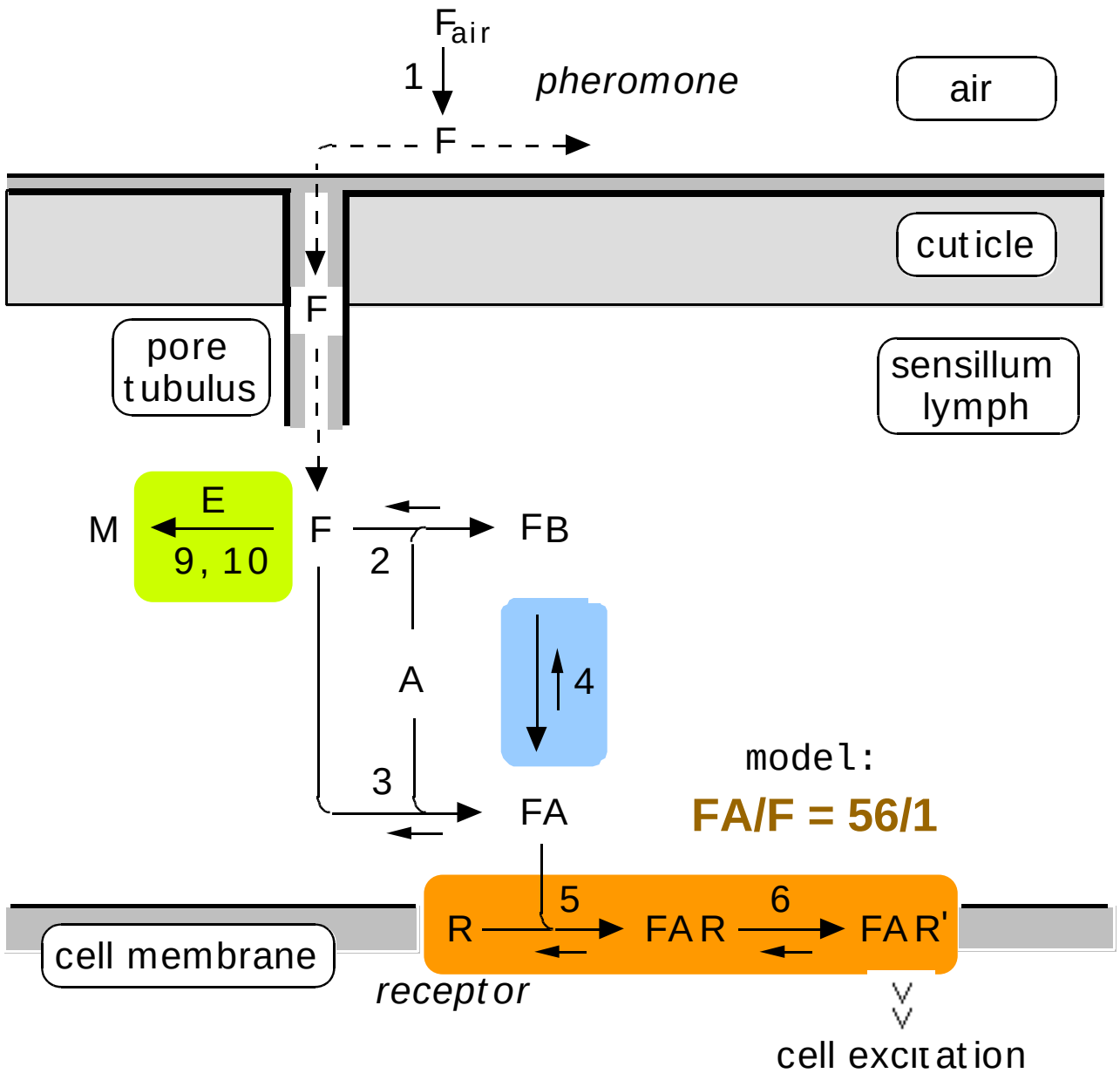
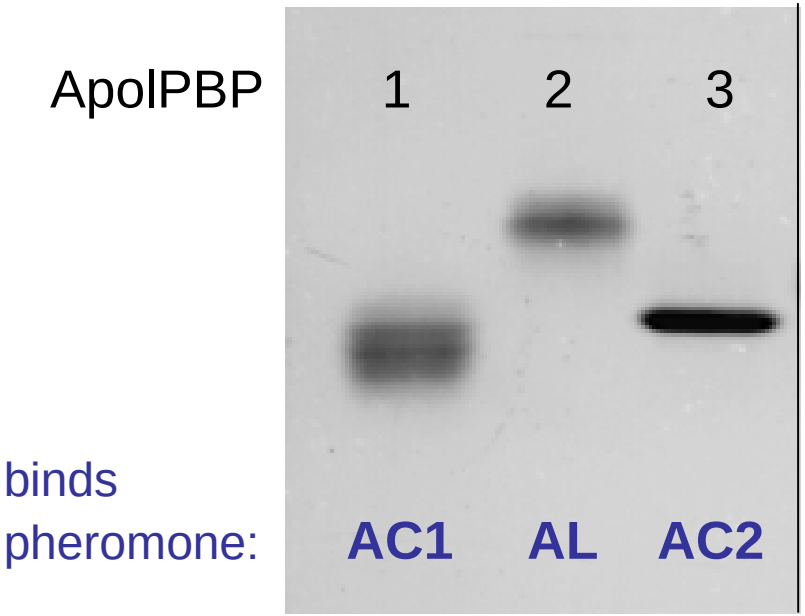
3 involved in pheromone-receptor **interaction** (Pophof)

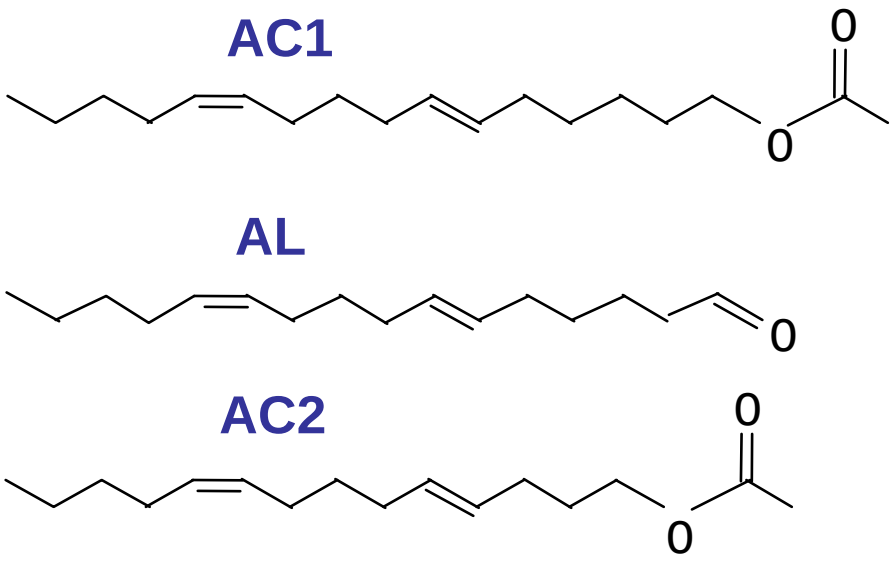
4

5


F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
M
E
9, 10
F
FB
2
A
4
3
FA
model:
FA/F = 56/1
5
6
R
FAR
FAR'
cell membrane
receptor
cell excitation

Antheraea polyphemus





Maida et al. 2003

rPBPs provided by J. Krieger

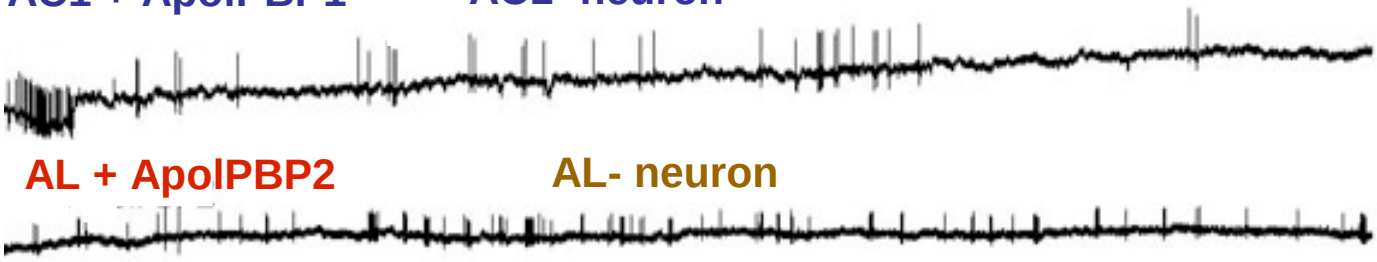
Antheraea polyphemus

Sensillum
perfusion with:

Spikes from:

AC1 + ApolPBP1

AC1- neuron



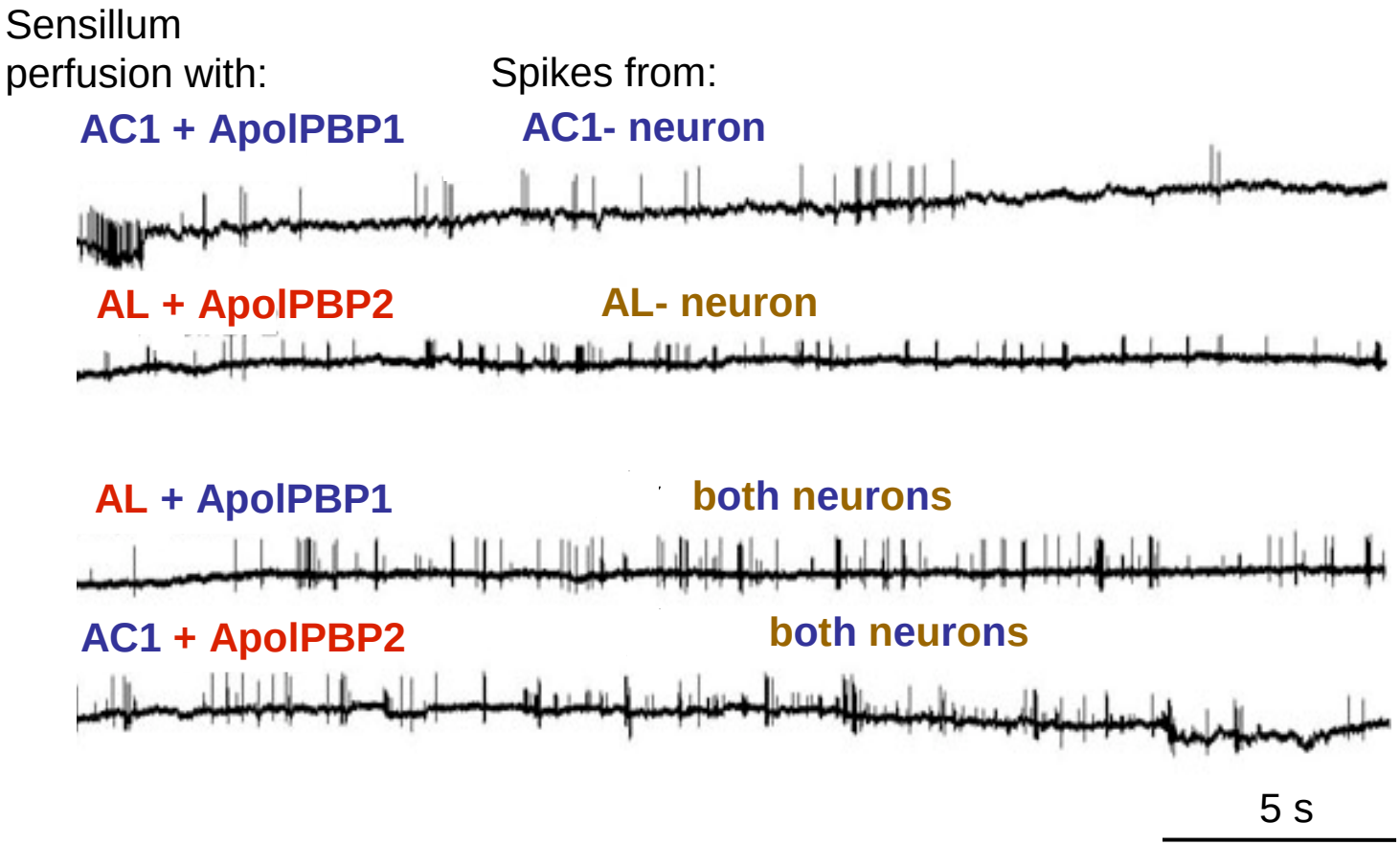
AL + ApolPBP2

AL- neuron

5 s

Pophof 2003

Antheraea polyphemus



Pophof 2003

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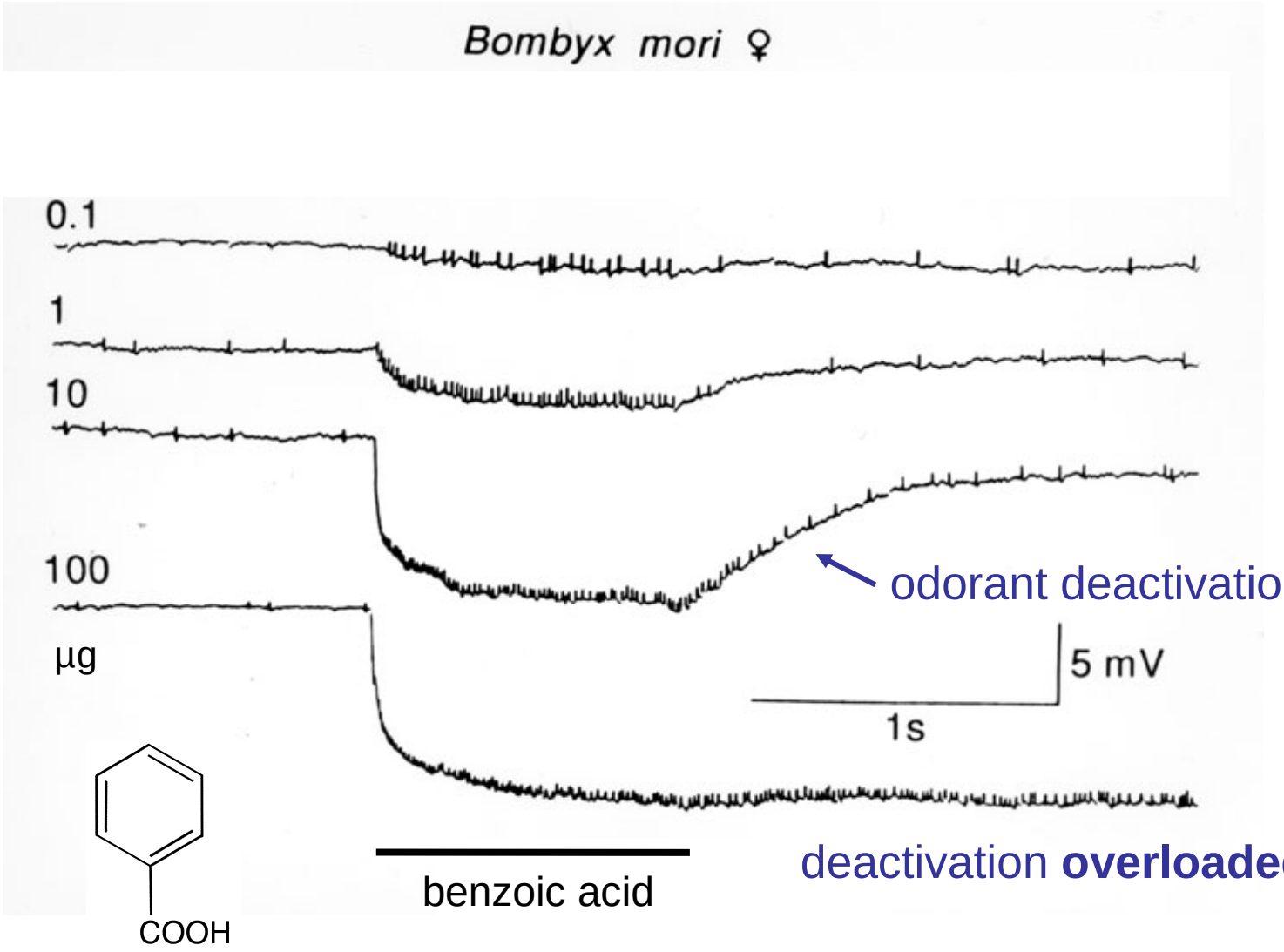
2 **protects** the pheromone from enzymatic degradation (Vogt)

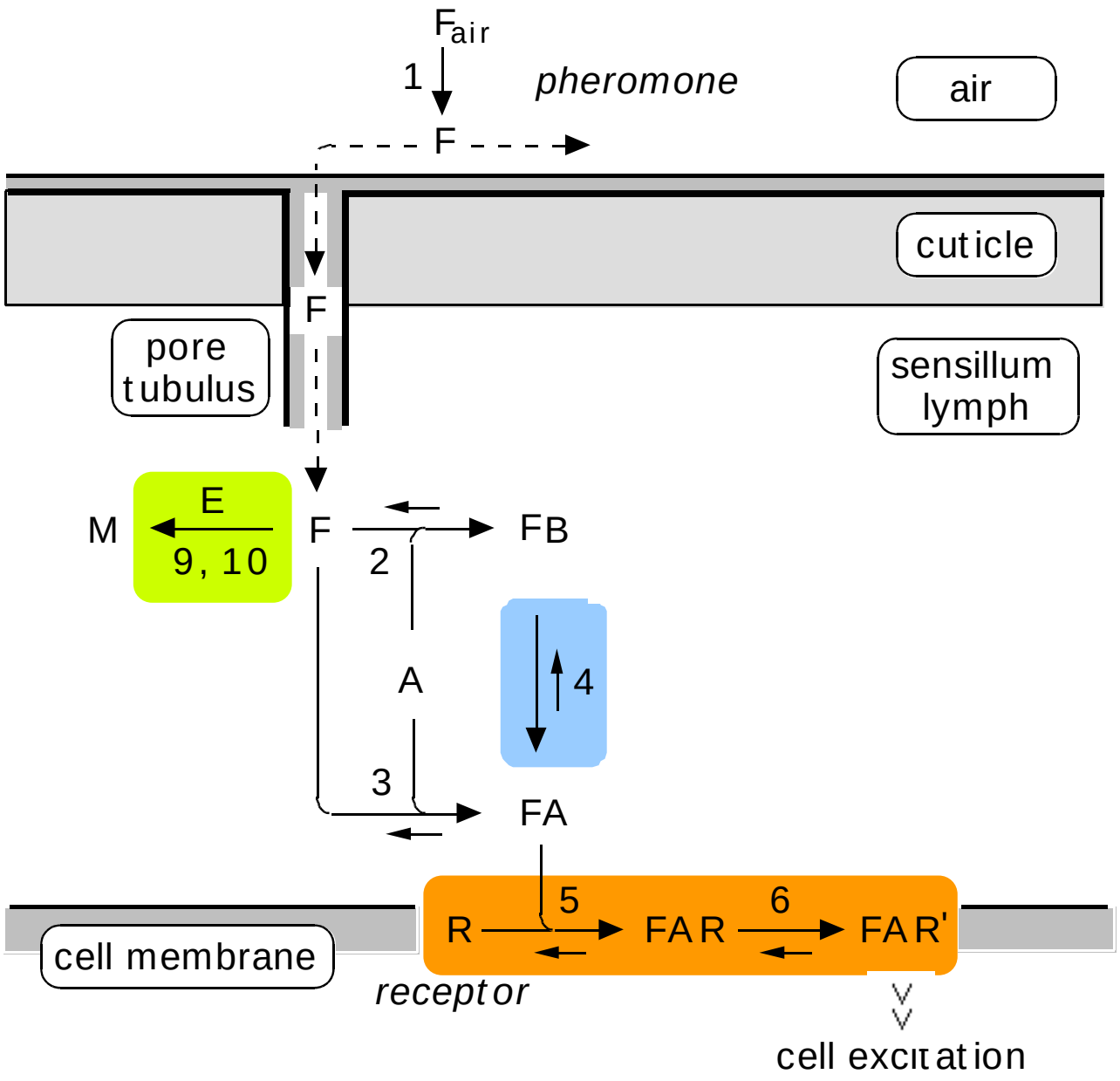
3 involved in pheromone-receptor **interaction** (Pophof)

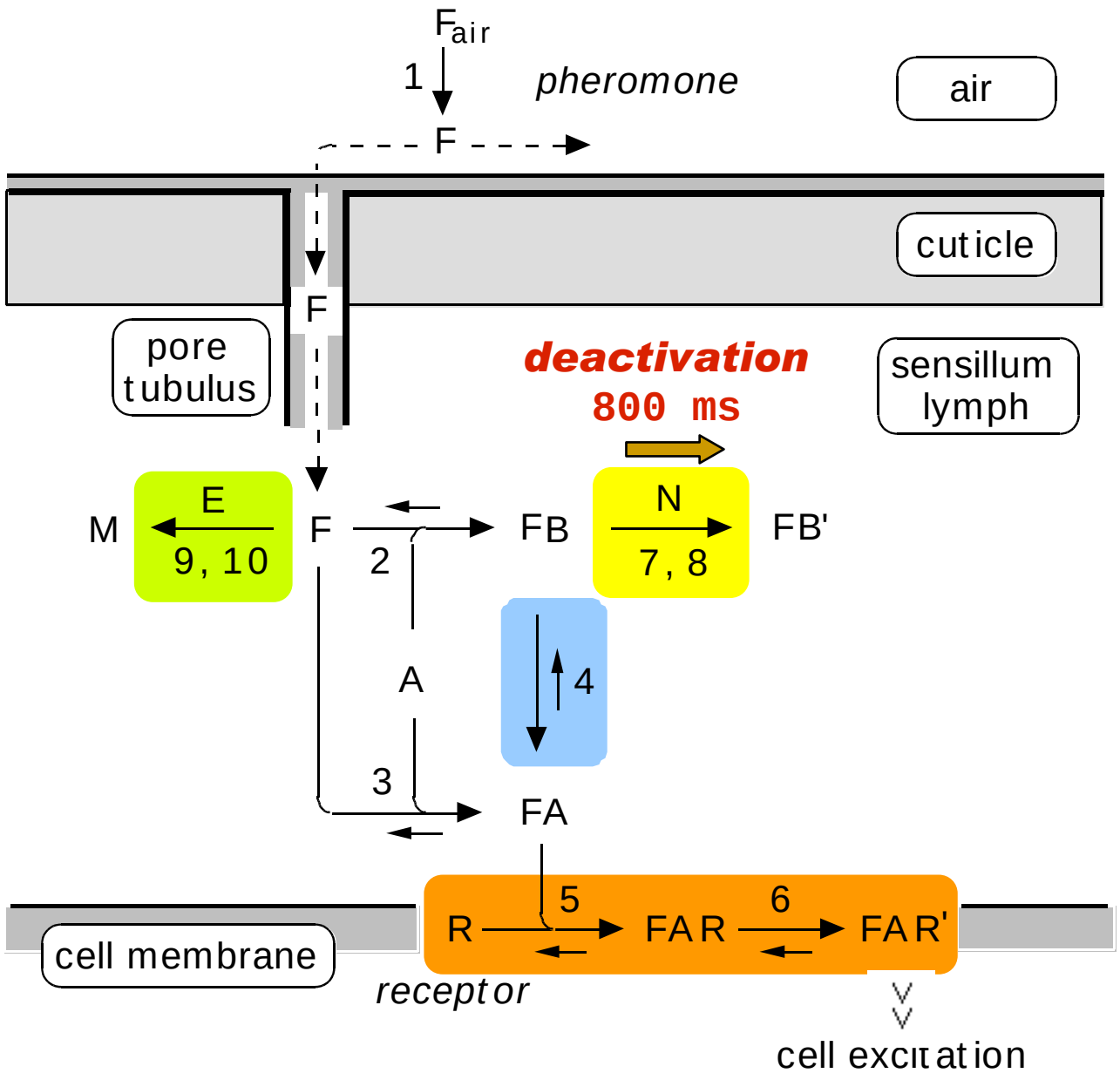
4 involved in pheromone deactivation (**scavenger**)

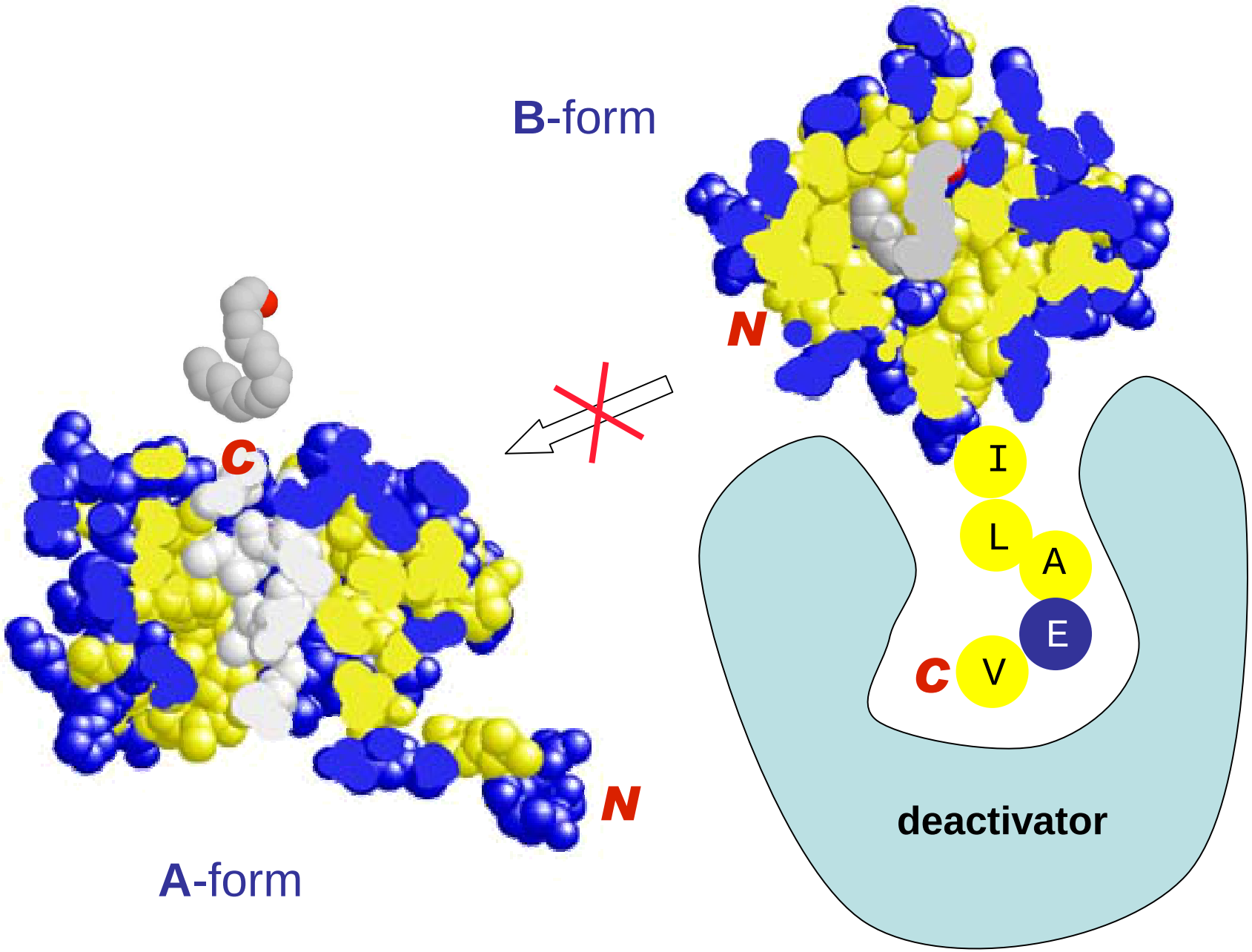
5

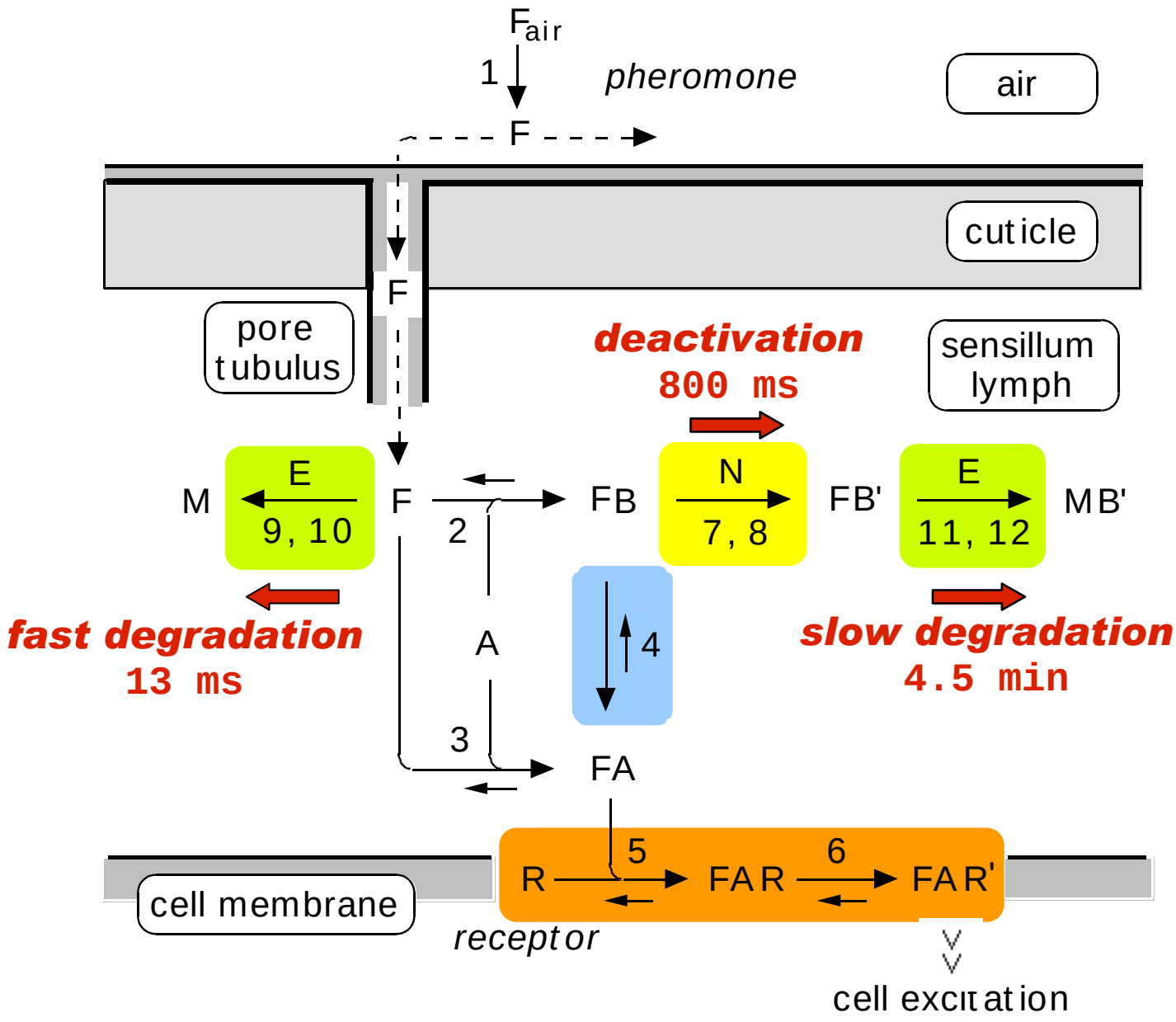


Kaissling 1987


F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
M
E
9, 10
F
FB
2
A
4
3
FA
5
R
FAR
6
FAR'
cell membrane
receptor
cell excitation

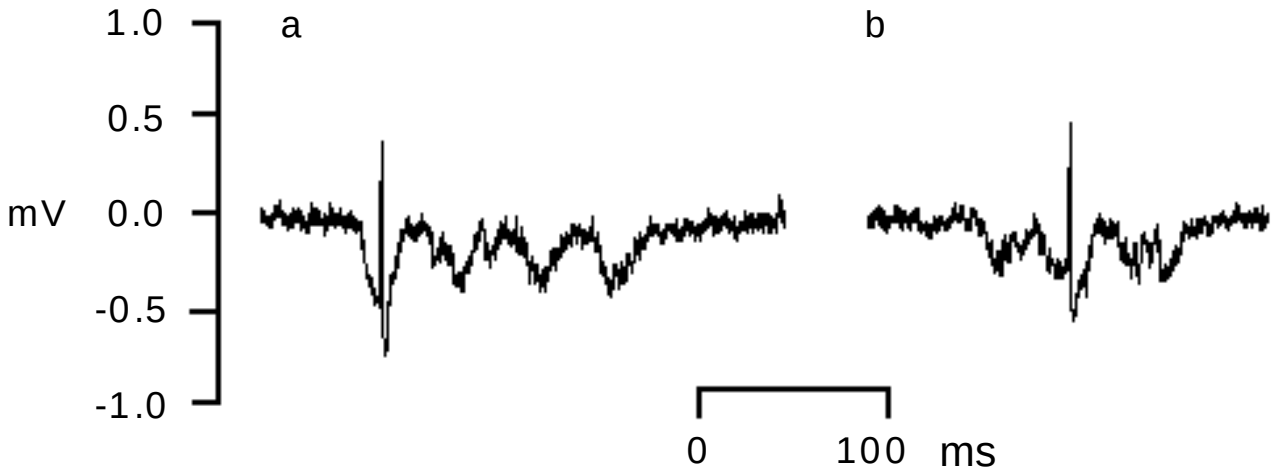

F air
1
pheromone
air
F
cuticle
F
pore
tubulus
deactivation
800 ms
sensillum
lymph
M
E
9, 10
F
2
FB
N
7, 8
FB'
A
4
3
FA
5
R
FAR
6
FAR'
cell membrane
receptor
cell excitation


B-form
N
C
I
L
A
E
C
V
deactivator
C
N
A-form


F air
1
pheromone
air
F
cuticle
F
pore tubulus
deactivation
800 ms
sensillum lymph
M
E
9, 10
F
2
FB
N
7, 8
FB'
E
11, 12
MB'
fast degradation
13 ms
A
4
slow degradation
4.5 min
3
FA
5
6
R
FAR
FAR'
cell membrane
receptor
cell excitation

Receptor activation
and
cell excitation

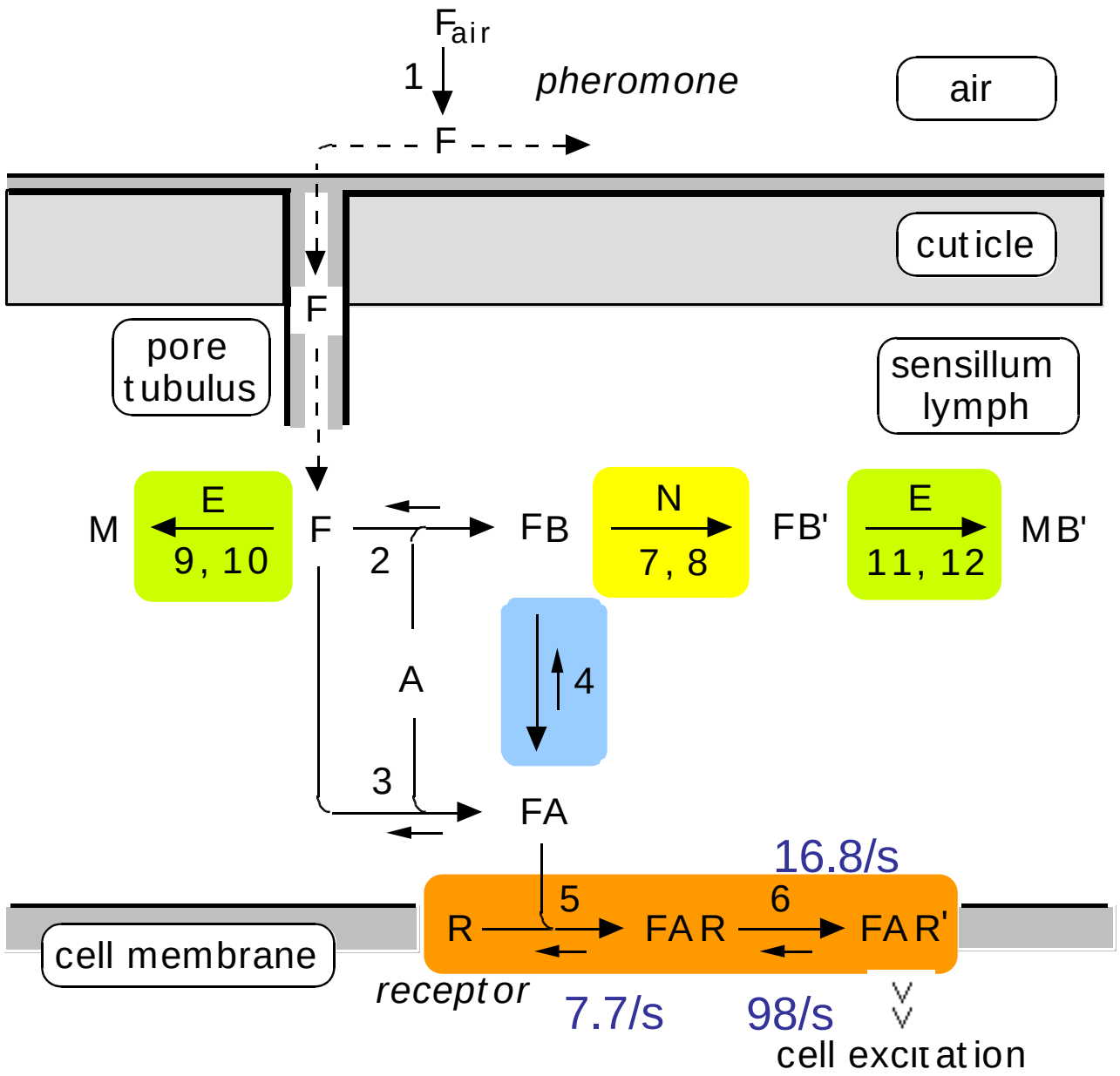
Elementary receptor potentials

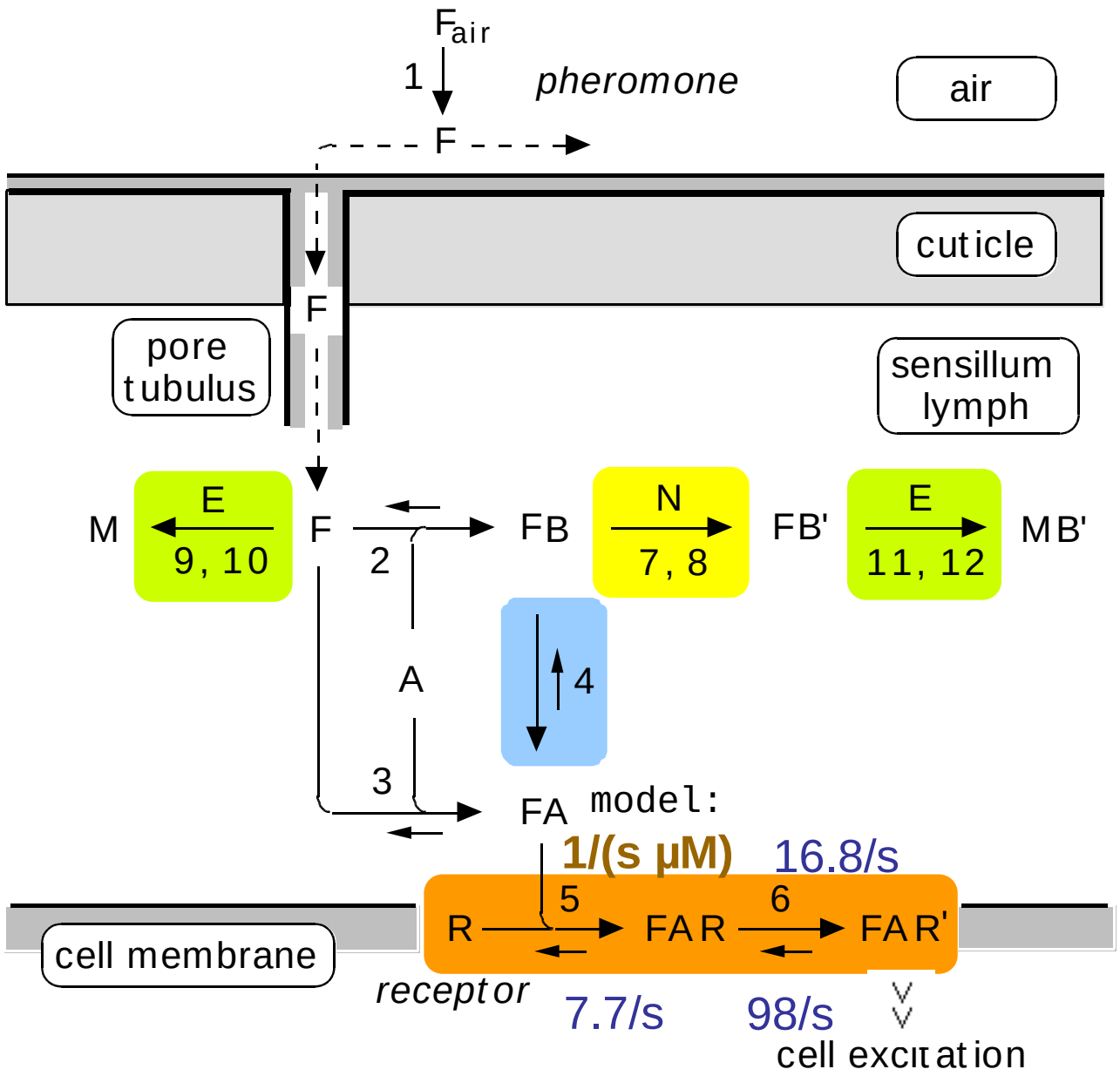


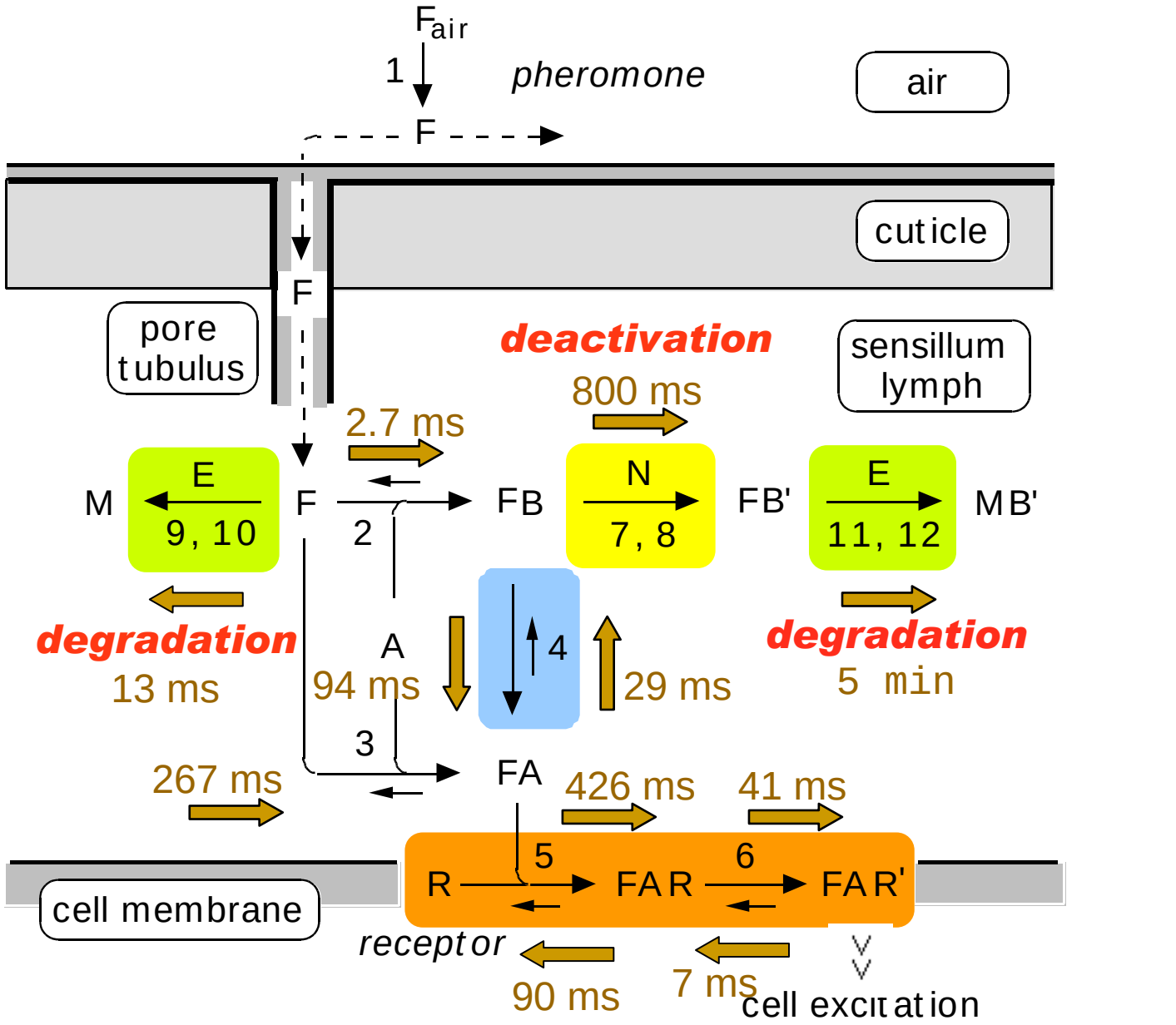
“bump“
(30 pS)

k_6 = 16.8/s
k_{-6} = 98/s
k_{-5} = 7.7/s

Kaissling and Thorson (1980); Minor and Kaissling (2003)

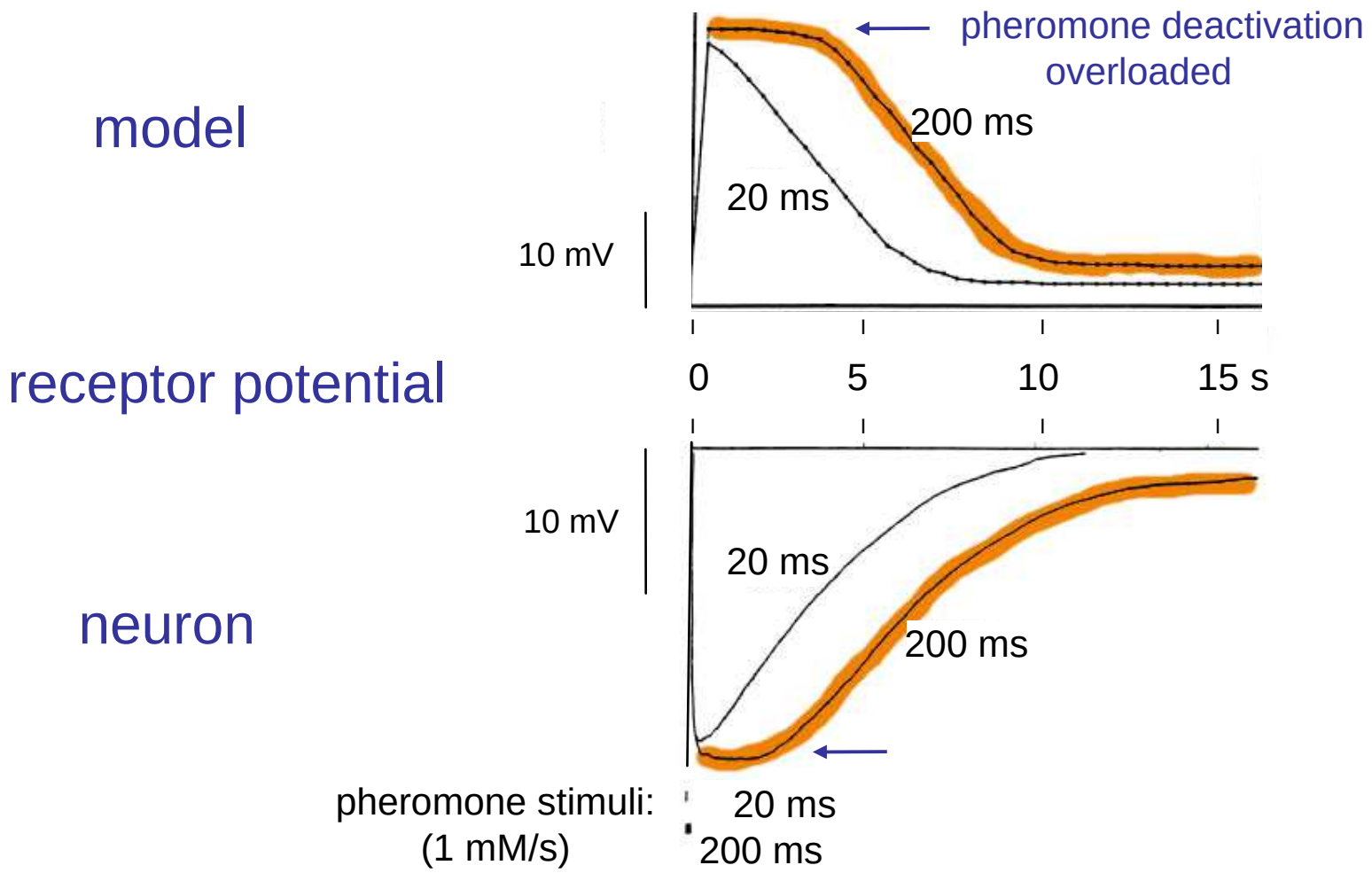

F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
M
E
9, 10
F
2
FB
N
7, 8
FB'
E
11, 12
MB'
A
4
3
FA
16.8/s
5
6
R
FAR
FAR'
cell membrane
receptor
7.7/s
98/s
cell excitation


F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
M
E
9, 10
F
2
FB
N
7, 8
FB'
E
11, 12
MB'
A
4
3
FA
model:
1/(s µM)
16.8/s
5
6
R
FAR
FAR'
cell membrane
receptor
7.7/s
98/s
cell excitation



Kaissling 2009

Antheraea polyphemus



Kaissling 2001

Rtot

$$R_{tot} = \frac{T_c\, U_{sat}\, Q_3}{Q_5} = 1.66 \text{ mM}$$

Kaissling 2009

density of receptor molecules = 6335/μm^2

$$T_c = \frac{1}{k_{-5}\, Q_4} = 153 \text{ ms}$$

Minor & Kaissling 2003

$k_{-5} = 7.7/s \quad k_6 = 16.8/s \quad k_{-6} = 98/s$

$Q_4 = k_{-6}/(k_6 + k_{-6}) = 0.854$

Kaissling 2009

$Q_5 = k_6/(k_{-5} + k_6) = 0.686$

$U_{sat} = 30$ mM/s

Kaissling 2001, 2009

$Q_3 = 0.25$

Kaissling & Priesner 1970

receptor molecules 6000

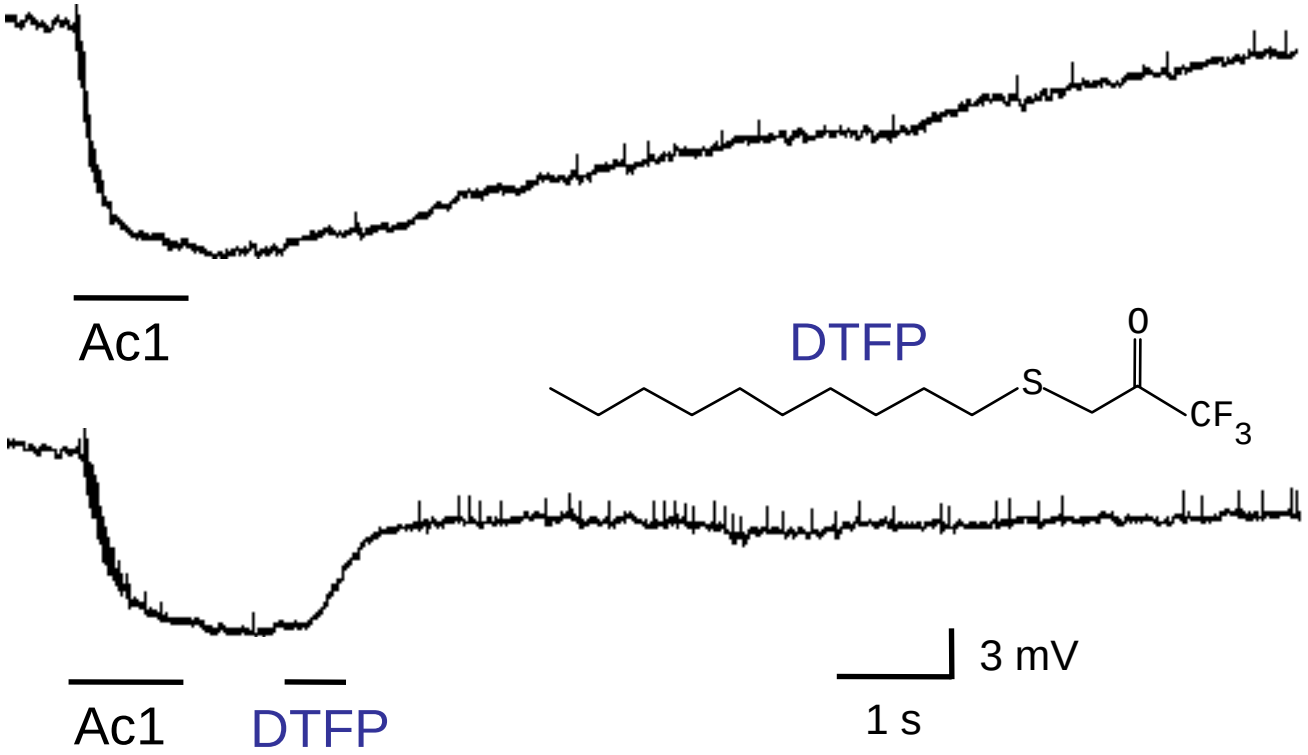
per μm^2 plasma membrane

relative numbers:

PBP molecules 14,000,000

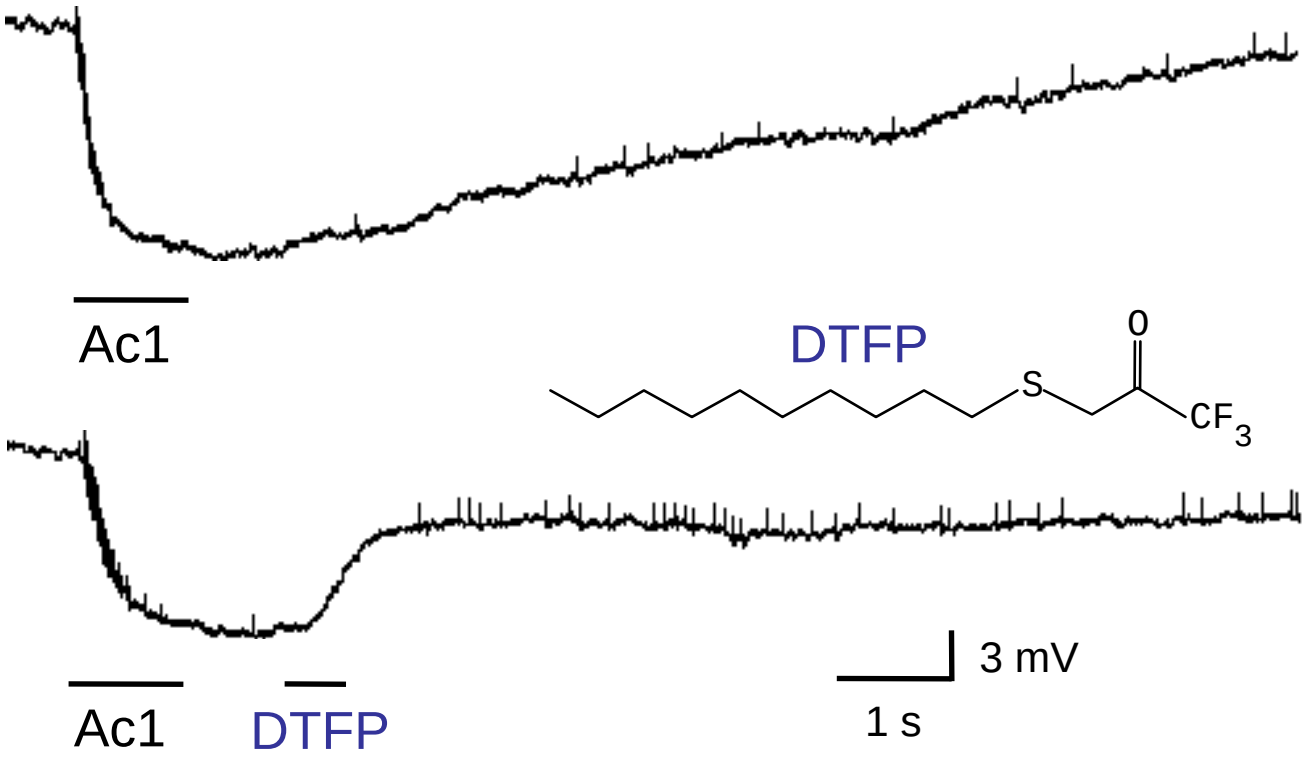
Antheraea polyphemus

Antheraea polyphemus.



Pophof 1998

Antheraea polyphemus.



Hypothesis:

DTFP - PBP complex blocks pheromone receptors

Pophof 1998

receptor molecules	6000

per μm^2 plasma membrane

relative numbers:

PBP molecules	14,000,000
DTFP for blocking	10,000

Antheraea polyphemus

biochemistry, radiometry

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Sudha Kanaujia
Gerhard Kasang
Rosario Maida
Therese Proebstl
Gunde Ziegelberger
Richard G. Vogt
Hans-Jürgen Bestmann
Jürgen Krieger
Walter S. Leal
Fred Damberger--NMR
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Alexei Redkozubov
Gabriela De Brito Sanchez
Romina Barrozo

modeling, computer programs

John Thorson
Alexander V. Minor

Thank you

estimated densities

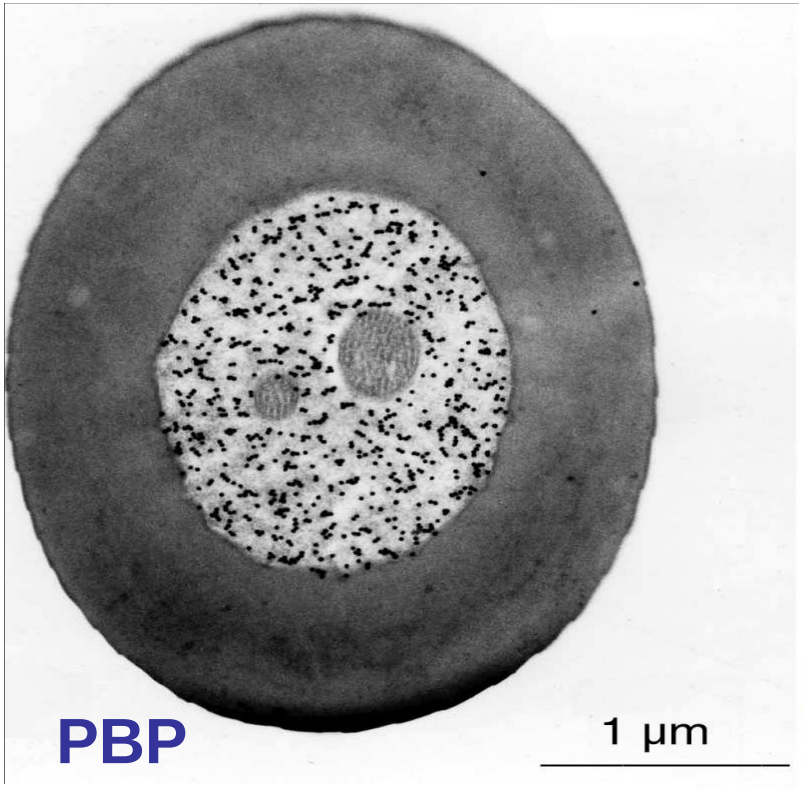
(per μm^2 plasma membrane)

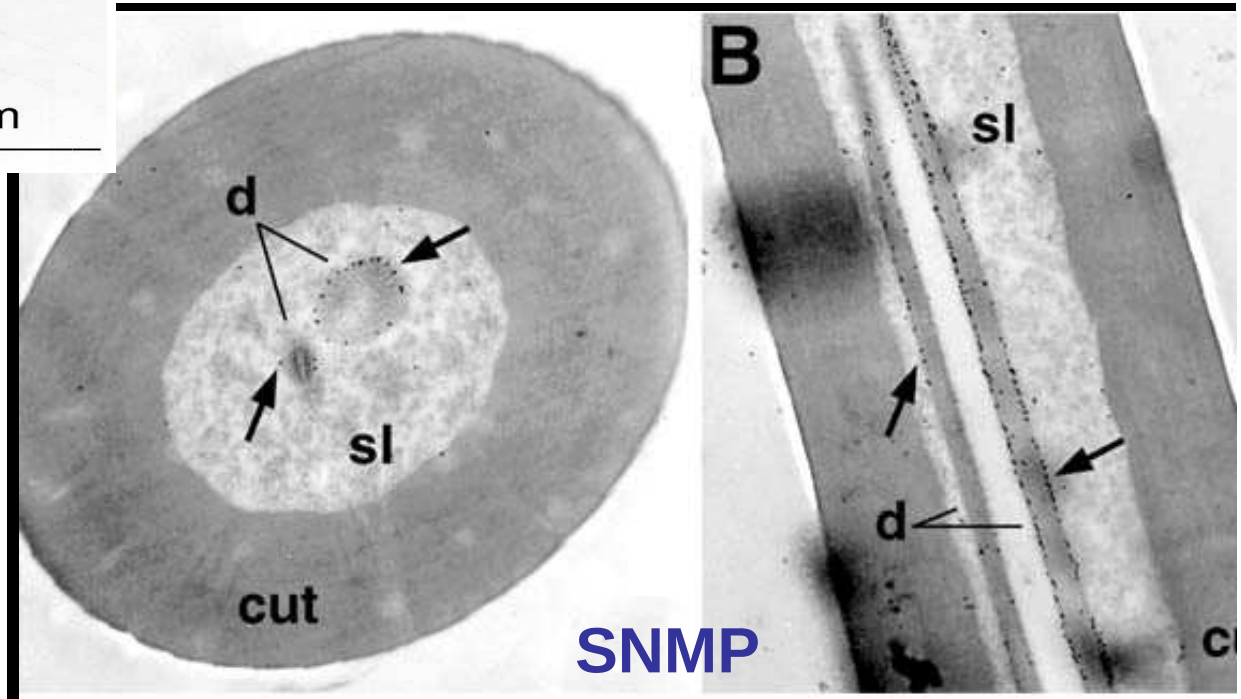
receptor molecules		6000
30-pS ion channels	(el. anal.)	>20
SNMP molecules	(gold lab.)	>300

relative numbers

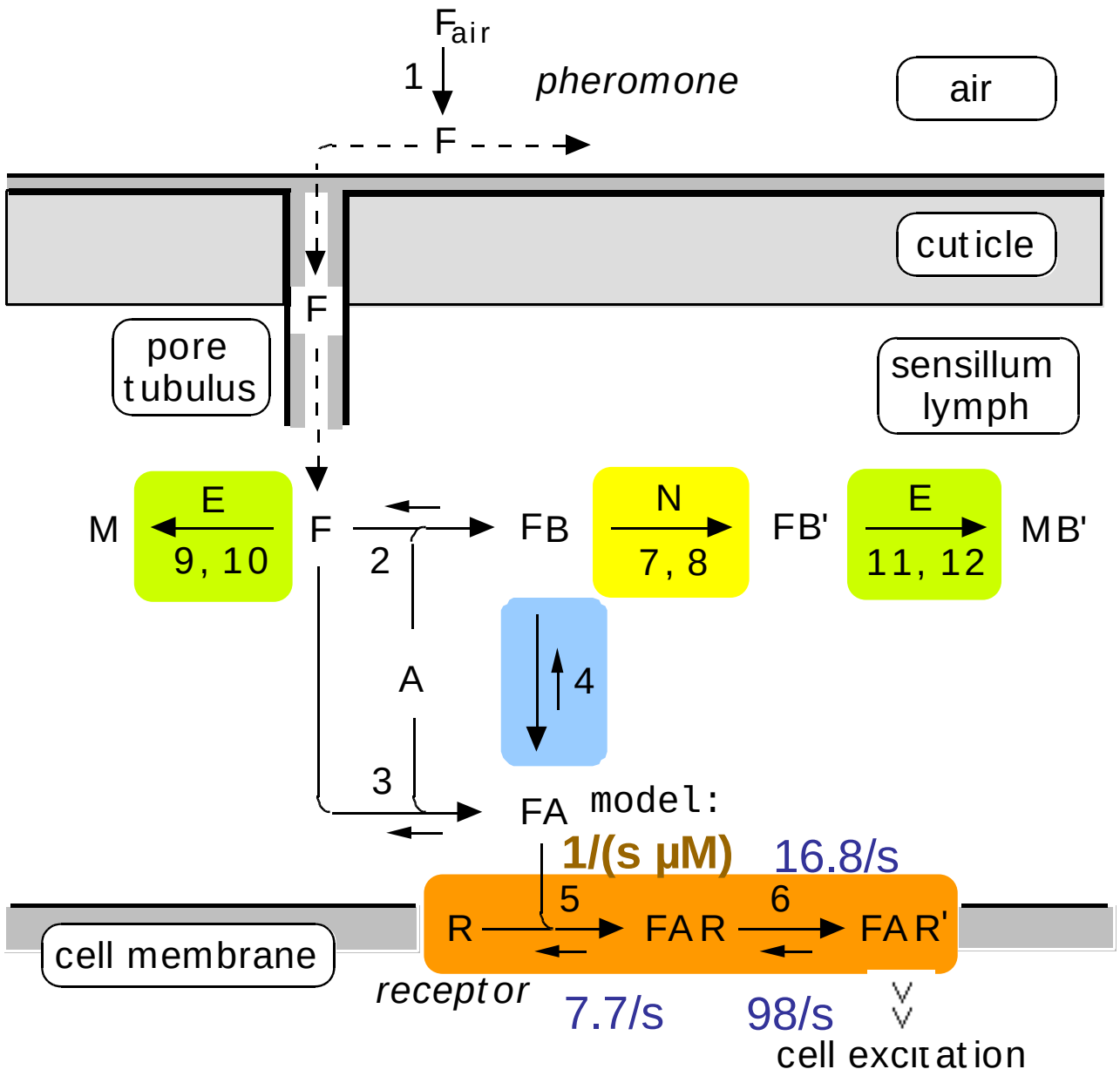
PBP molecules	14,000,000
DTFP for blocking	10,000

Antheraea polyphemus



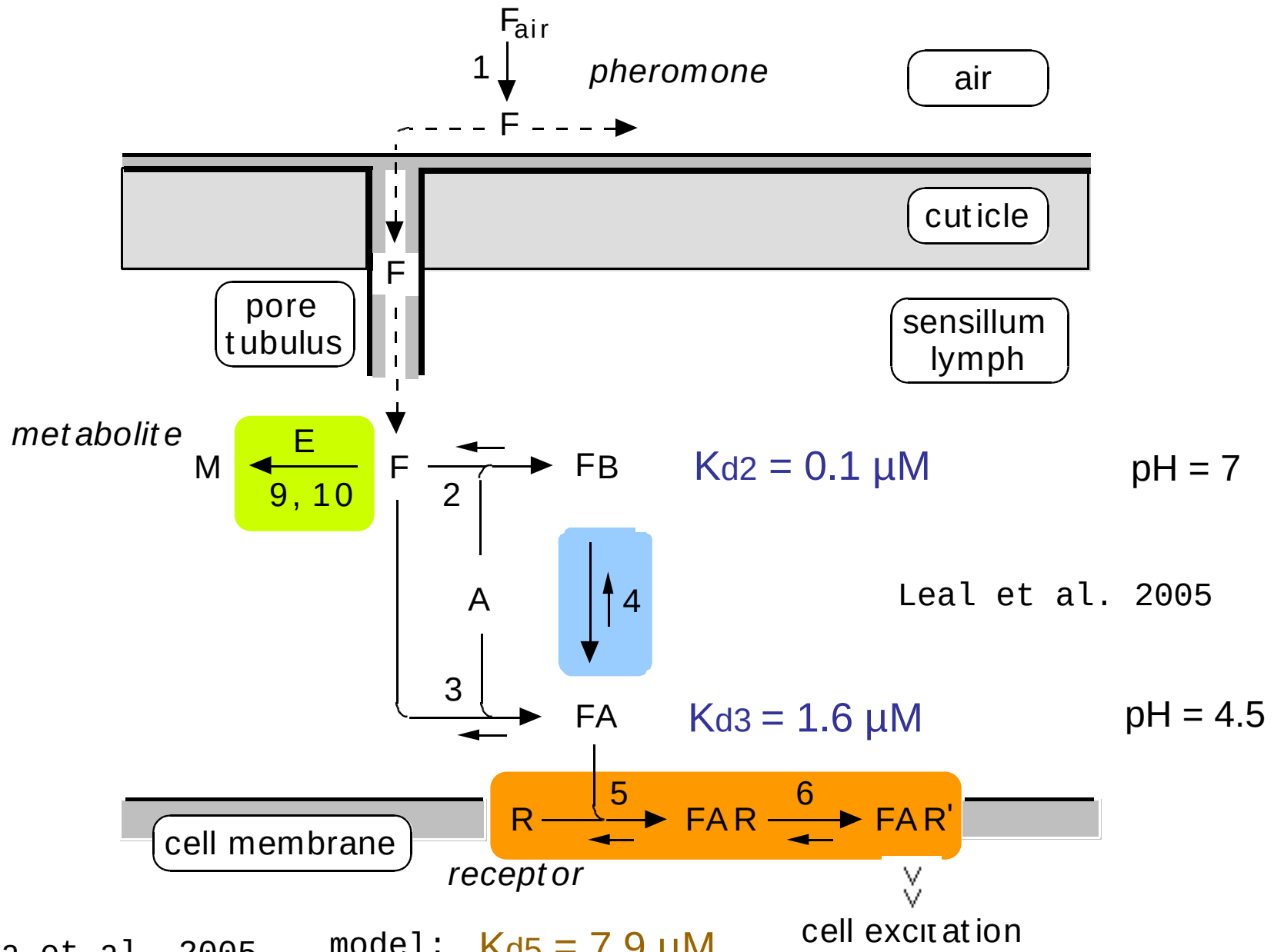


Steinbrecht et al. 1995


F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
M
E
9, 10
F
2
FB
N
7, 8
FB'
E
11, 12
MB'
A
4
3
FA
model:
1/(s µM)
16.8/s
5
6
R
FAR
FAR'
cell membrane
receptor
7.7/s
98/s
cell excitation

$$k_5 = \frac{k_{-5} \cdot Q_4 \cdot k_{fall}}{U_{sat} \cdot Q_1 \cdot Q_6} = \frac{0.974}{s \cdot mM}$$

$k_{-5} = 7.7/s$	Minor & Kaissling 2003
$Q_4 = k_{-6}/(k_6 + k_{-6}) = 0.854$	
$k_{fall} = \ln 2 / t_{1/2\,FAR'\,fall} = 0.87/s$	Kaissling 2009
$U_{sat} = 30\ mM/s$	
$Q_1 = 0.83$	Kasang 1971 - 1989
$Q_6 = 1/(K_4 \cdot C + 1) = 0.236$	Kaissling 2009
$K_4 = \frac{k_{-4}}{k_4} = \frac{24/s}{74/s} = 0.32$	Leal et al., 2005
$C = 10$	Kaissling 2009, Keil 1984

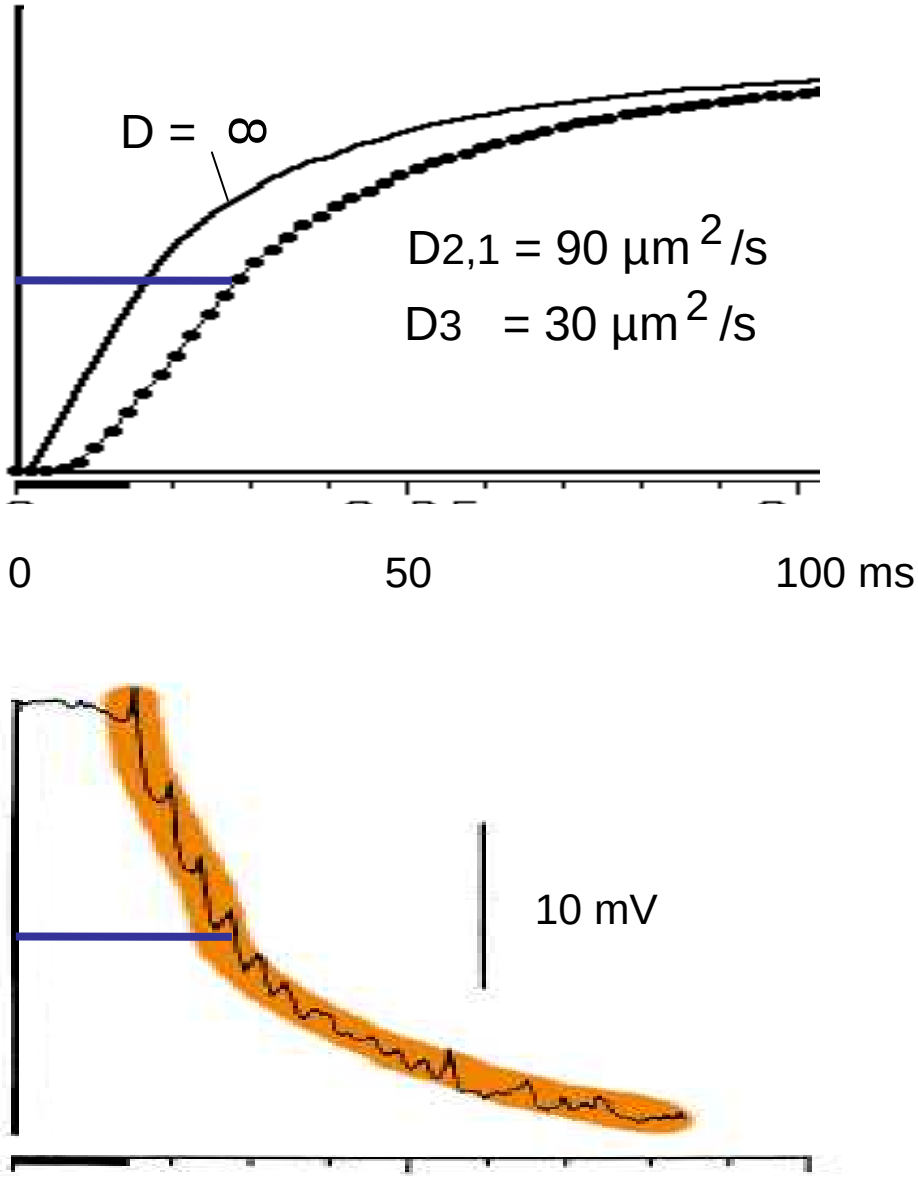

F air
1
pheromone
air
F
cuticle
F
pore tubulus
sensillum lymph
metabolite
M
E
9, 10
F
FB
Kd2 = 0.1 µM
pH = 7
2
A
4
Leal et al. 2005
3
FA
Kd3 = 1.6 µM
pH = 4.5
5
6
R
FAR
FAR'
cell membrane
receptor
cell excitation
Nakagawa et al. 2005
EC50 = 1.5 µM
model:
Kd5 = 7.9 µM
(EC50 = 6.8 µM)
Kaissling 2001

Antheraea polyphemus

model

receptor potential

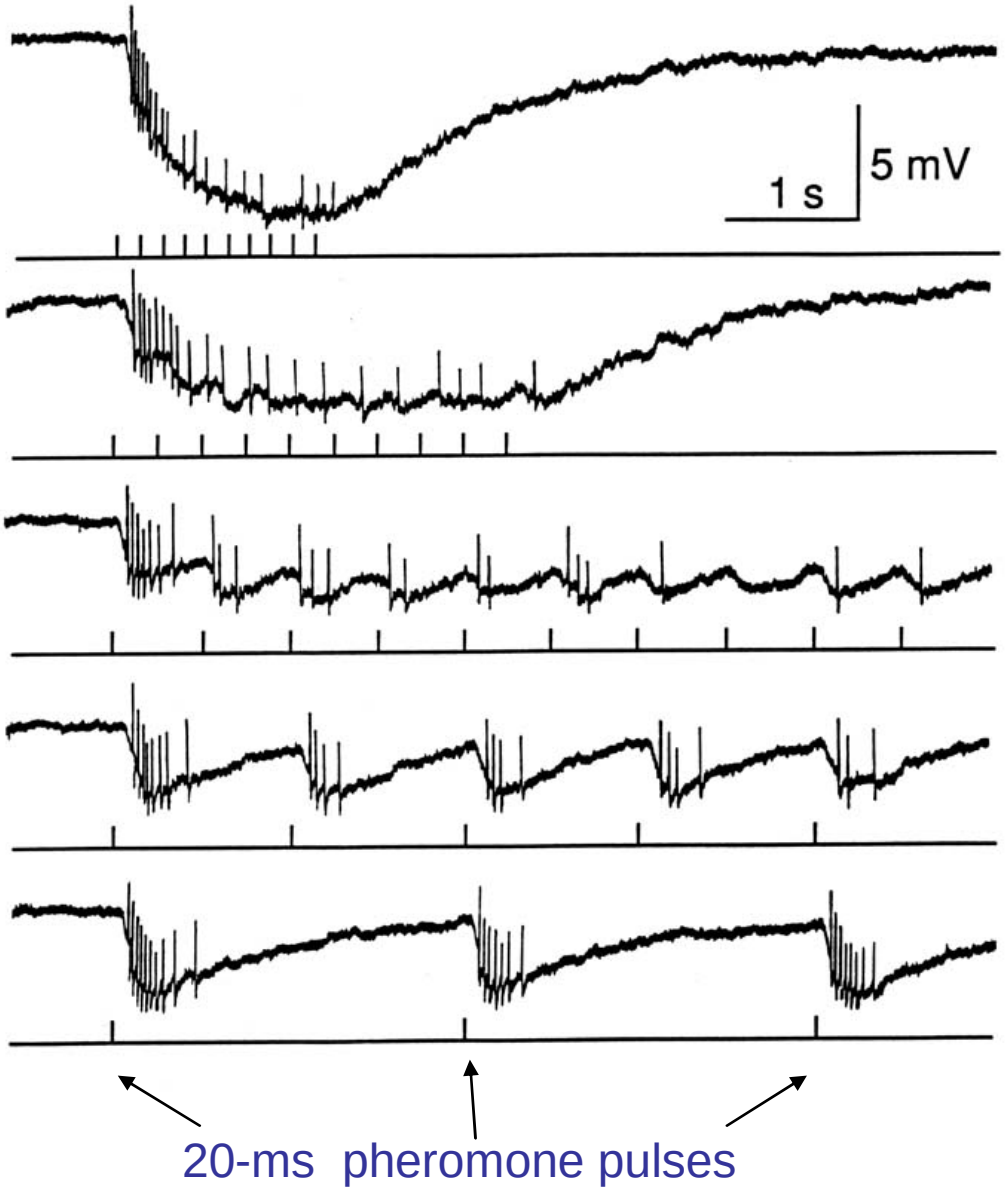
neuron



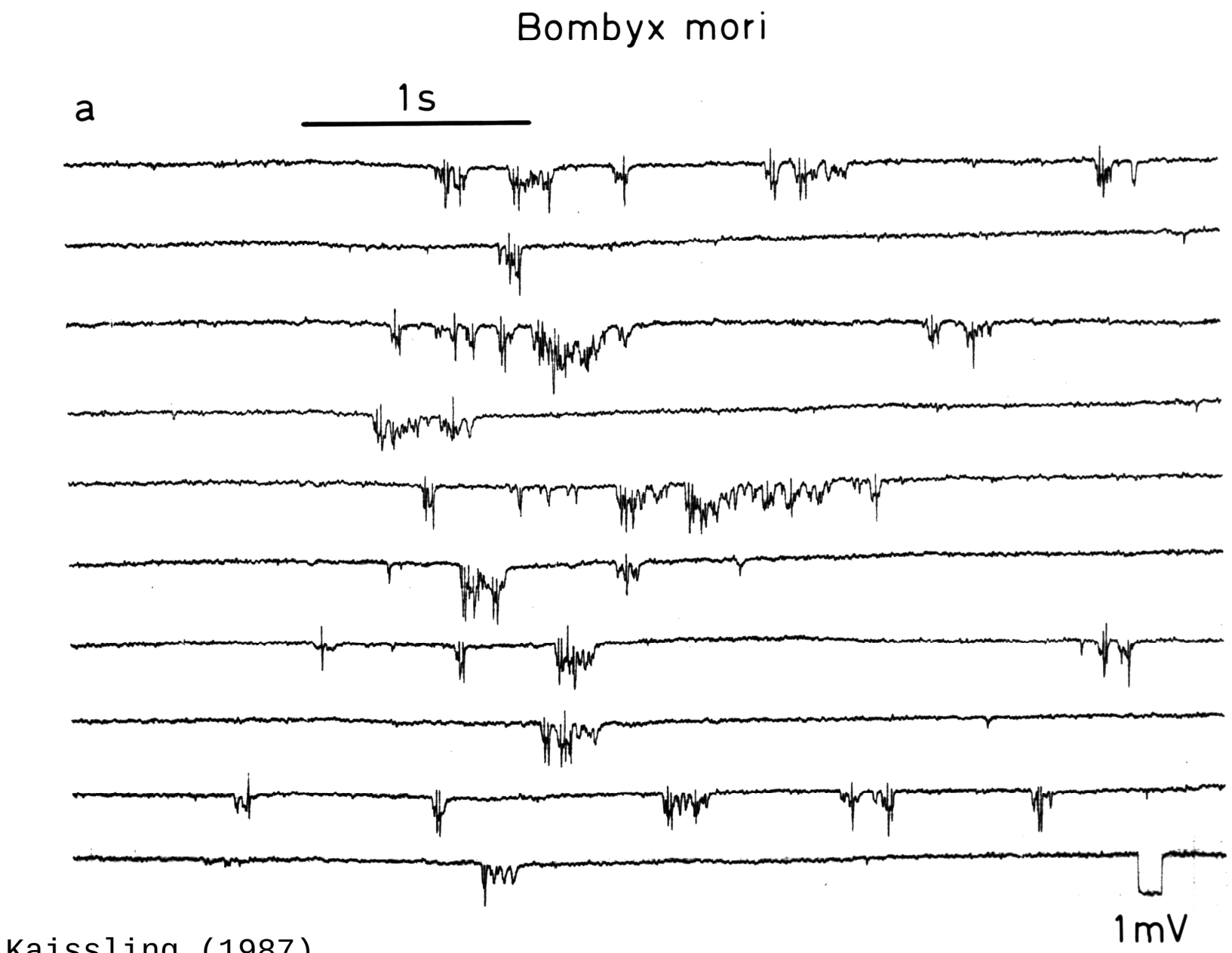
pheromone stimulus: 15 ms

Kaissling 2001

Antheraea pernyi

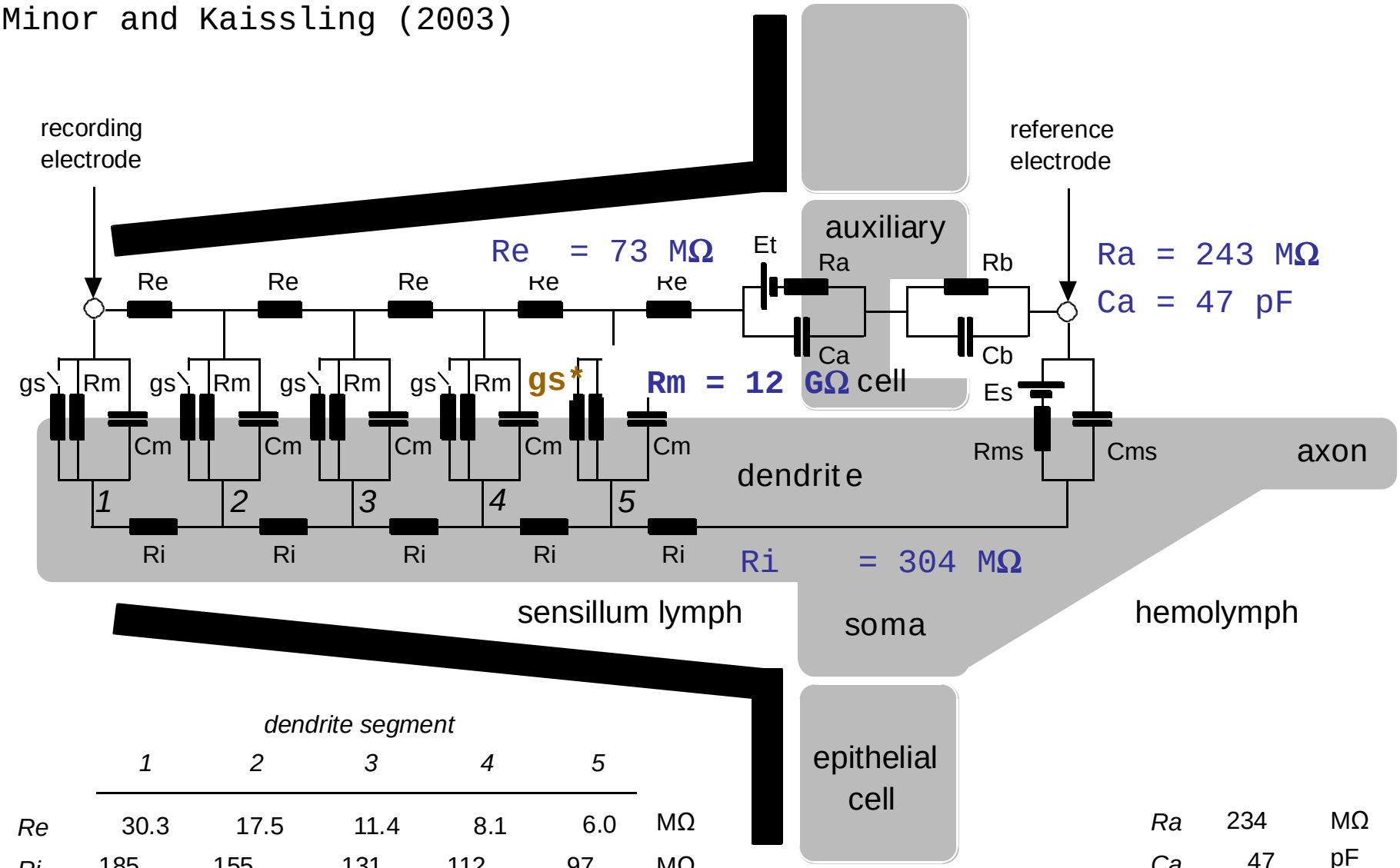


B. Pophof



Kaissling (1987)

Minor and Kaissling (2003)



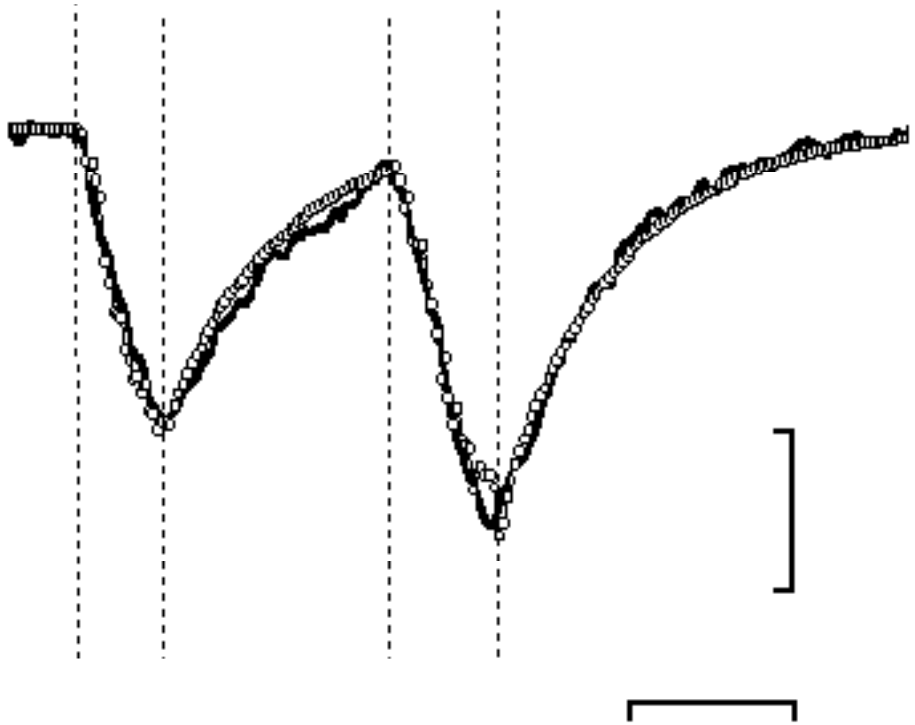
dendrite segment

	1	*2*	*3*	*4*	*5*	
Re	30.3	17.5	11.4	8.1	6.0	MΩ
Ri	185	155	131	112	97	MΩ
Rm	71	65	60	56	52	GΩ
Cm	0.105	0.115	0.125	0.135	0.145	pF

*gs** conductance increase during one bump **gs* = 30 pS**

Rms	440	MΩ
Cms	2.3	pF
Es	46	mV

Ra	234	MΩ
Ca	47	pF
Rb	16	MΩ
Cb	19	pF
Et	30	mV



$k_{23} = \mathbf{p}/\mathbf{D_2}$

$k_{32} = 1/\mathbf{D_3}$

$k_{21} = (1-\mathbf{p})/\mathbf{D_2}$

$\mathbf{p} = 1 - 1/N$

3

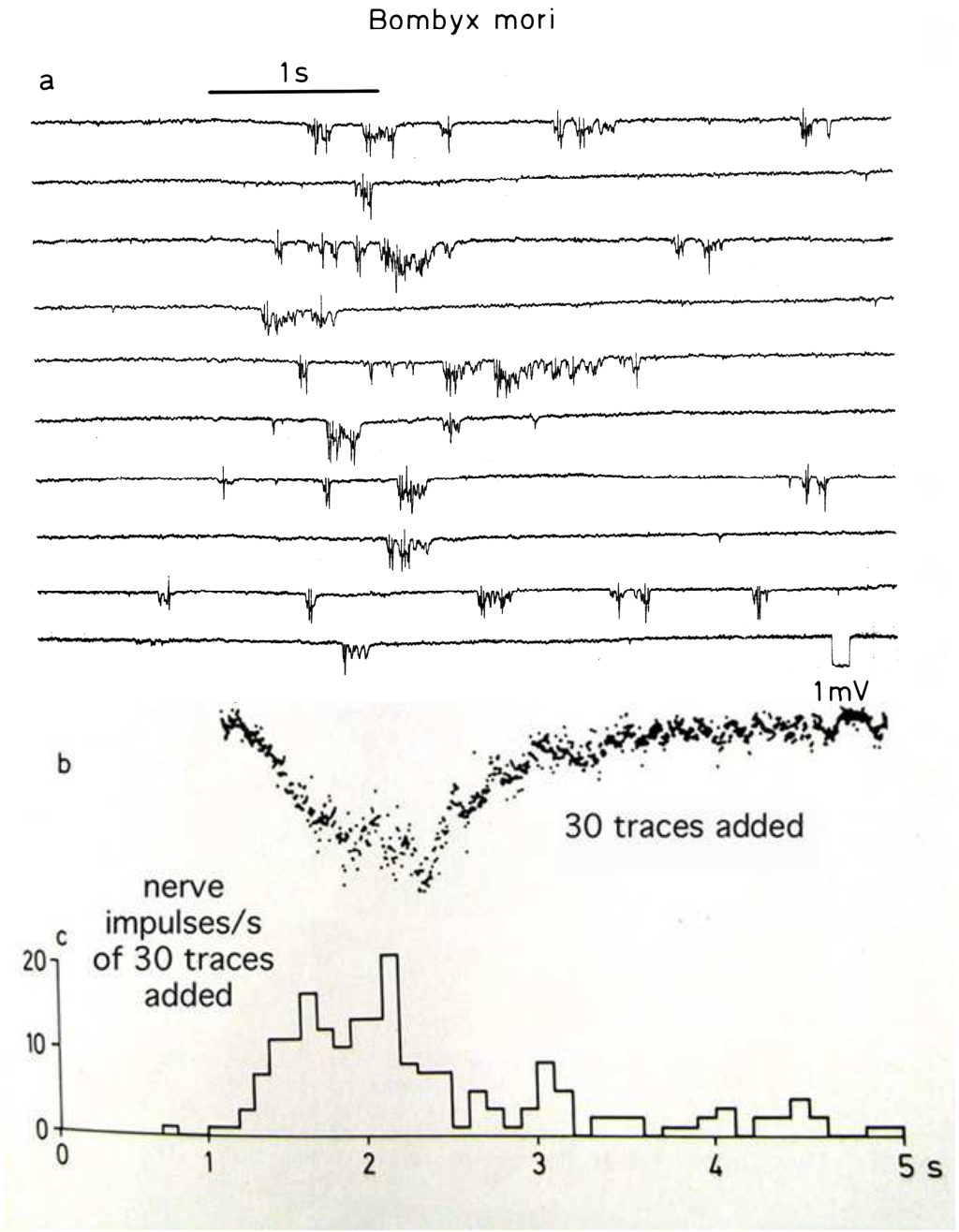
2

1

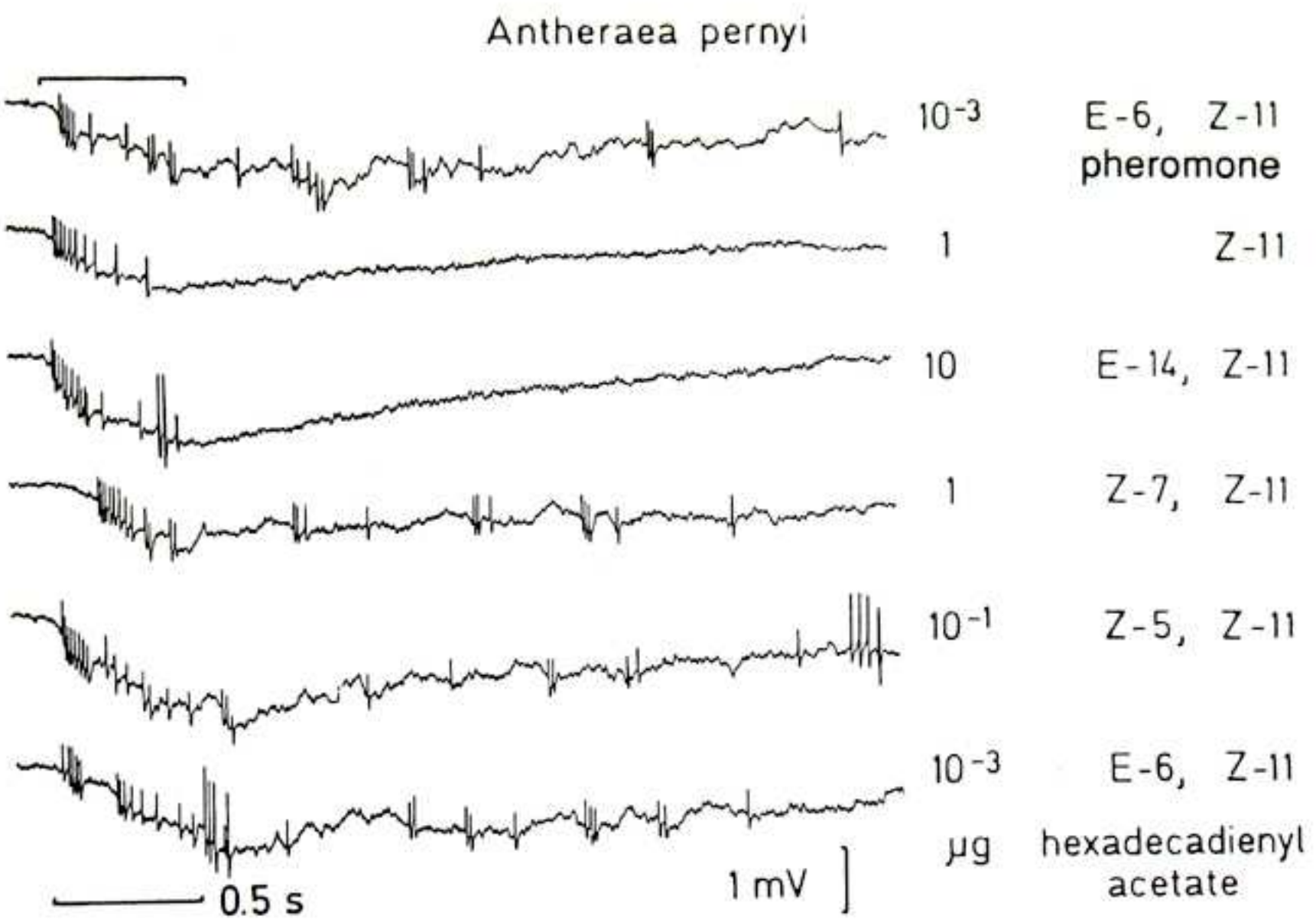
$t_c = (D_2 + pD_3)/(1 - p)$

$= 153$ ms

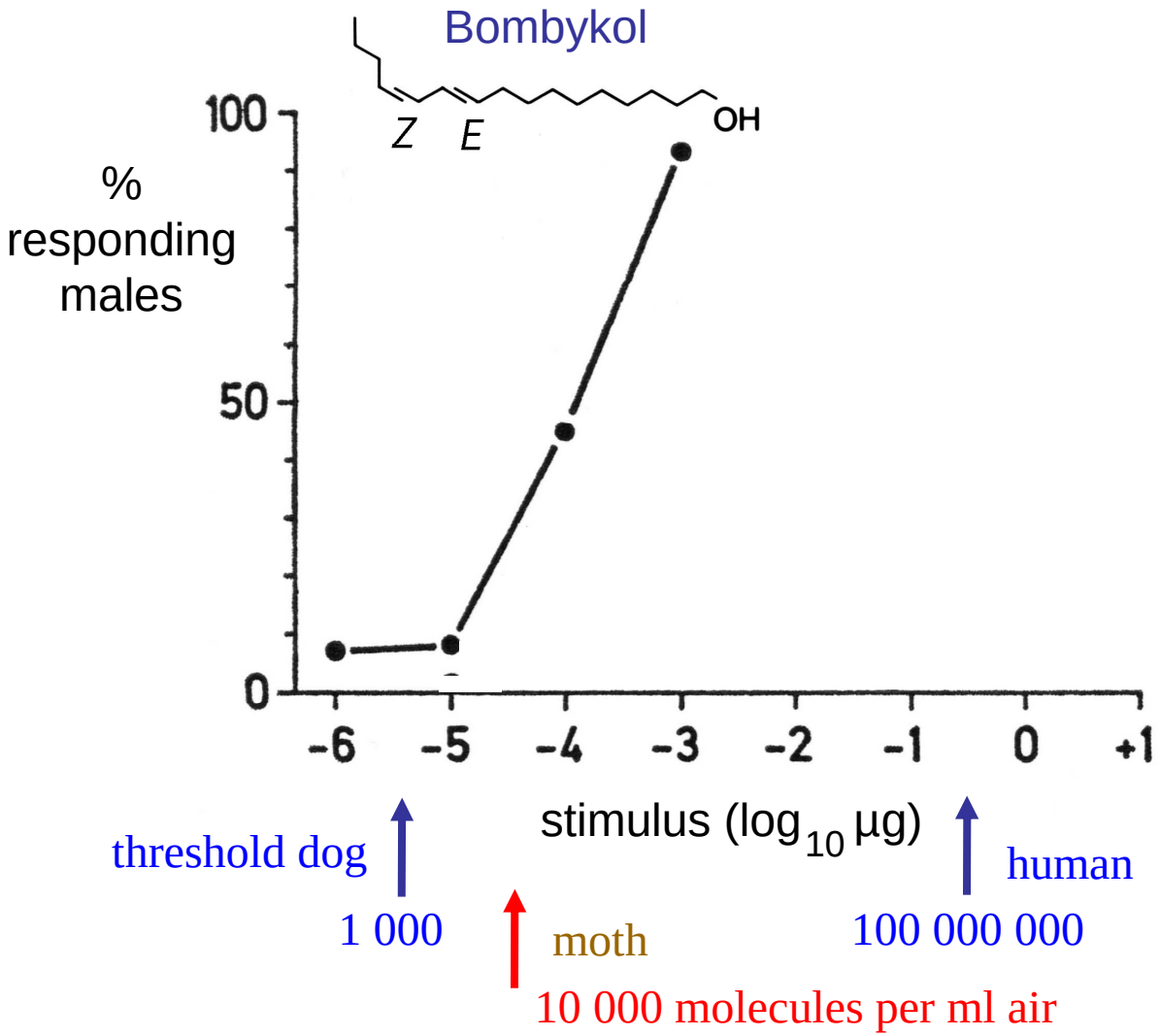
Minor & Kaissling 2003

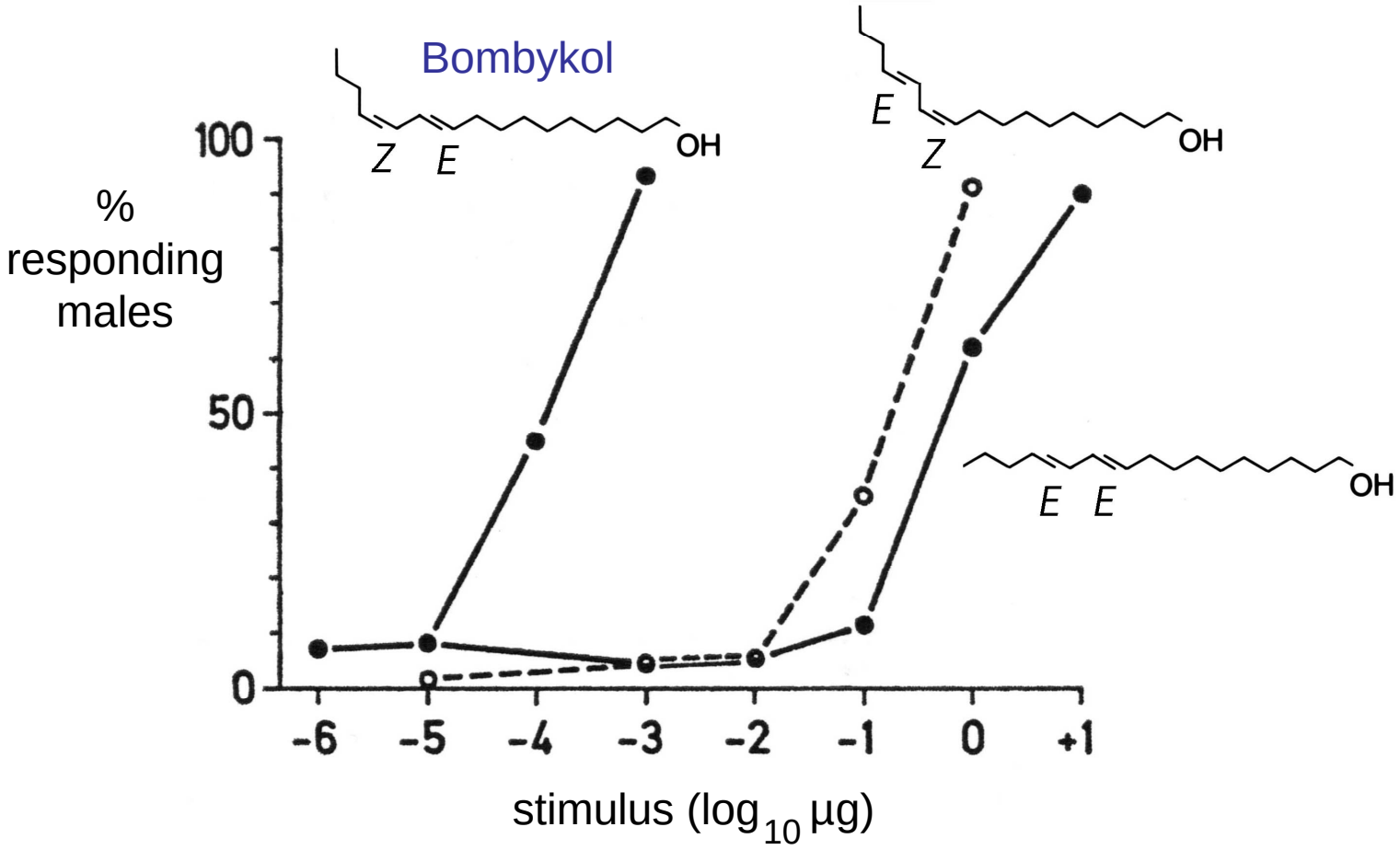


Kaissling 1987



Kaissling 1977


Bombykol
Z E
OH
100
50
0
% responding males
-6 -5 -4 -3 -2 -1 0 +1
stimulus (log10 µg)
threshold dog
1 000
moth
10 000 molecules per ml air
human
100 000 000



Kaissling 1987

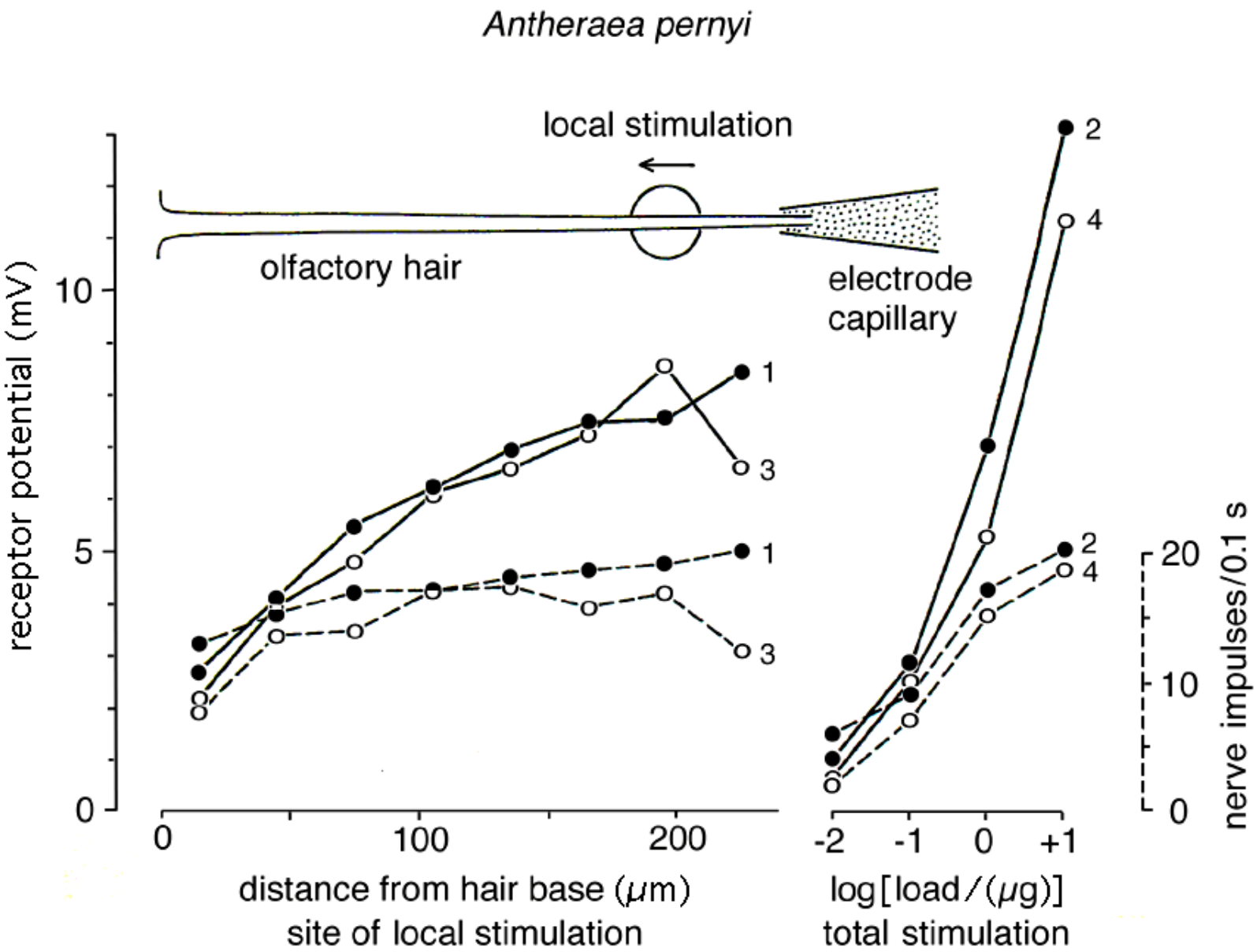
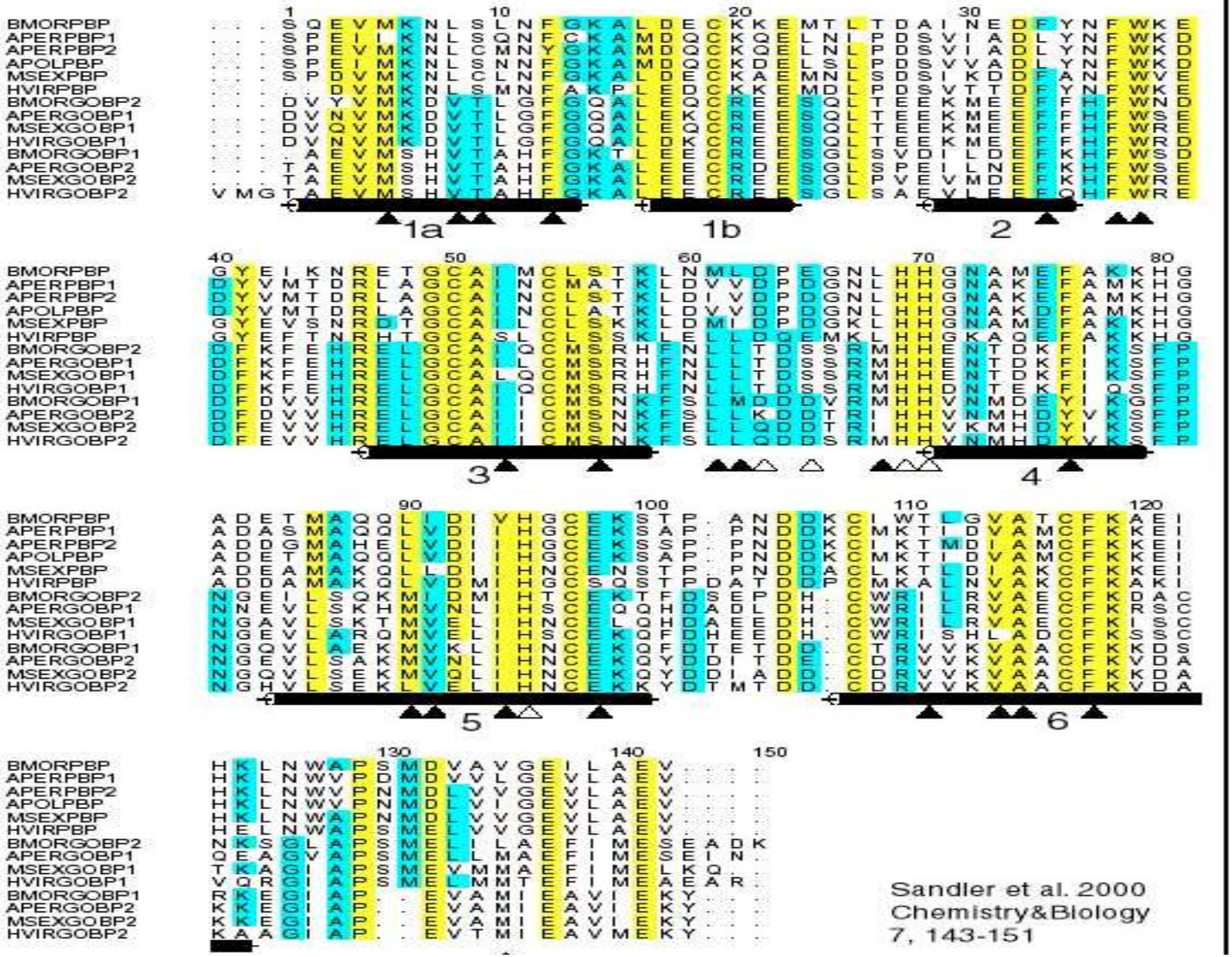
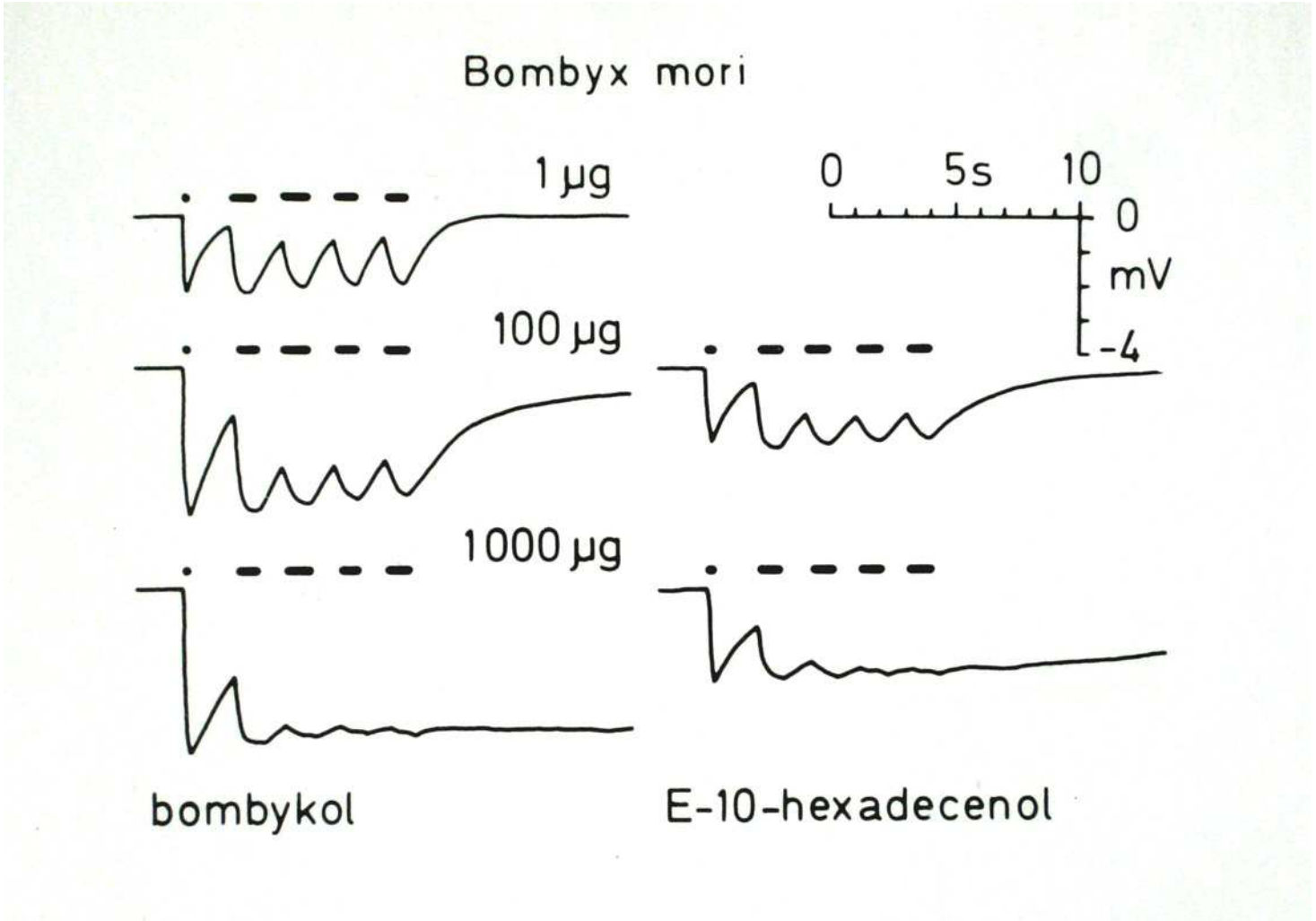


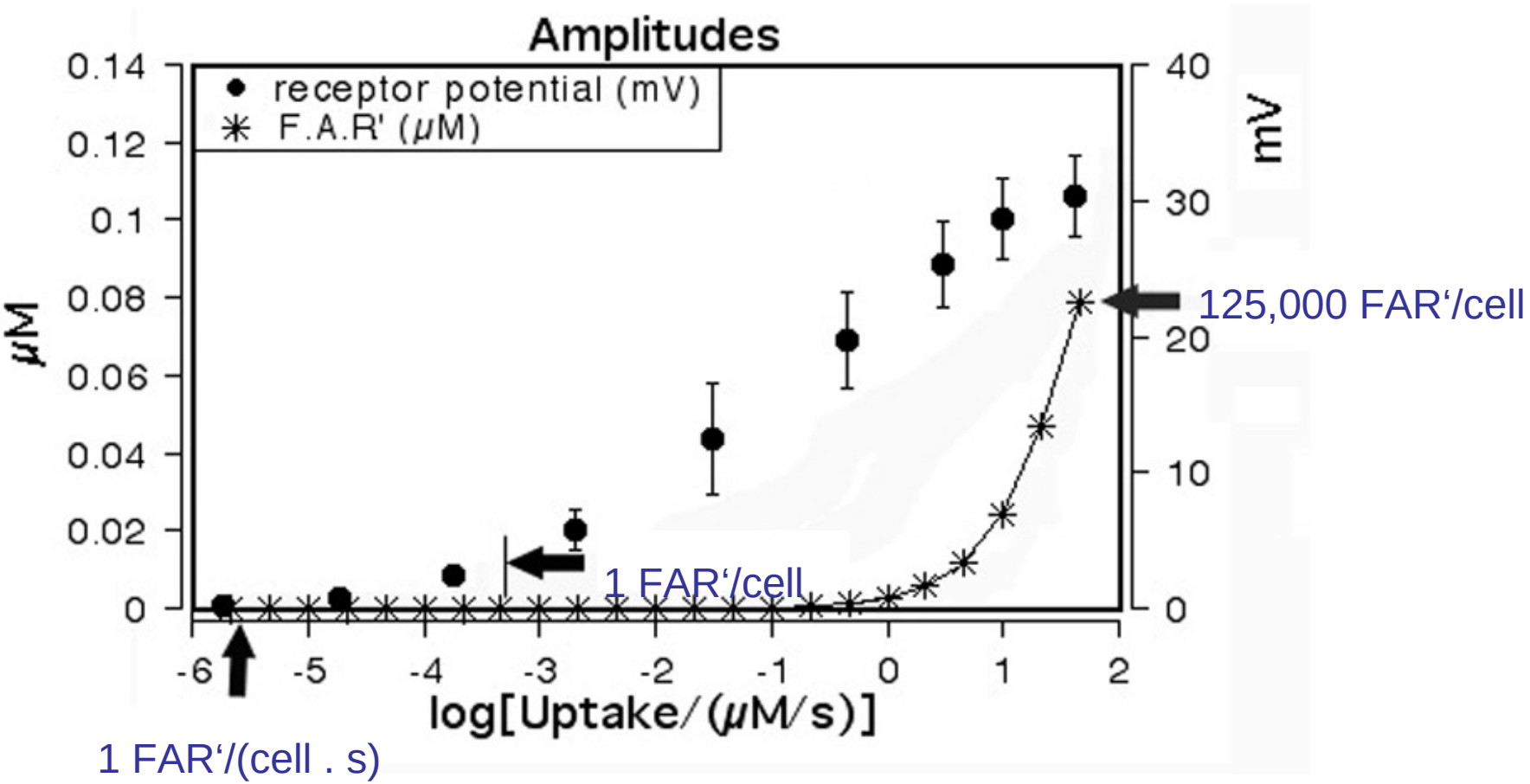
Fig. 13

Kaissling 2009


BMORPBP
APERPBP1
APERPBP2
APOLPBP
MSEXPBP
HVIRPBP
BMORGOBP2
APERGOBP1
MSEXGOBP1
HVIRGOBP1
BMORGOBP1
APERGOBP2
MSEXGOBP2
HVIRGOBP2
1a
1b
2
3
4
5
6
Sandler et al. 2000
Chemistry&Biology
7, 143-151



Kaissling 1971


Amplitudes
receptor potential (mV)
F.A.R' (μM)
μM
mV
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
40
30
20
10
0
-6
-5
-4
-3
-2
-1
0
1
2
log[Uptake/(μM/s)]
1 FAR'/cell
125,000 FAR'/cell
1 FAR'/(cell . s)

stimulus source	males responding	conc. in air	within 2 s	within integration time (0.2 s)	
µg	%	molecules per ml	spikes per one cell	spikes per 17,000 cells	
				backgr.	noise
0		0	0.17	289	17
10^{-5}	40%	2,600	+ 0.03	signal + 51	
10^{-4}	80%	26,000	+ 0.309	signal + 525	

Possible functions of the
pheromone binding protein (PBP)

1 **solubilizes** the pheromone (Van den Berg, Ziegelberger)

a **transports** it through the sensillum lymph (**carrier**)

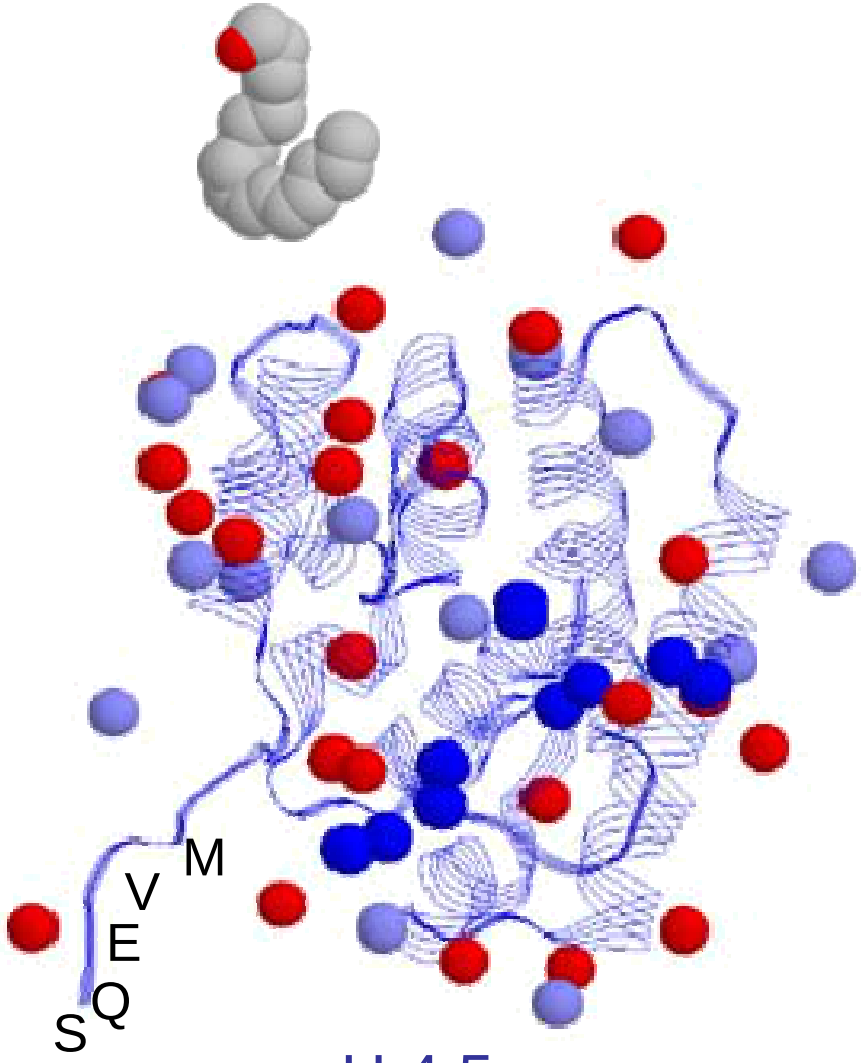
b **prevents** it from entering the cell membrane

2 **protects** the pheromone from enzymatic degradation (Vogt)

3 involved in pheromone-receptor **interaction** (Pophof)

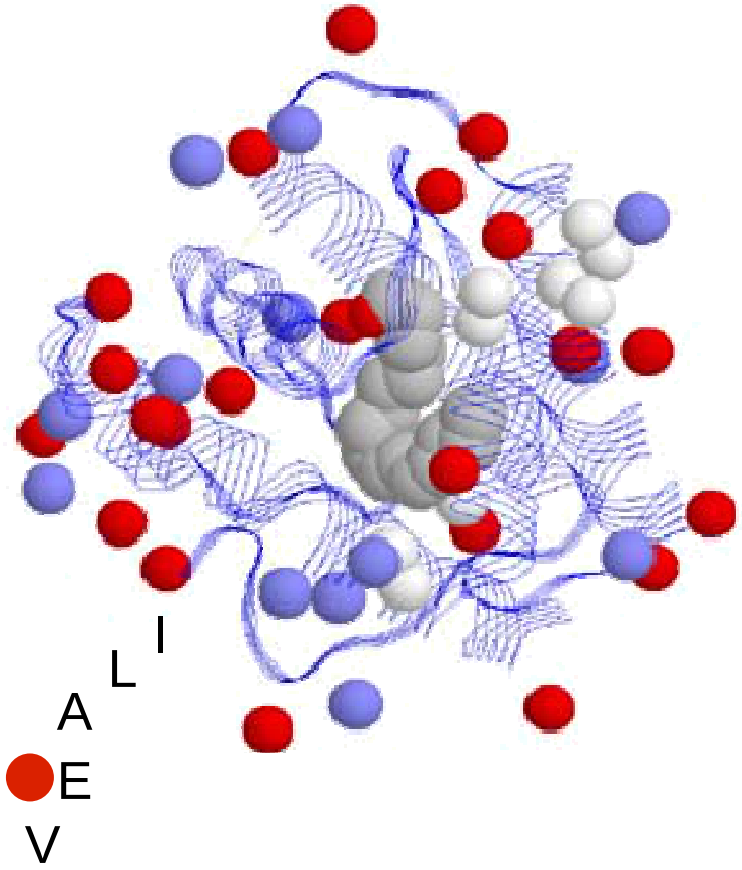
4 involved in pheromone deactivation (**scavenger**)

5 provides **organic anions** to the sensillum lymph



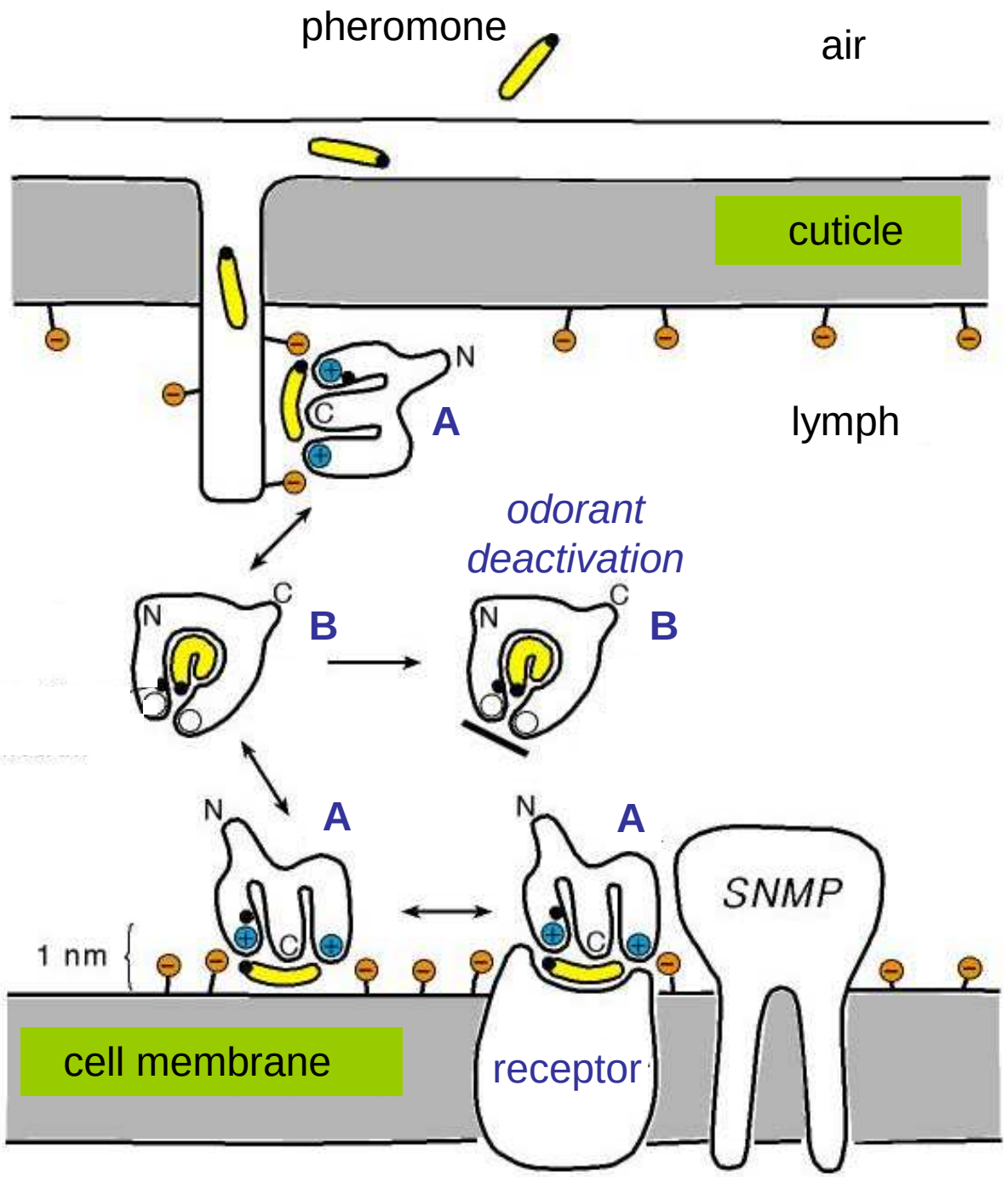
pH 4.5

22 - 19 +



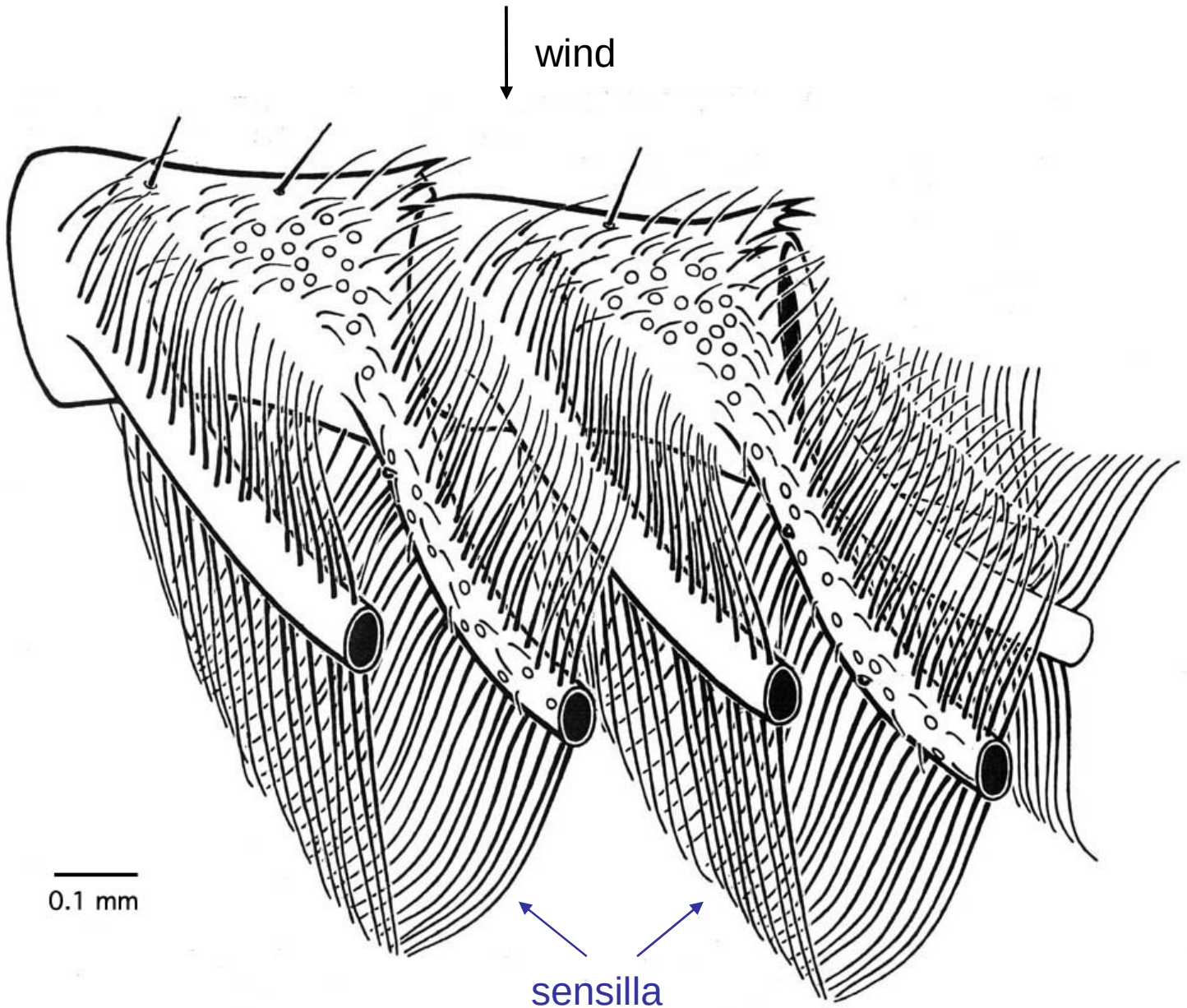
pH 7

22 - 14 +



Kaissling
& Leal 2004


E. Priesner


wind
0.1 mm
sensilla
tuned to pheromone