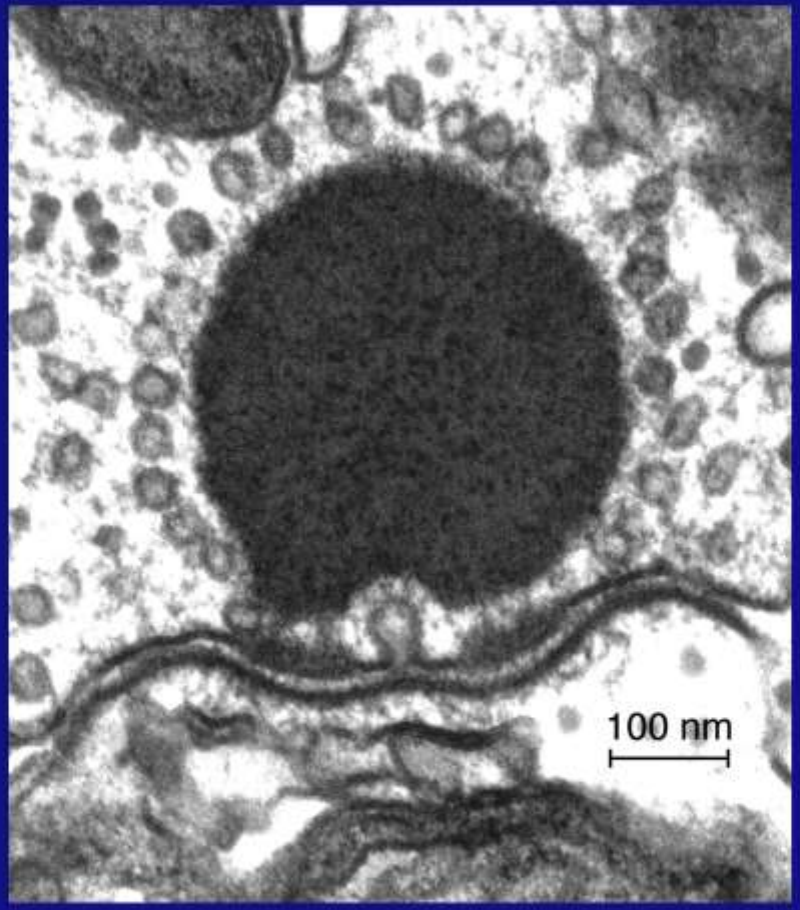
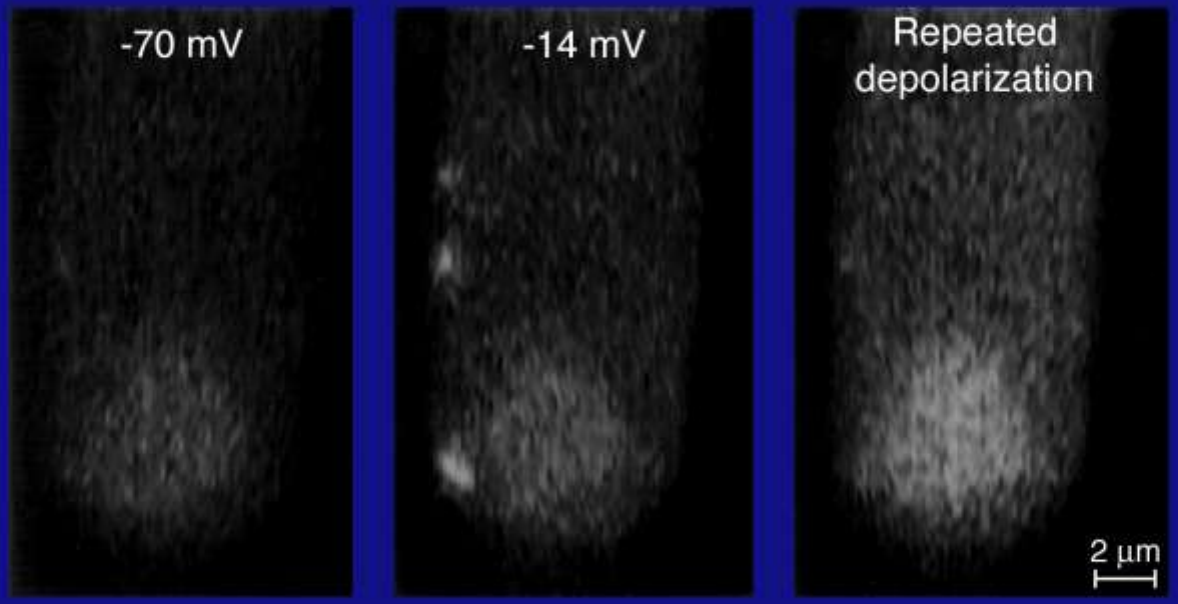
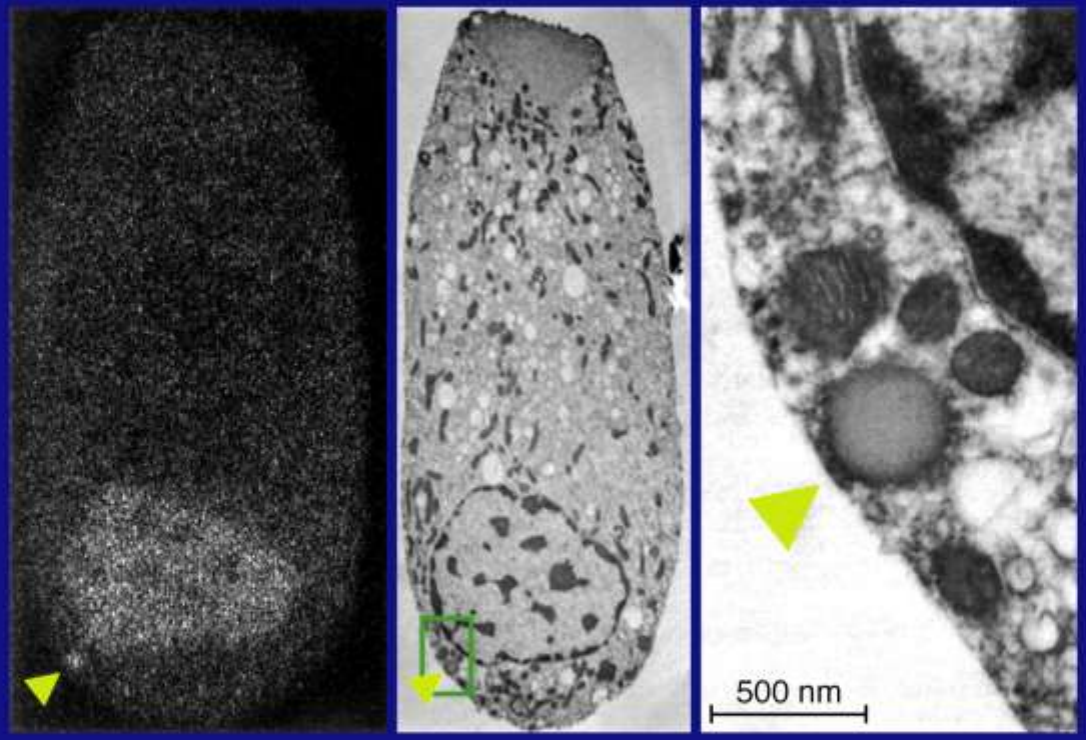
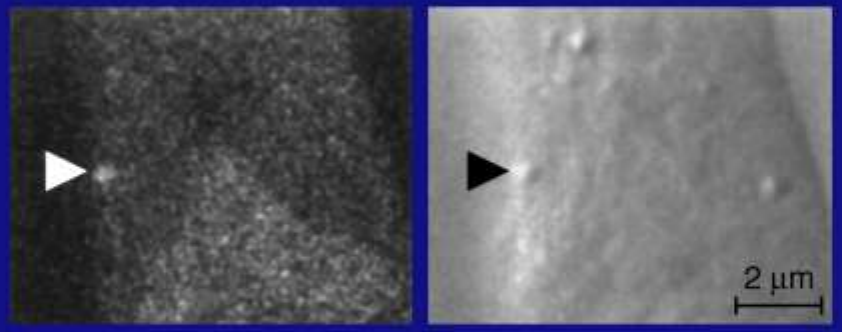
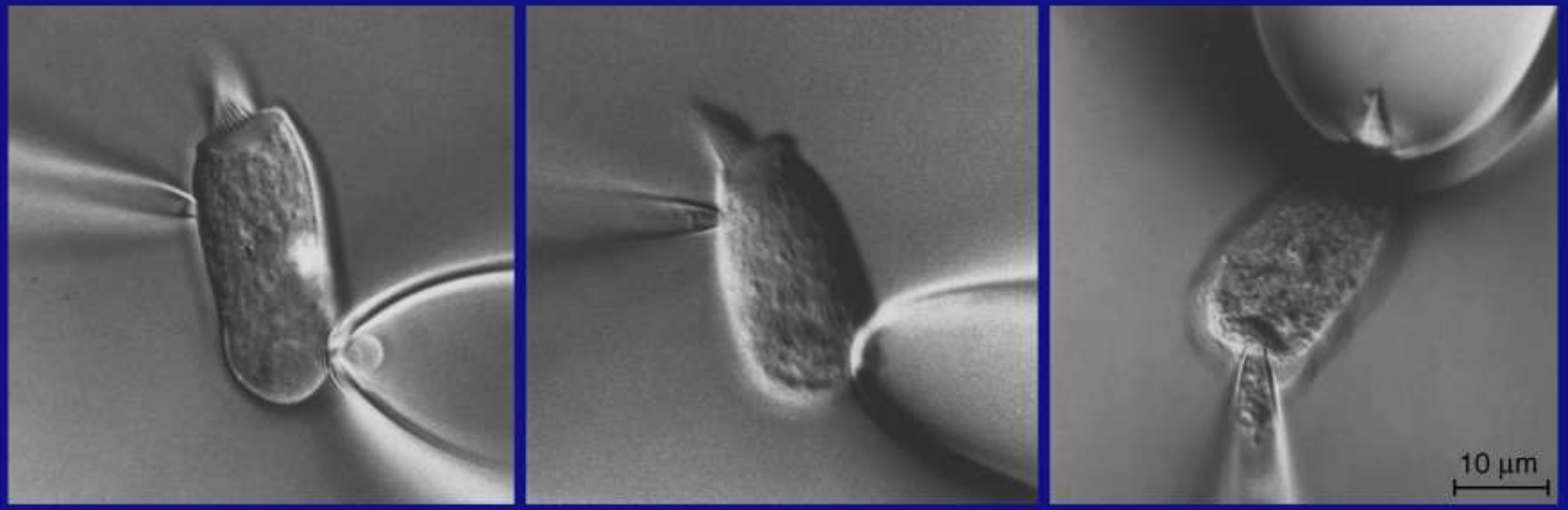
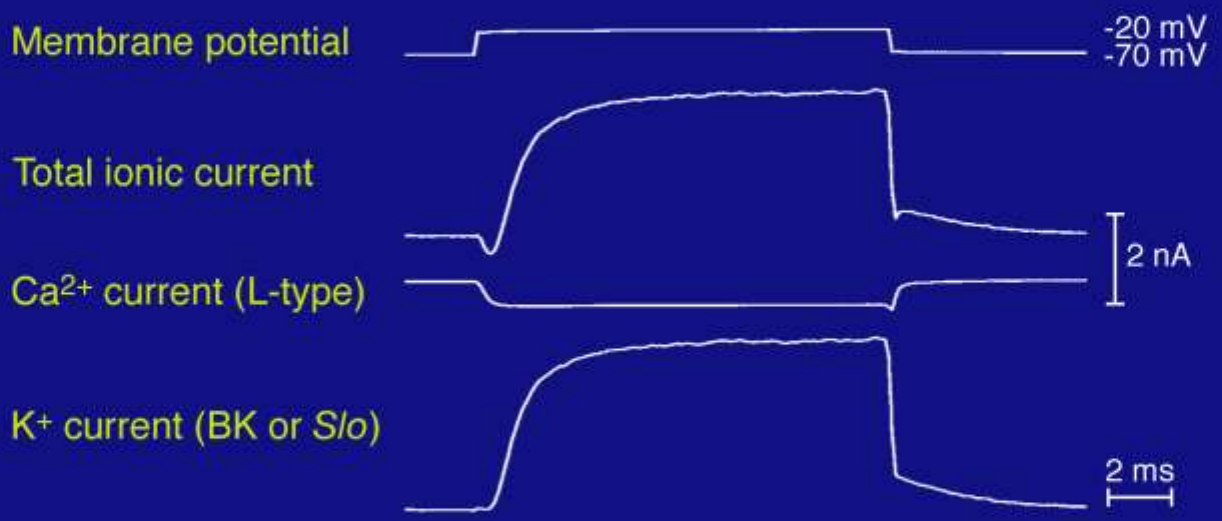

100 nm


-70 mV
-14 mV
Repeated
depolarization
2 µm

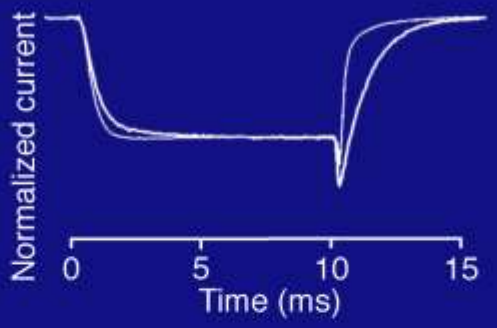

500 nm

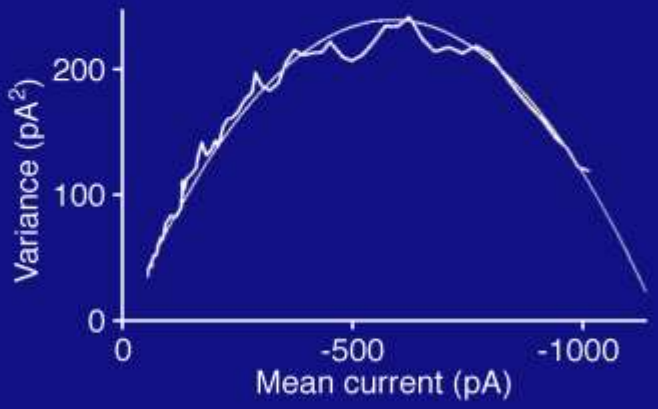

2 µm


10 µm


Membrane potential
-20 mV
-70 mV
Total ionic current
2 nA
Ca2+ current (L-type)
K+ current (BK or Slo)
2 ms

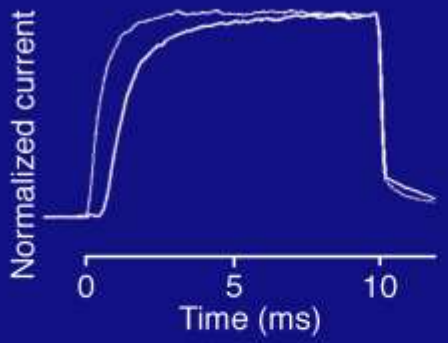
Ca^{2+} current

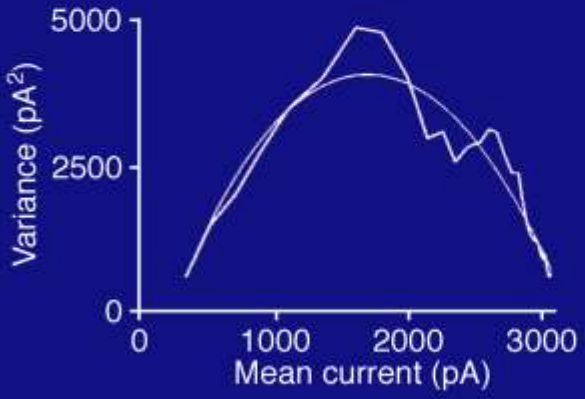




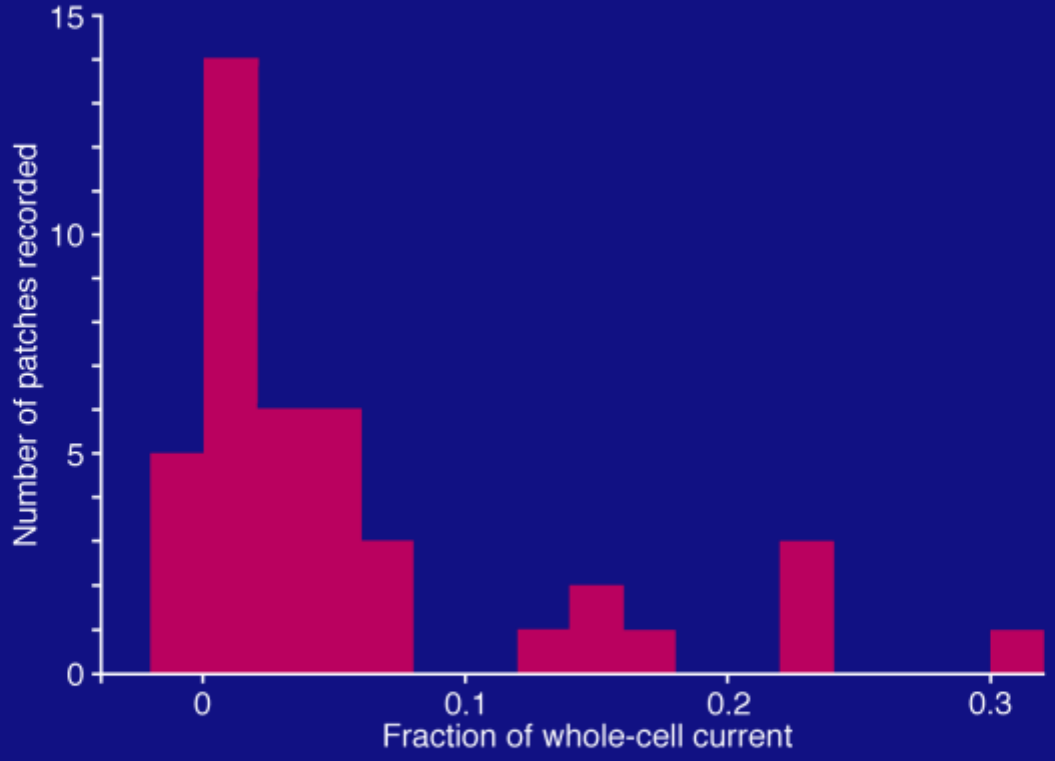
~1800 Ca^{2+} channels
per hair cell

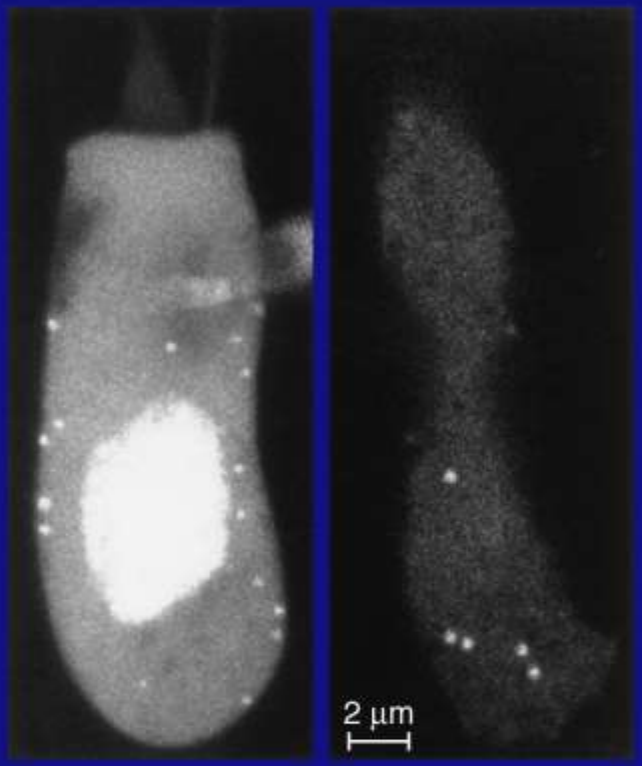
K^{+} current

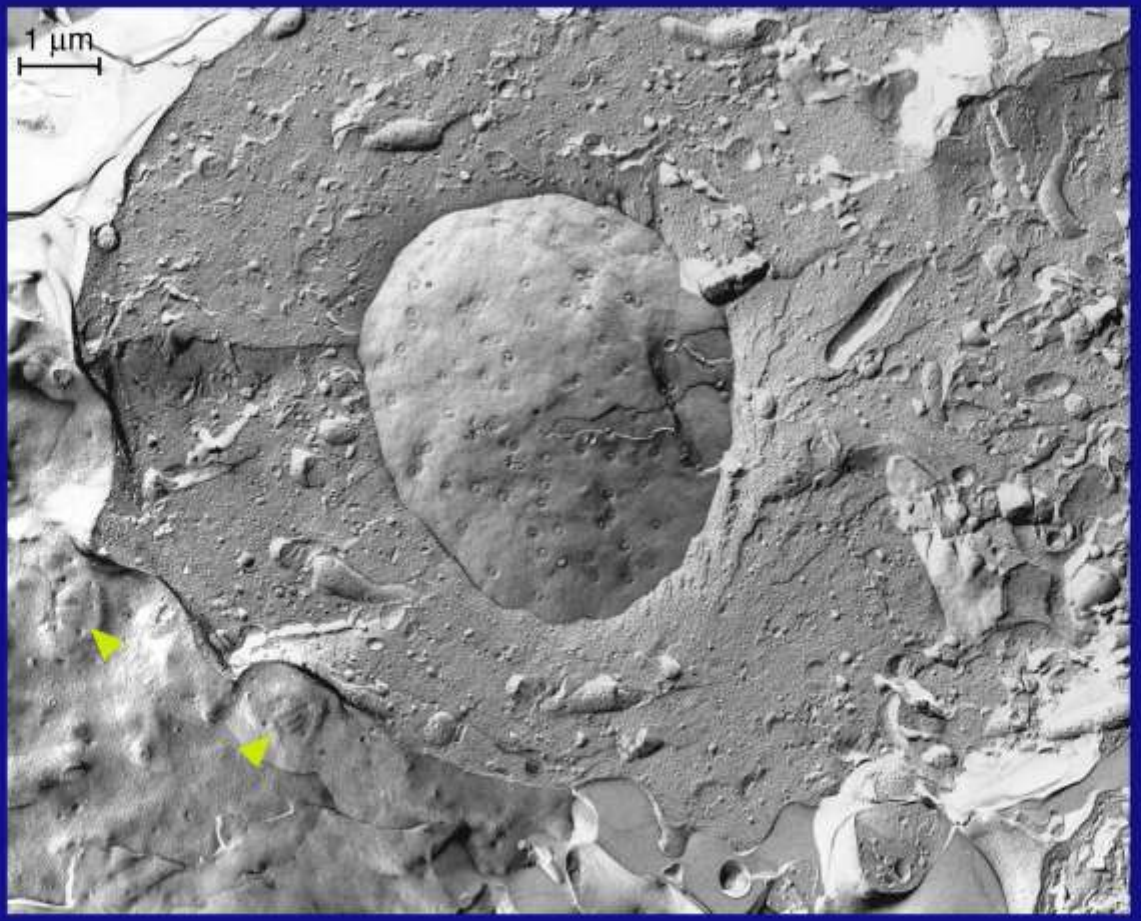


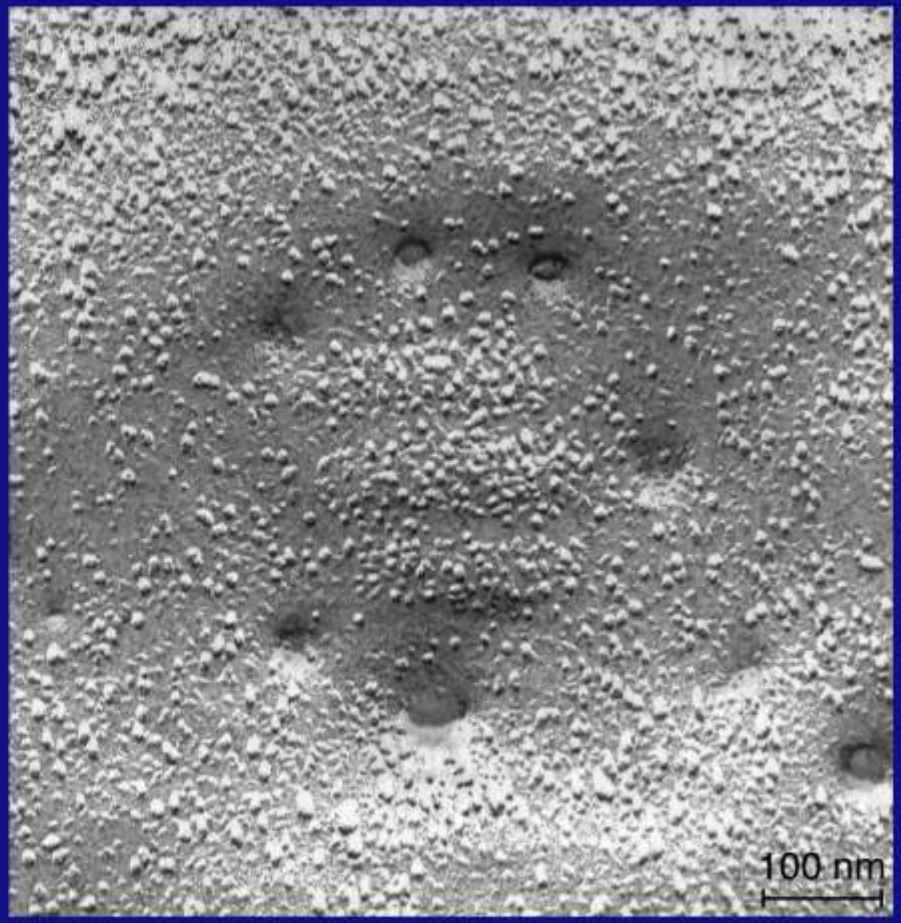


~800 K^{+} channels
per hair cell


15
10
5
0
Number of patches recorded
0
0.1
0.2
0.3
Fraction of whole-cell current

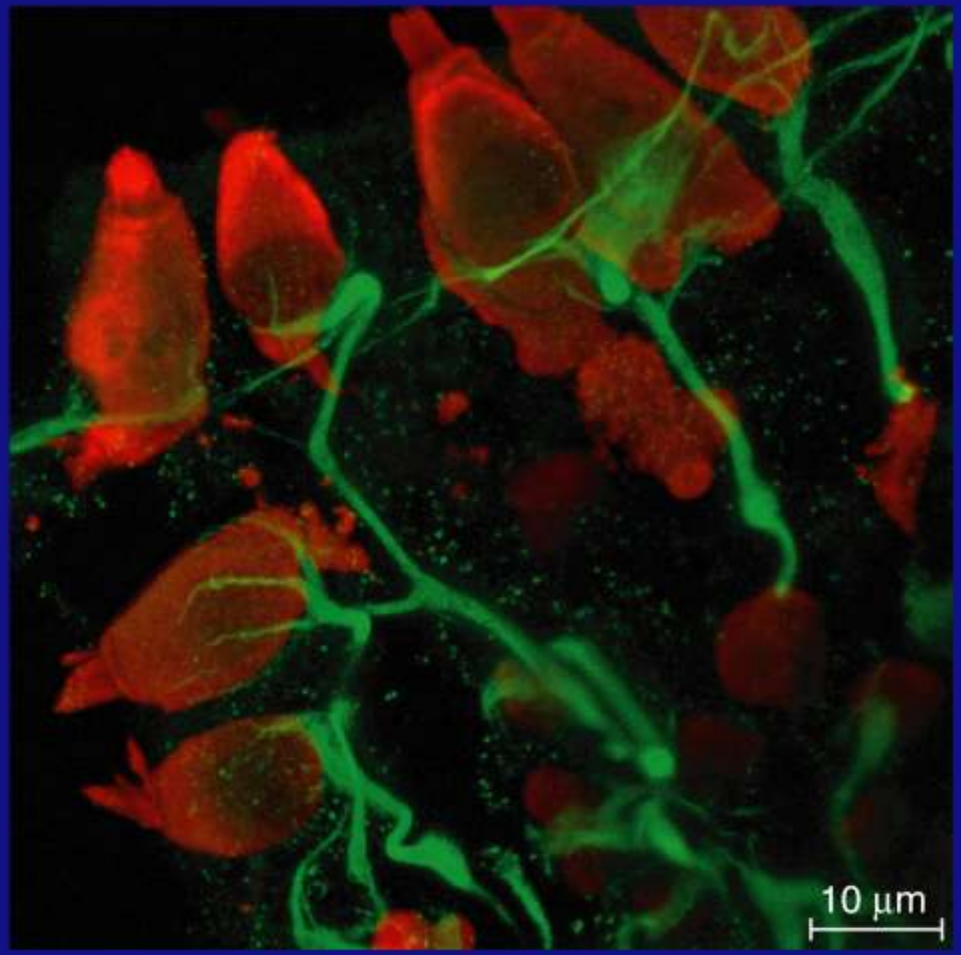

2 µm

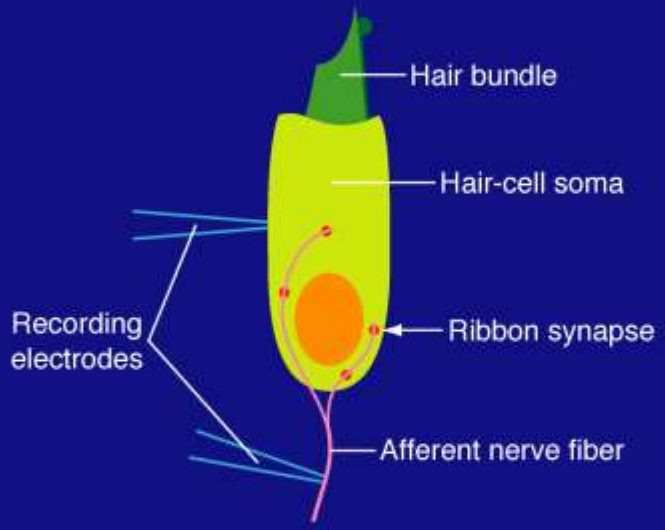

1 µm

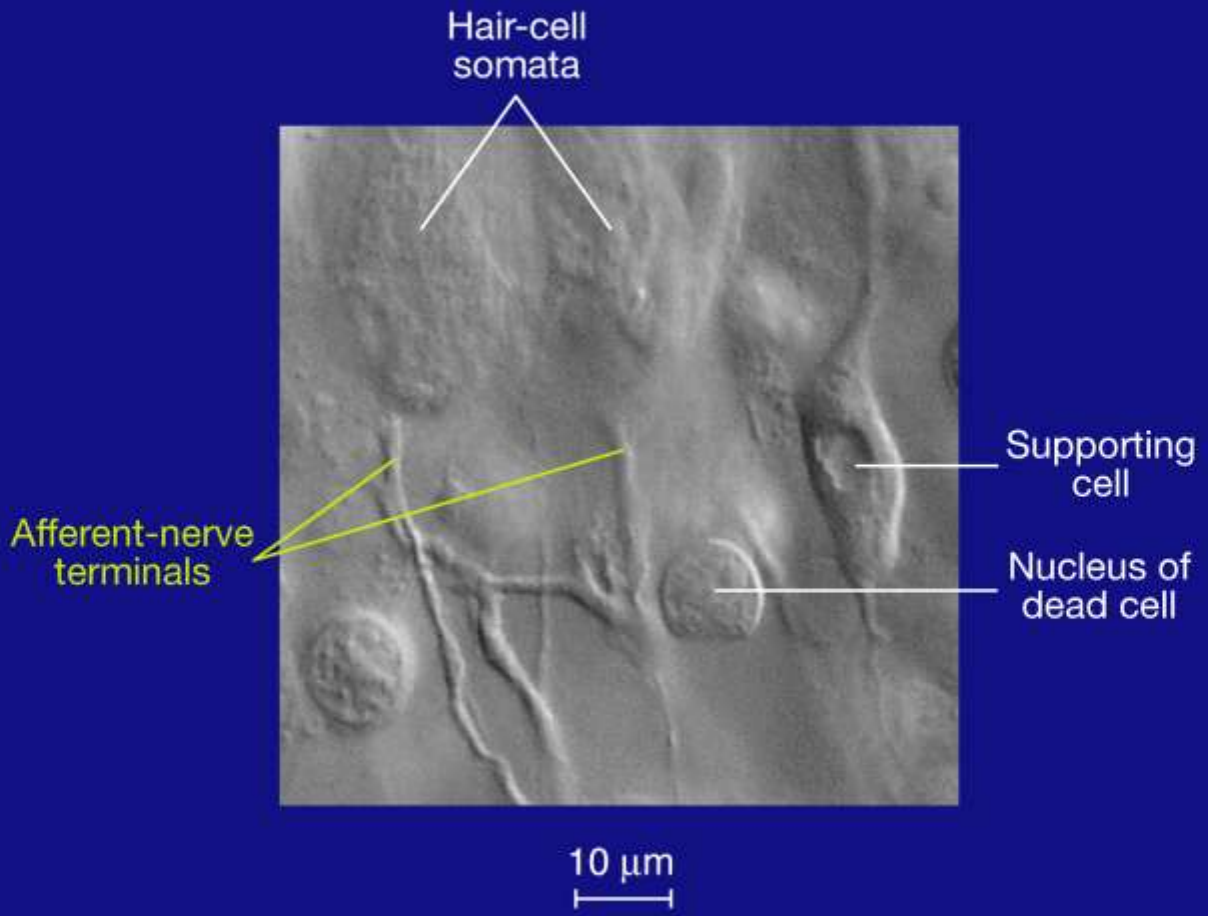


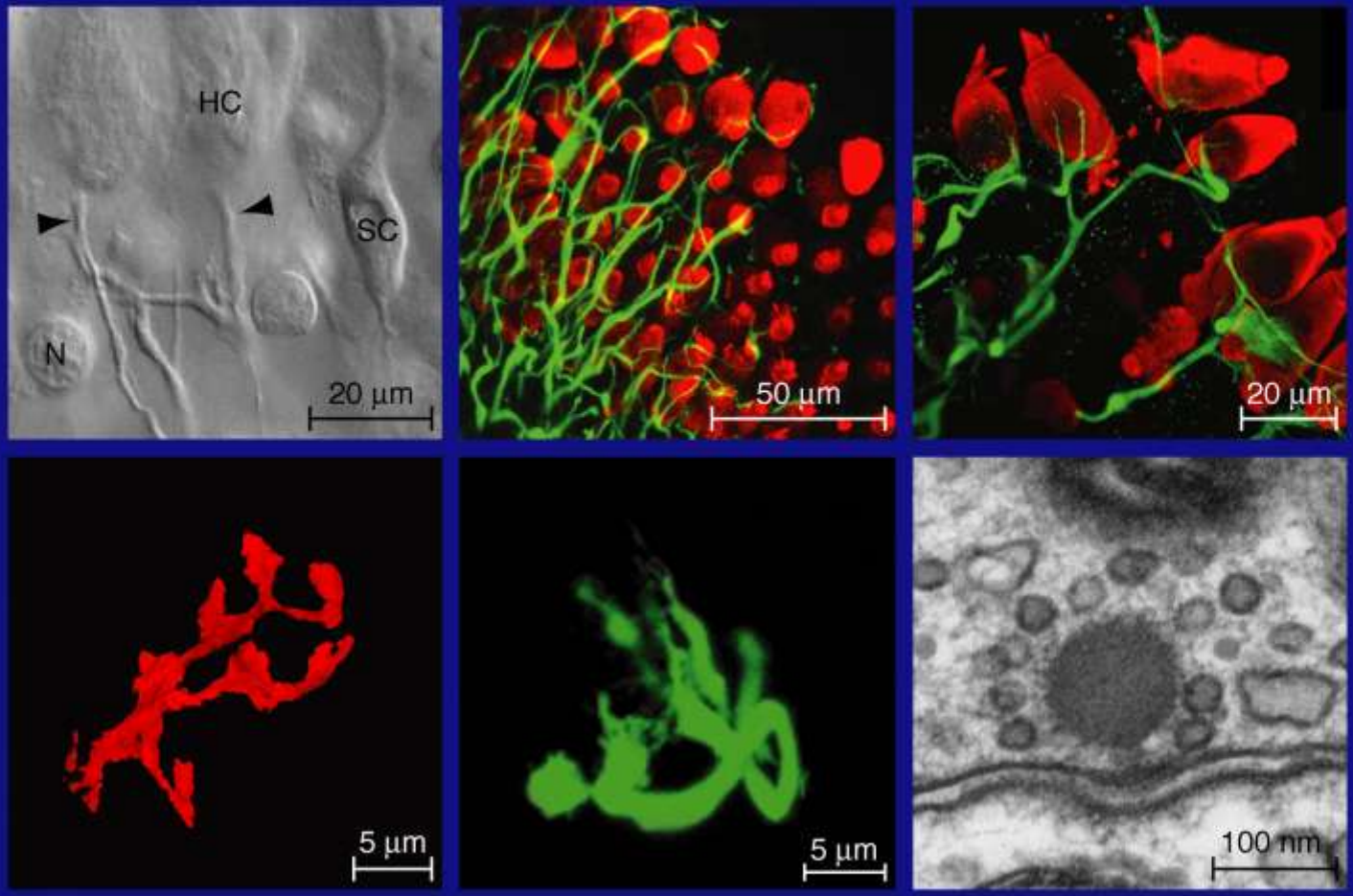
~90 Ca^{2+} channels per presynaptic active zone

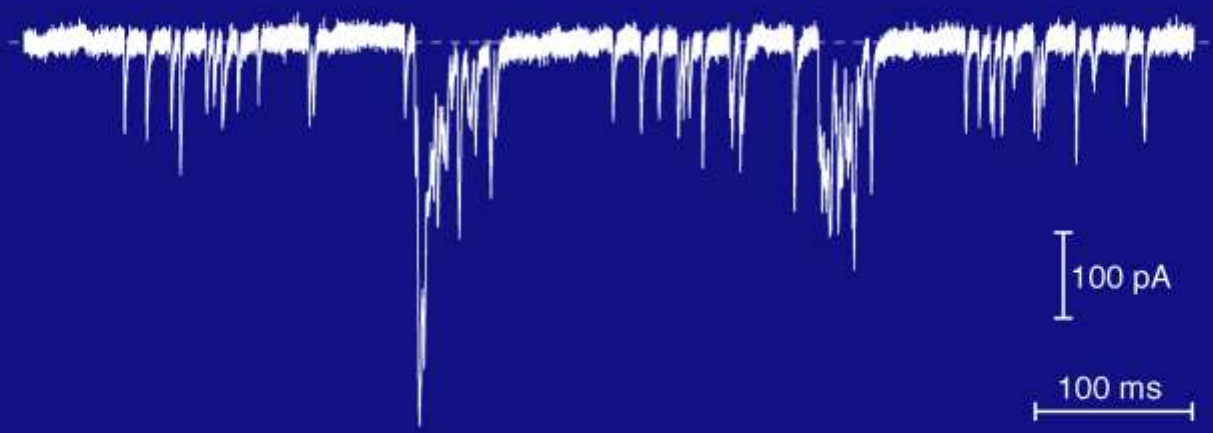
~40 K^{+} channels per presynaptic active zone

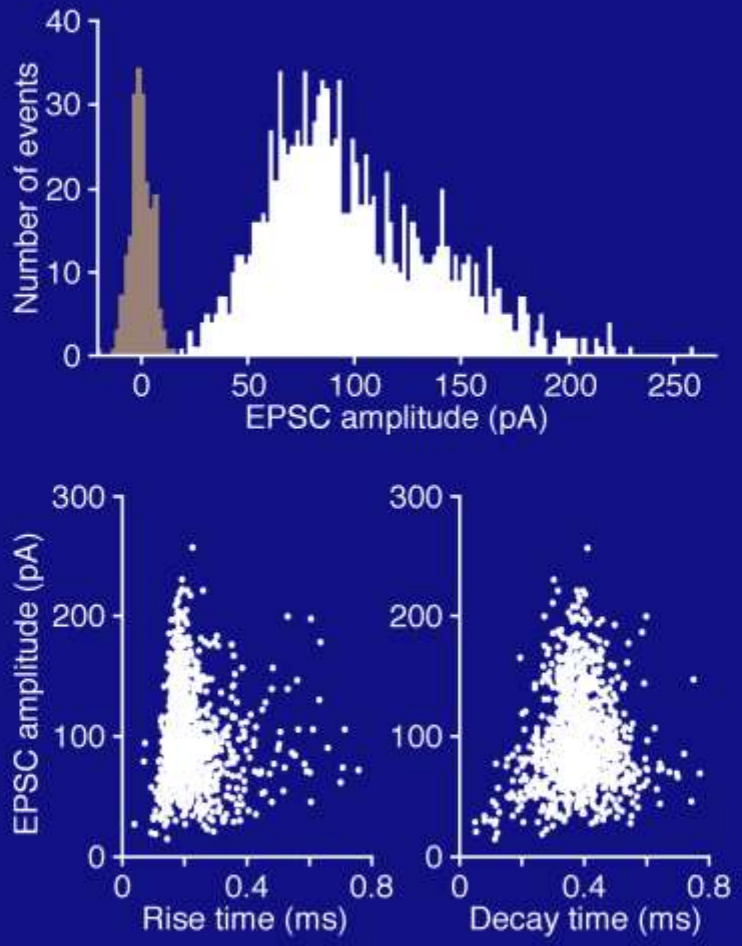

10 µm

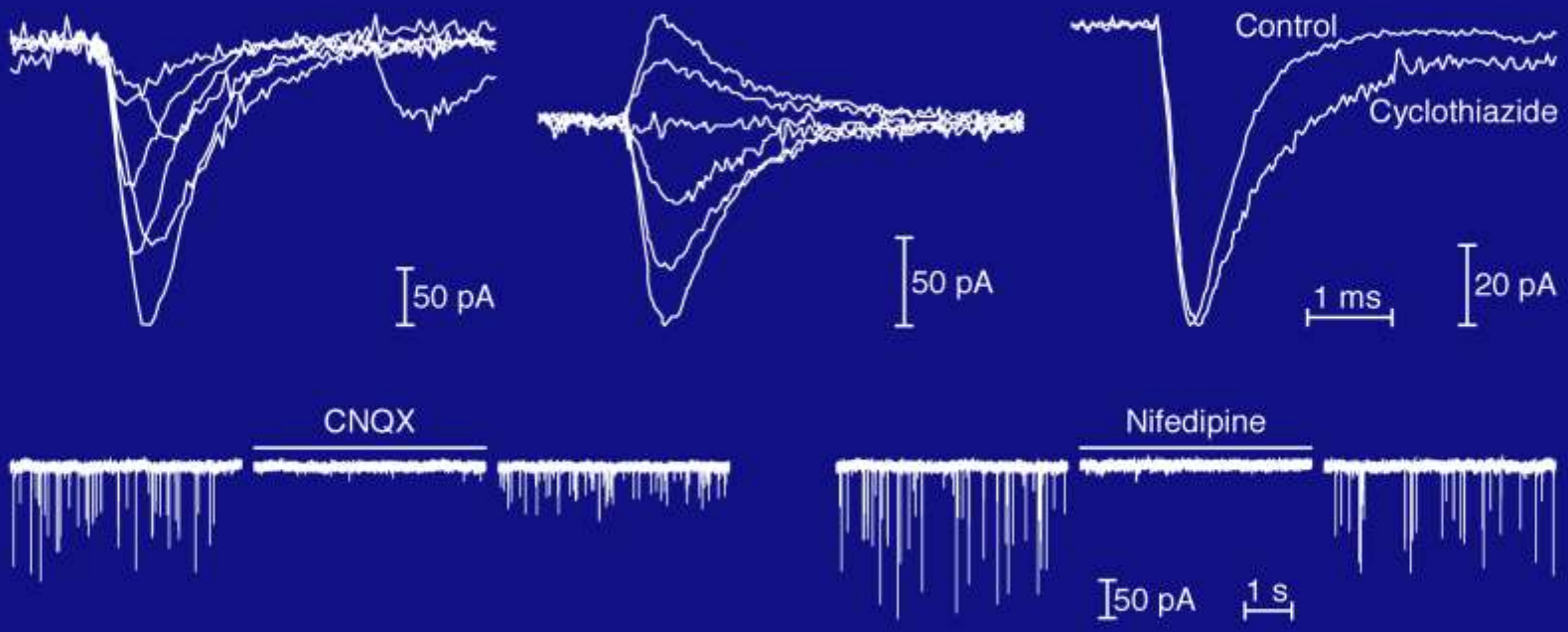

Hair bundle
Hair-cell soma
Recording electrodes
Ribbon synapse
Afferent nerve fiber

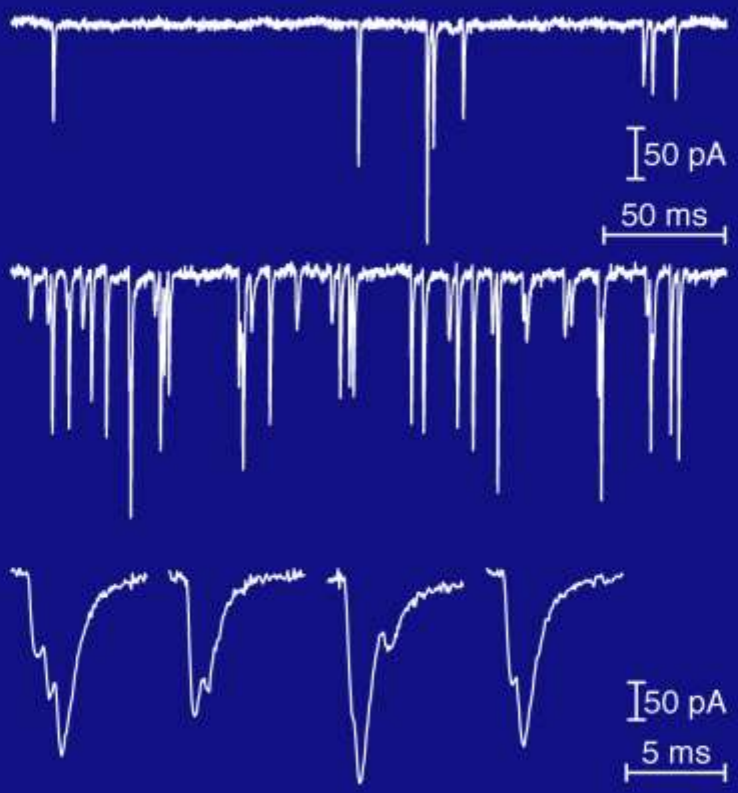

Hair-cell somata
Supporting cell
Afferent-nerve terminals
Nucleus of dead cell
10 µm

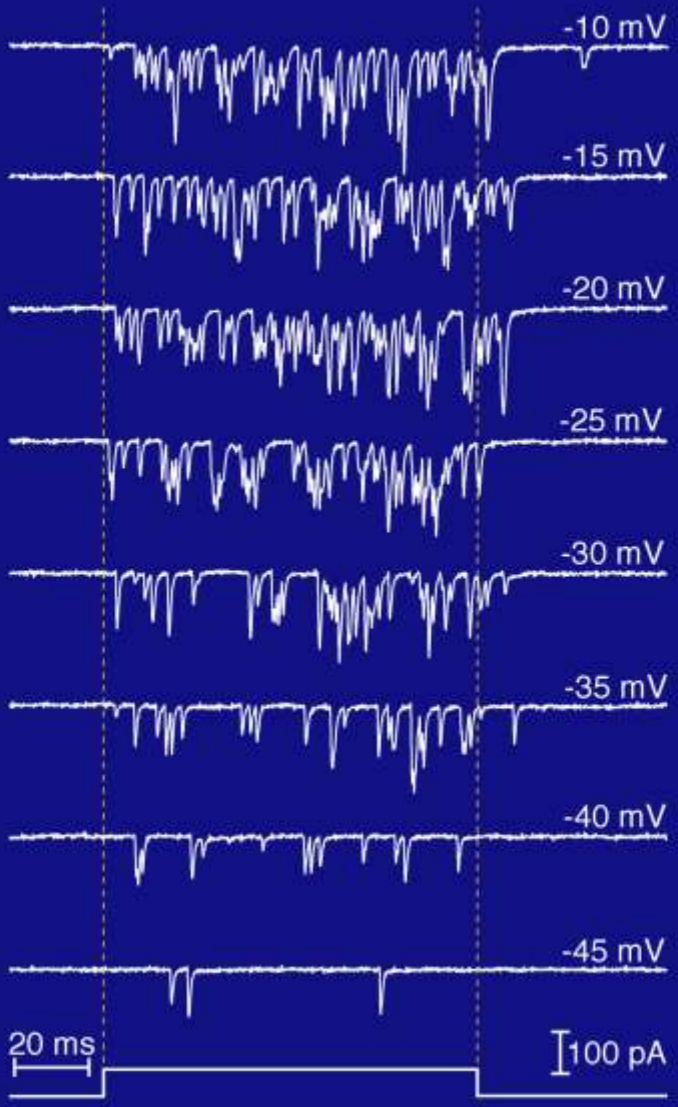

HC
SC
N
20 µm
50 µm
20 µm
5 µm
5 µm
100 nm

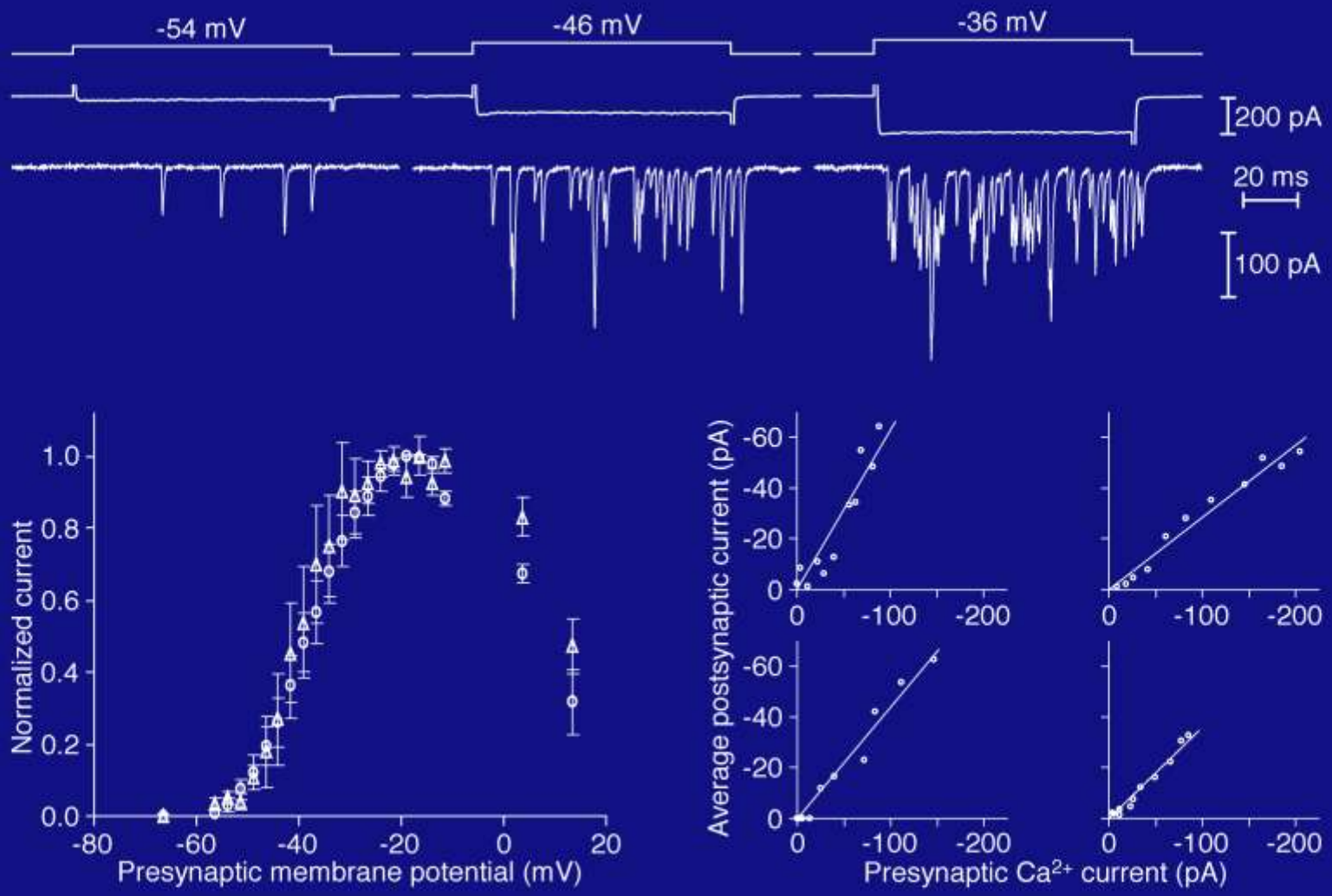

100 pA
100 ms


40
30
20
10
0
Number of events
0
50
100
150
200
250
EPSC amplitude (pA)
300
200
100
0
EPSC amplitude (pA)
0
0.4
0.8
Rise time (ms)
300
200
100
0
0
0.4
0.8
Decay time (ms)

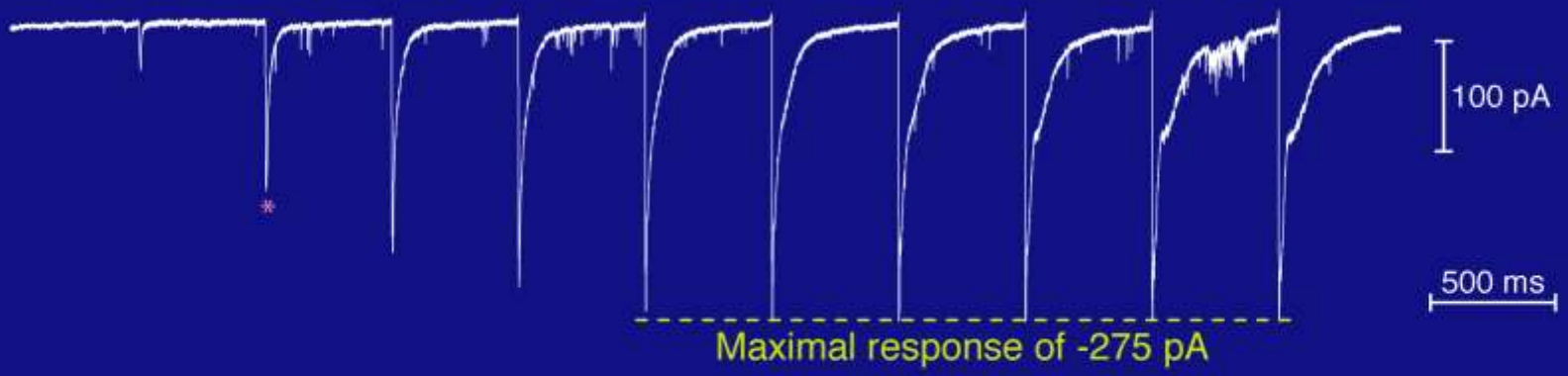

50 pA
50 pA
Control
Cyclothiazide
1 ms
20 pA
CNQX
Nifedipine
50 pA
1 s


50 pA
50 ms
50 pA
5 ms


-10 mV
-15 mV
-20 mV
-25 mV
-30 mV
-35 mV
-40 mV
-45 mV
20 ms
100 pA

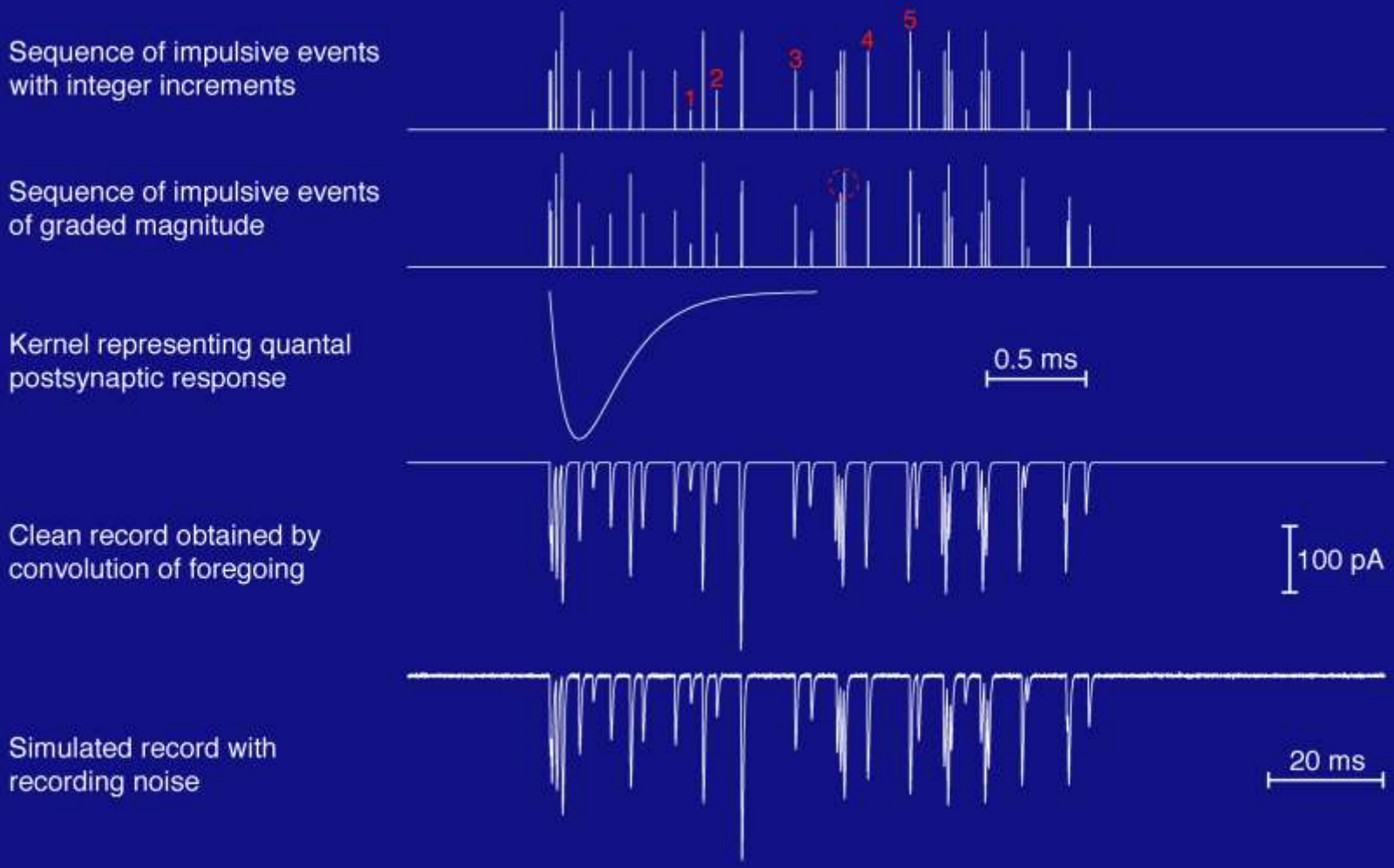

-54 mV
-46 mV
-36 mV
200 pA
20 ms
100 pA
Normalized current
1.0
0.8
0.6
0.4
0.2
0.0
-80
-60
-40
-20
0
20
Presynaptic membrane potential (mV)
Average postsynaptic current (pA)
-60
-40
-20
0
-100
-200
Presynaptic Ca2+ current (pA)

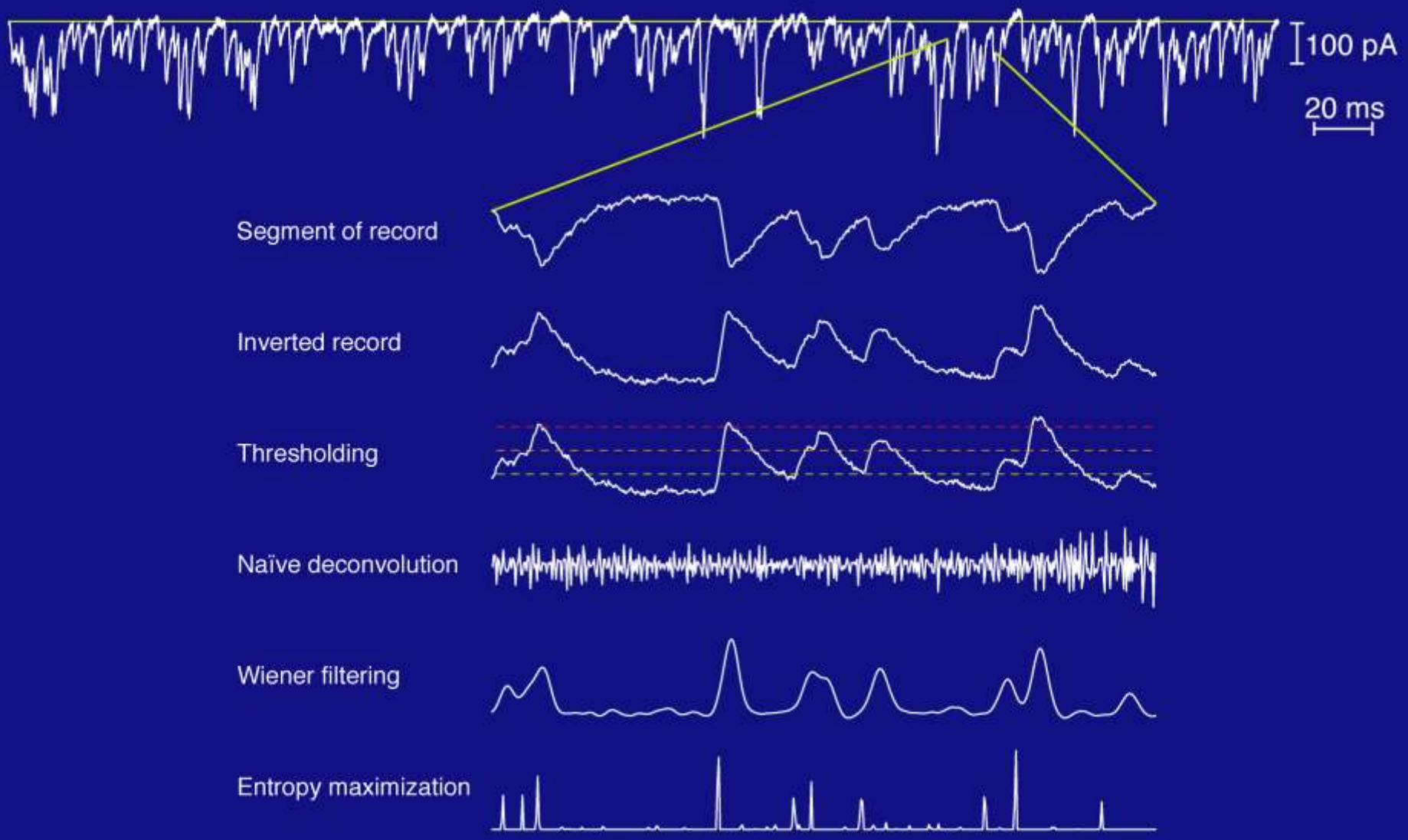
Iontophoretic application of glutamate 1 µm from a single afferent terminal;
10 nA holding current plus 20-ms pulses of current from -10 nA to -100 nA

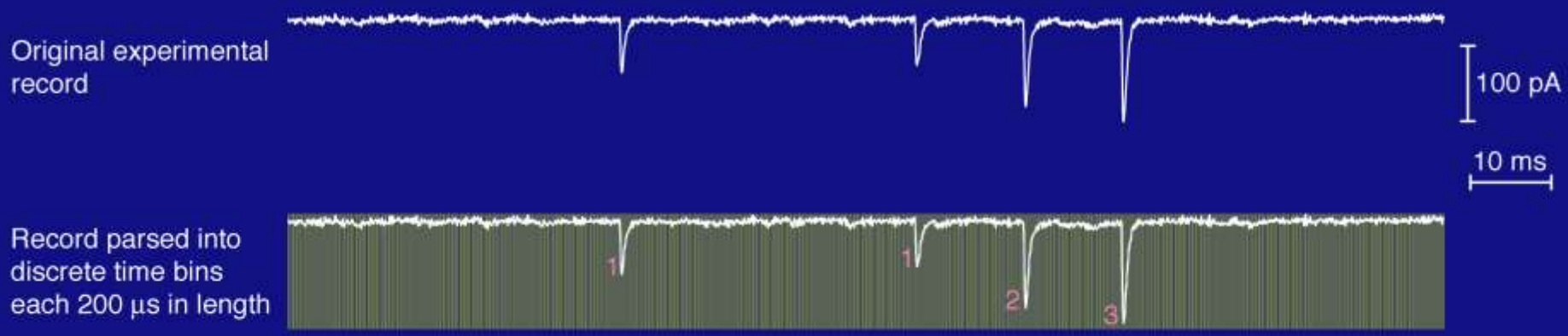


At a holding potential of -90 mV and for a single-channel conductance of AMPA receptors of 20 pS, the maximal response corresponds to the activation of about 150 receptors.

* For a current of -10 nA, a transference number $t = 0.32$, a valence $z = -1$, and a diffusion coefficient $D = 760 \cdot 10^{-12}$ $m^2 \cdot s^{-1}$, the glutamate concentration at the terminal is approximately 3.5 mM.


Sequence of impulsive events with integer increments
1
2
3
4
5
Sequence of impulsive events of graded magnitude
Kernel representing quantal postsynaptic response
0.5 ms
Clean record obtained by convolution of foregoing
100 pA
Simulated record with recording noise
20 ms


100 pA
20 ms
Segment of record
Inverted record
Thresholding
Naïve deconvolution
Wiener filtering
Entropy maximization



If the occurrence of each unitary (quantal) event is independent, for example if each originates at a distinct active zone:

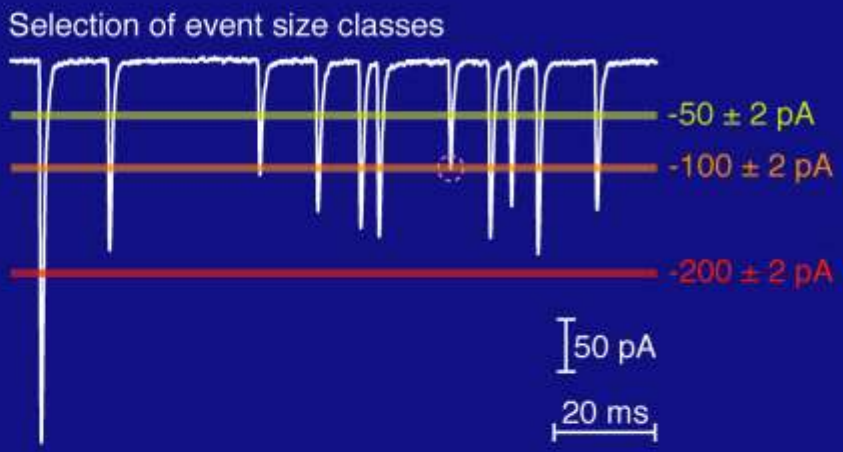
Probability of an event in any particular 200-µs interval = (1 + 1 + 2 + 3 events)/(750 bins in 150 ms) = $p \approx 0.0093$

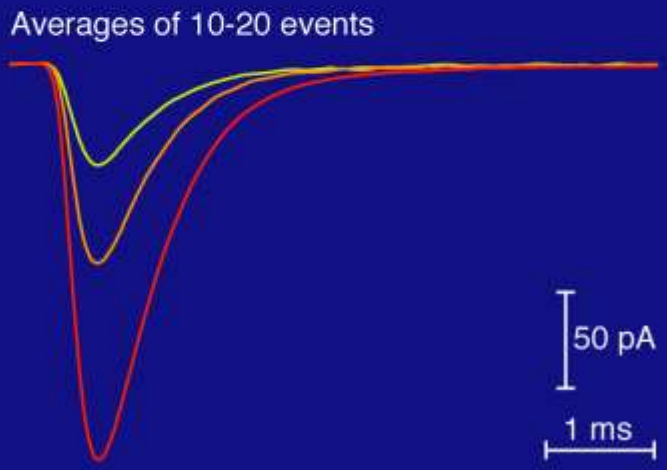
Probability of a triple event in any particular 200-µs interval = $p^3 \approx 0.0000008$

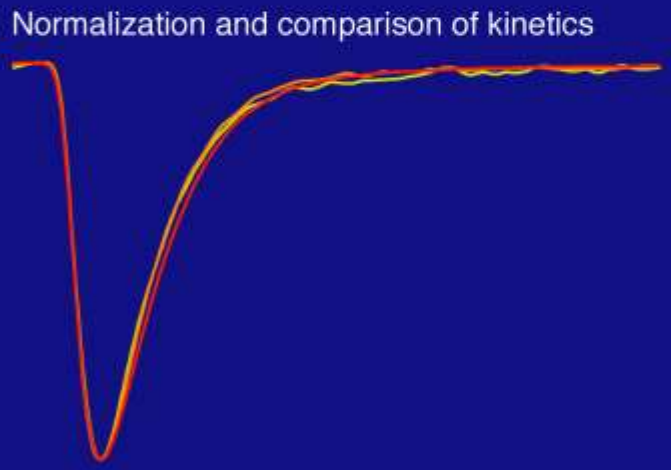
Probability of *no* triple event in any particular 200-µs interval = $1 - p^3 \approx 0.9999992$

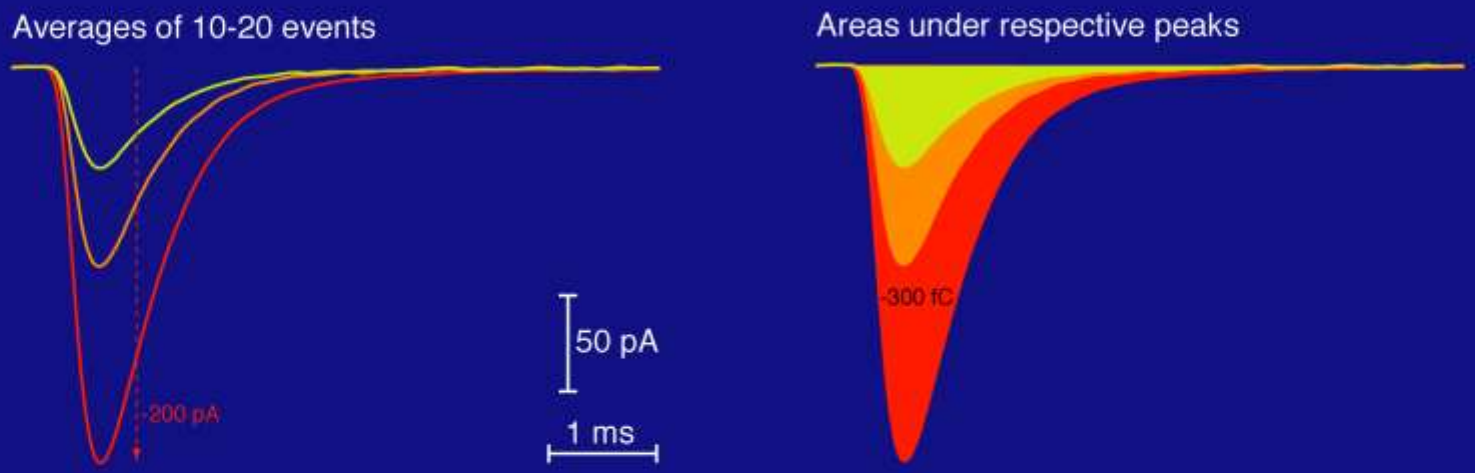
Probability of *no* triple event in all 750 intervals = $(1 - p^3)^{750} \approx 0.99939$

Probability of *at least one* triple event in all 750 intervals = $1 - (1 - p^3)^{750} \approx 0.0006$

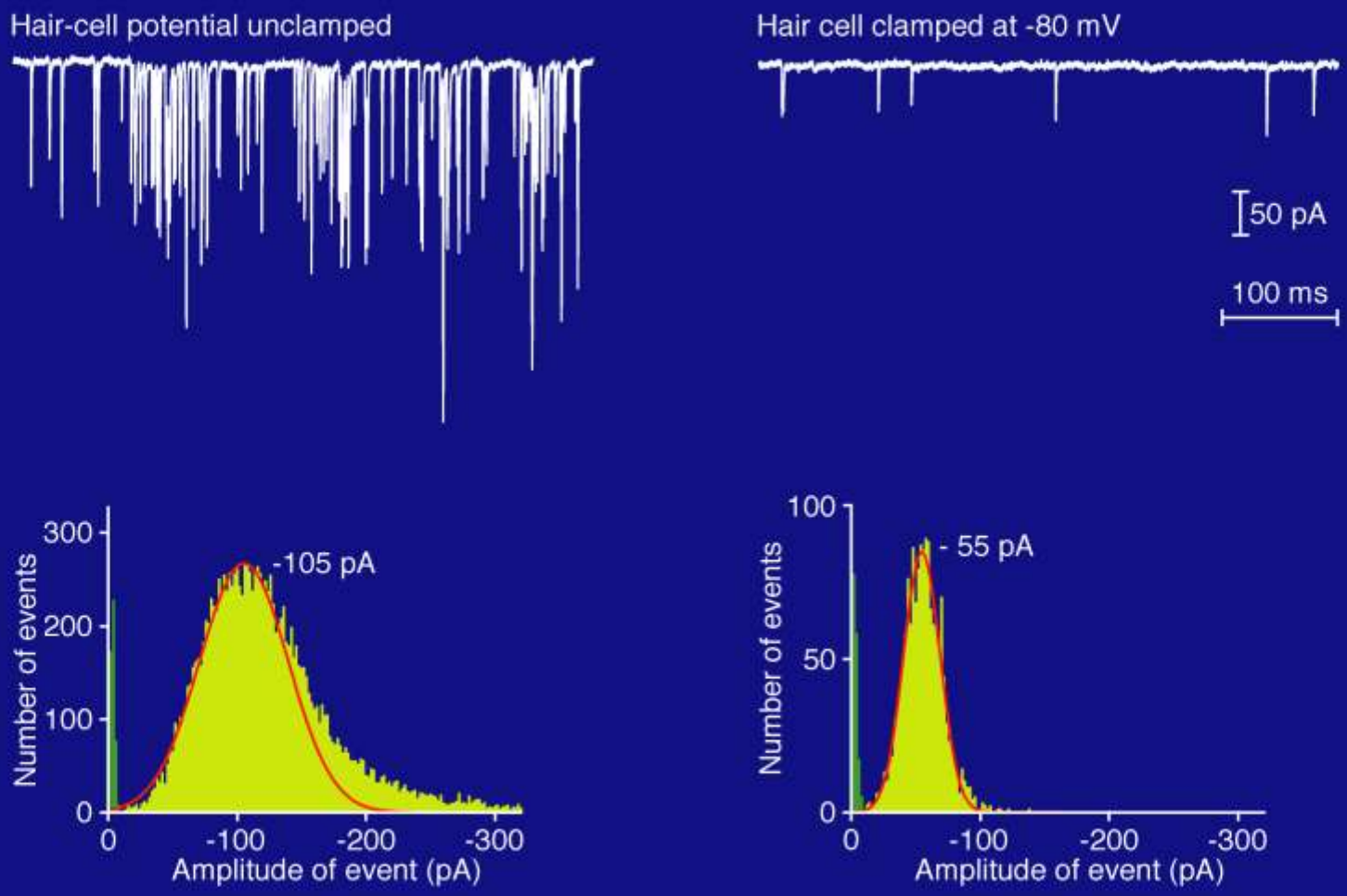

Selection of event size classes
-50 ± 2 pA
-100 ± 2 pA
-200 ± 2 pA
50 pA
20 ms

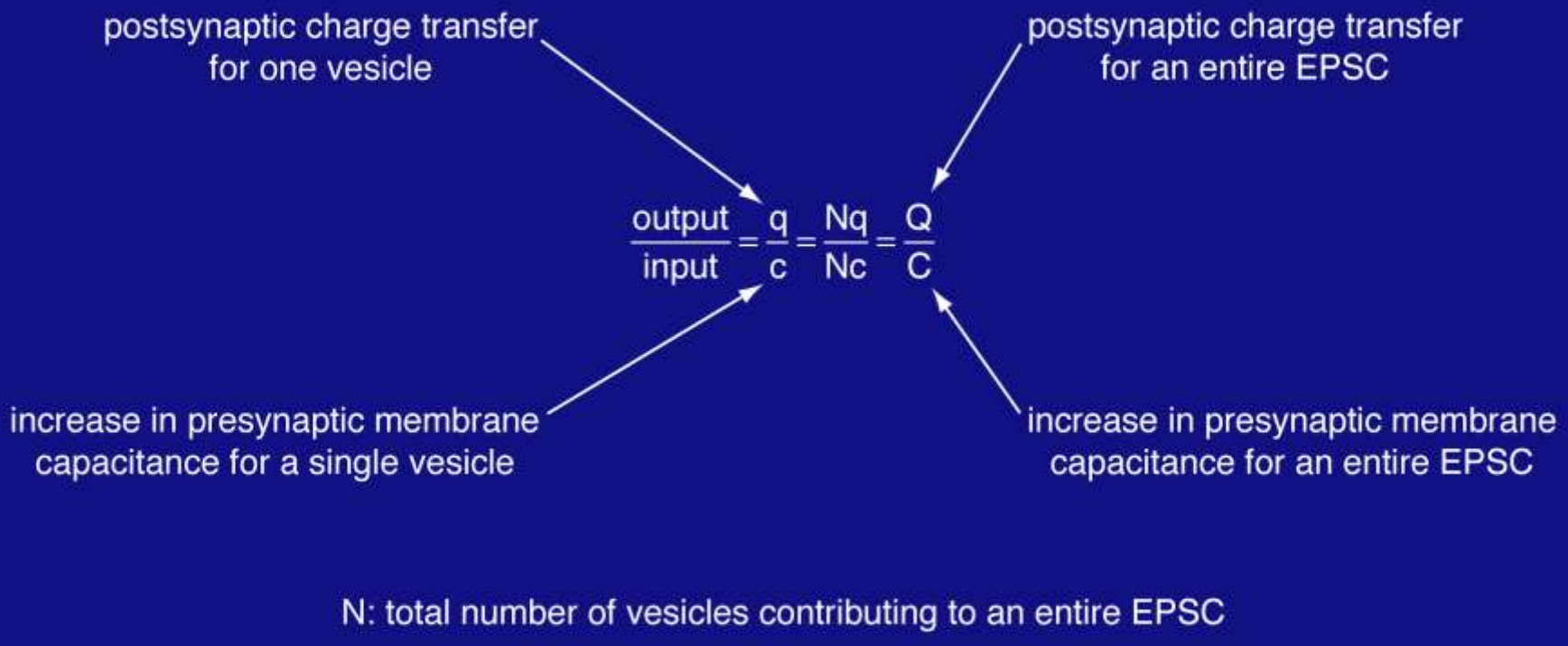

Averages of 10-20 events
50 pA
1 ms


Normalization and comparison of kinetics

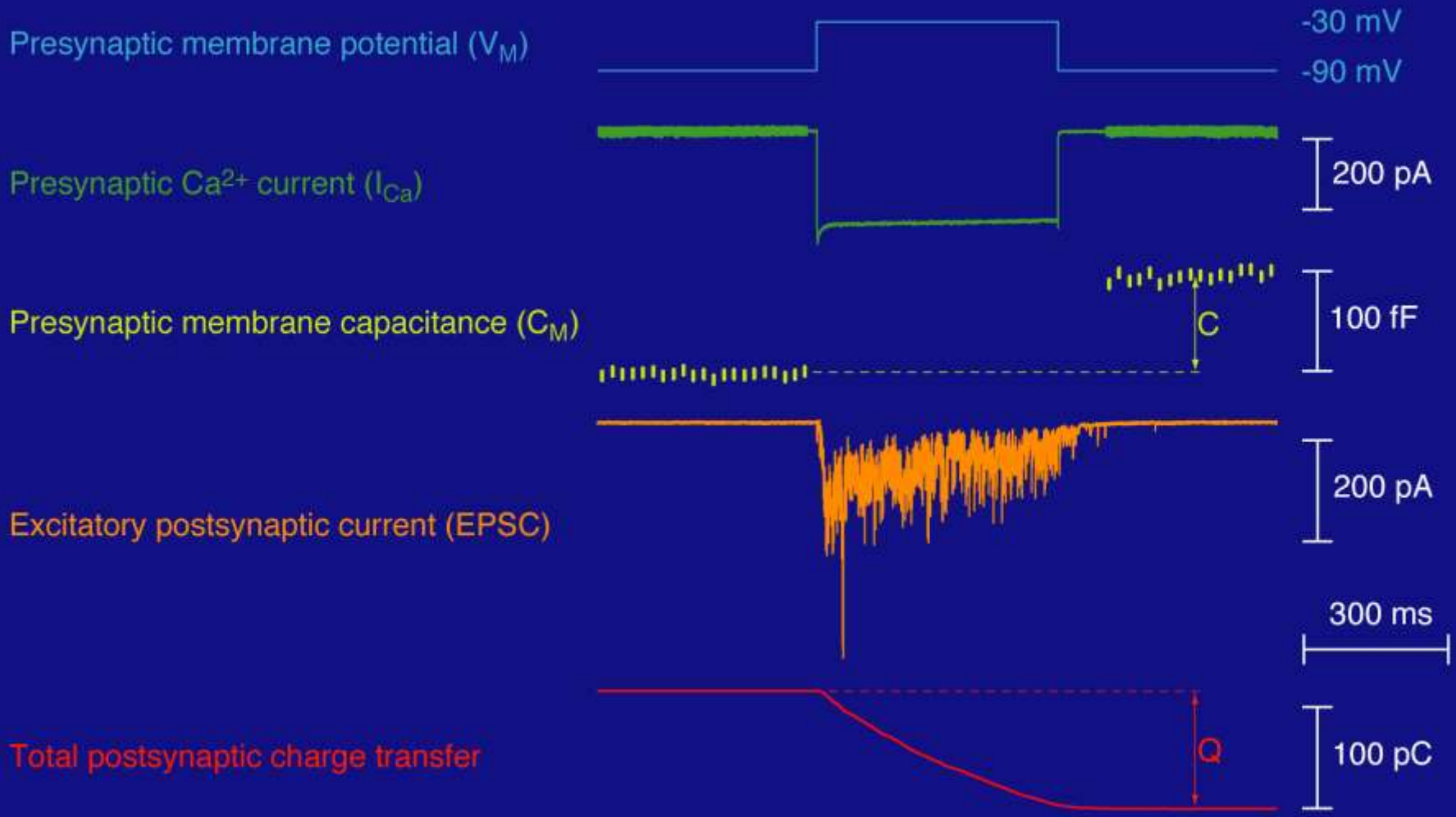


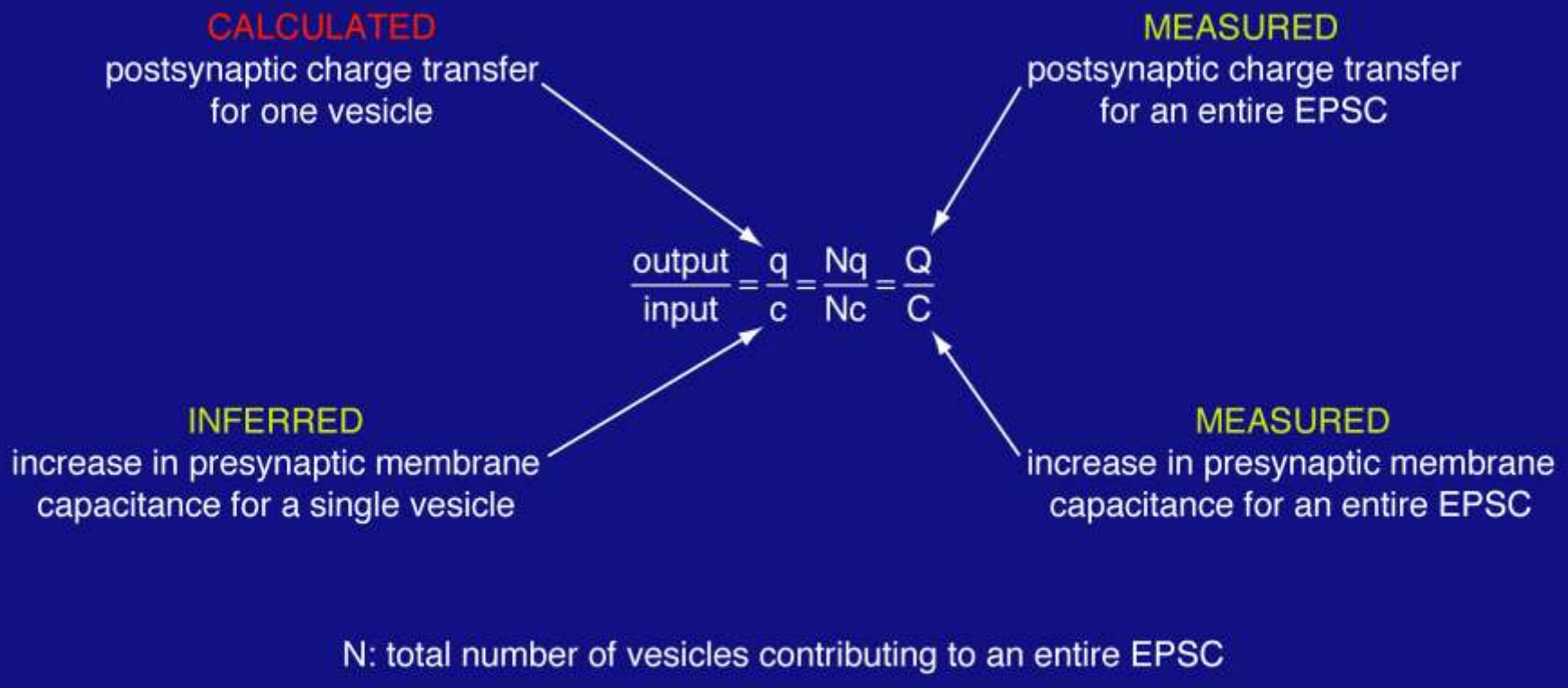
Postsynaptic charge transfer = 1.5 $mC \cdot A^{-1}$


Hair-cell potential unclamped
Hair cell clamped at -80 mV
50 pA
100 ms
300
200
100
0
Number of events
-105 pA
0
-100
-200
-300
Amplitude of event (pA)
100
50
0
Number of events
- 55 pA
0
-100
-200
-300
Amplitude of event (pA)

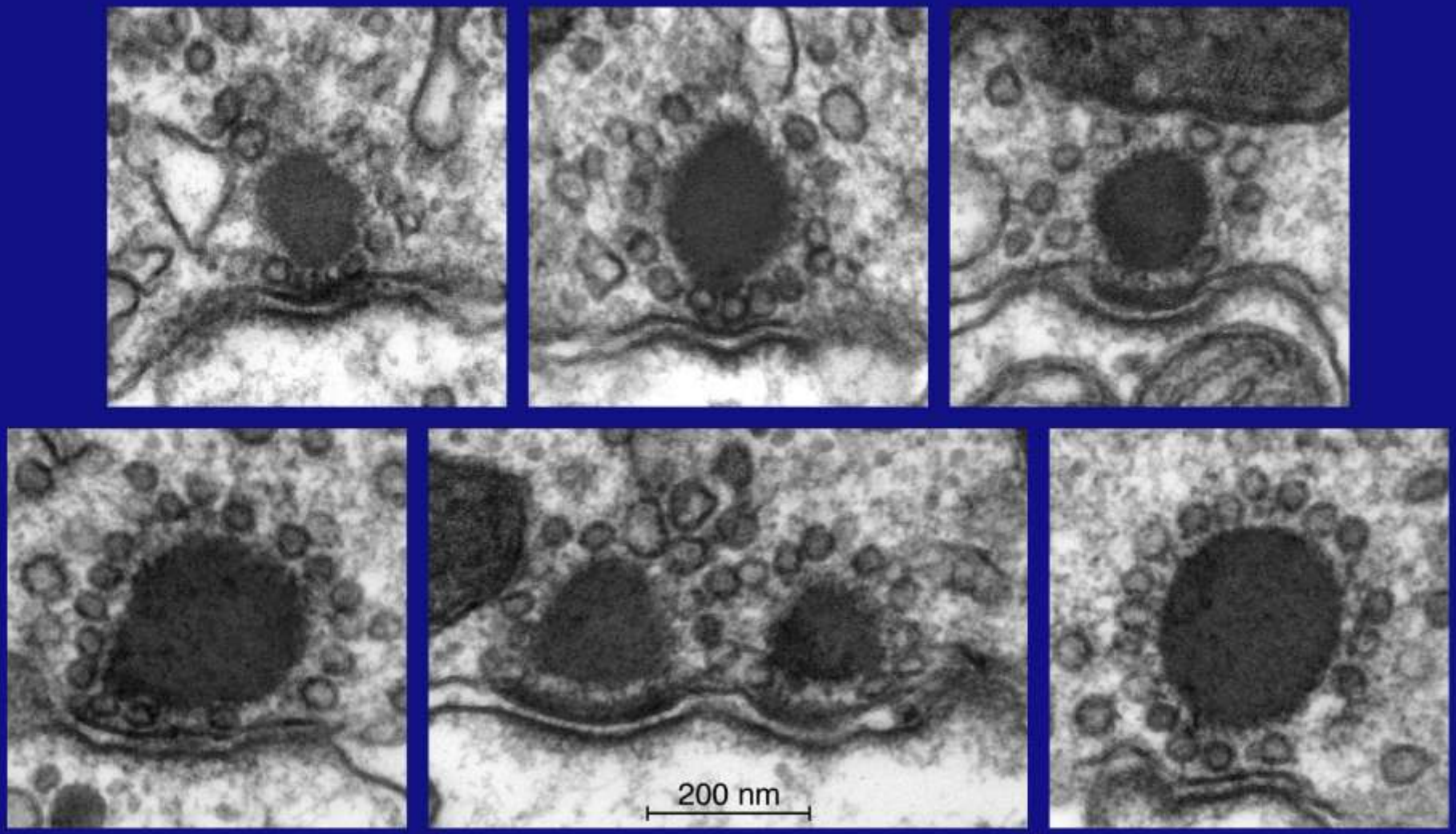


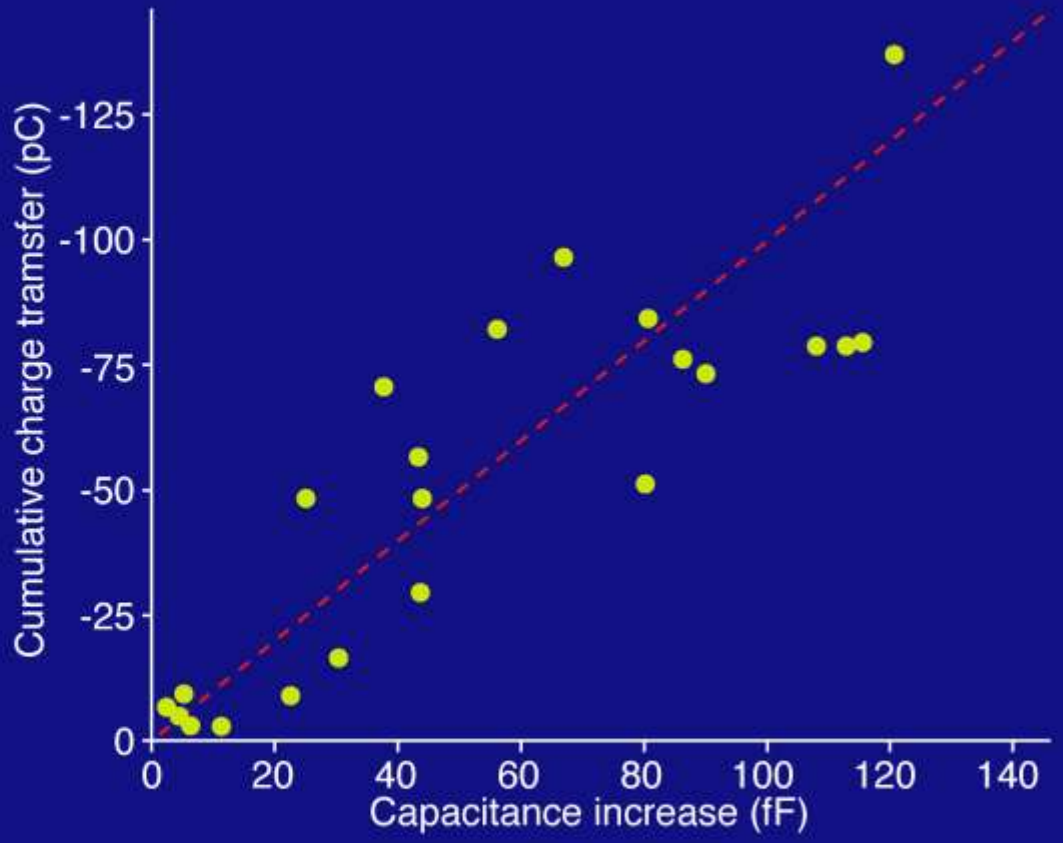
N: total number of vesicles contributing to an entire EPSC


Presynaptic membrane potential (V_M)
-30 mV
-90 mV
Presynaptic Ca^{2+} current (I_{Ca})
200 pA
Presynaptic membrane capacitance (C_M)
C
100 fF
Excitatory postsynaptic current (EPSC)
200 pA
300 ms
Total postsynaptic charge transfer
Q
100 pC



N: total number of vesicles contributing to an entire EPSC


200 nm



$$\frac{Q}{C} = 1.1\ kC \cdot F^{-1}$$

$$q = \left(\frac{\pi d^2 \boldsymbol{C} P}{R}\right)\frac{Q}{C}$$

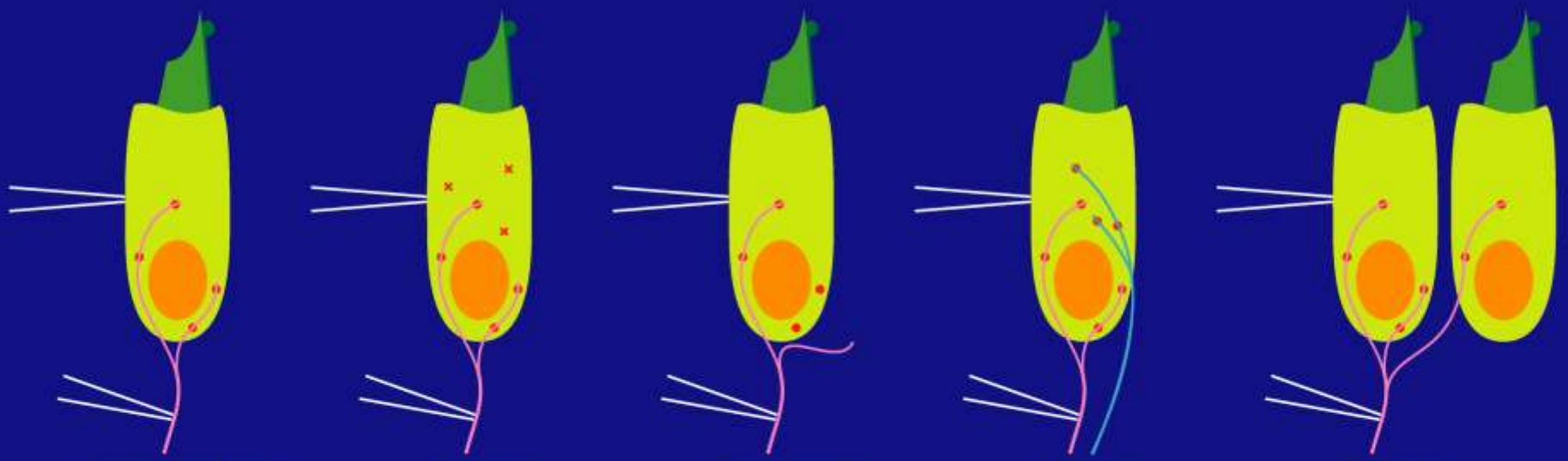
$d = 38\ nm$

$\boldsymbol{C} = 10\ mF \cdot m^{-2}$

$q = -75\ fC$

$1.5\ mC \cdot A^{-1}$

-50 pA per quantal event



Ideal situation
P=1
R=1

Ectopic
vesicle fusion
P<1
R=1

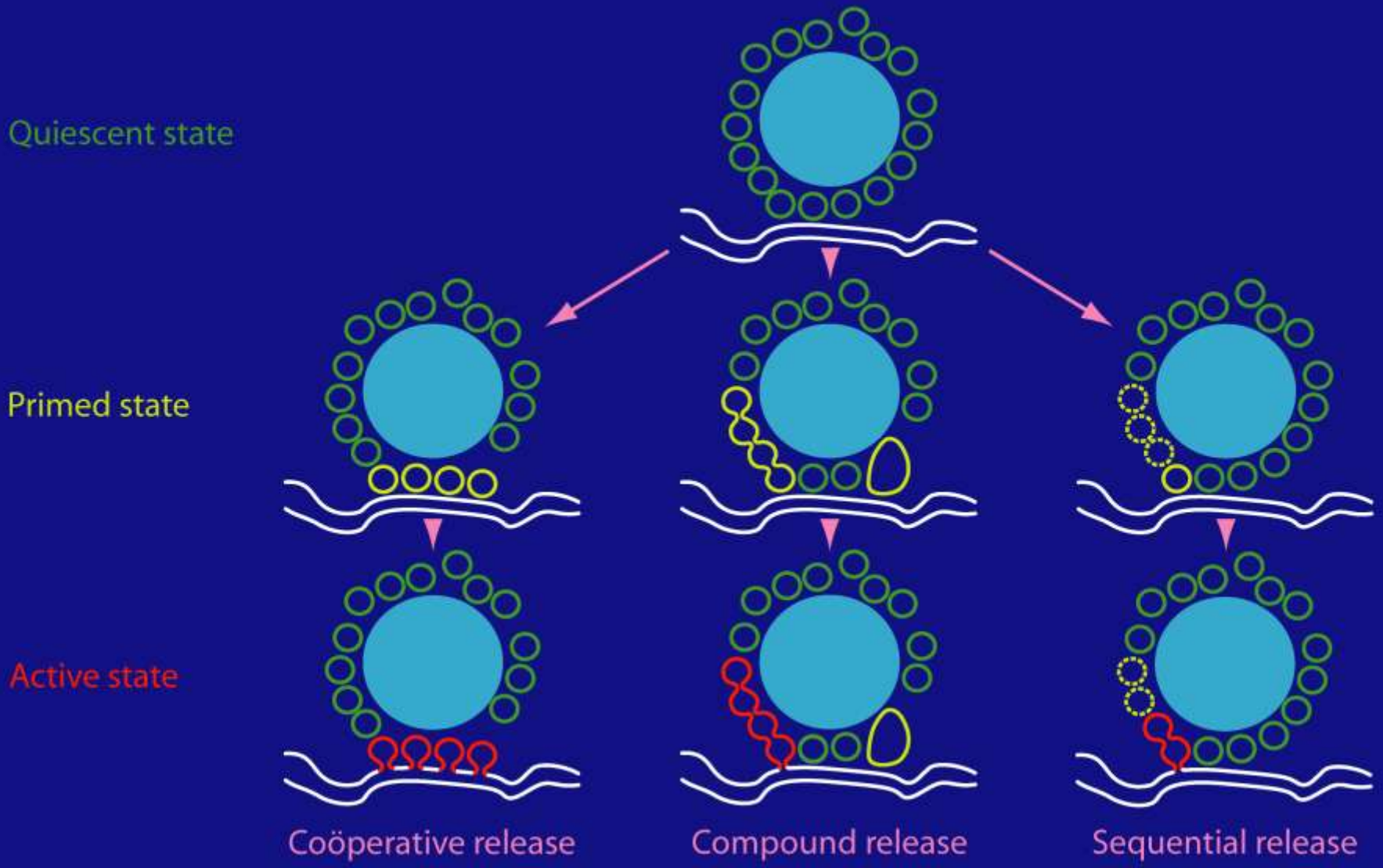
Displaced
nerve fiber
P<1
R=1

Multiple
innervation
of hair cell
P=1
R>1

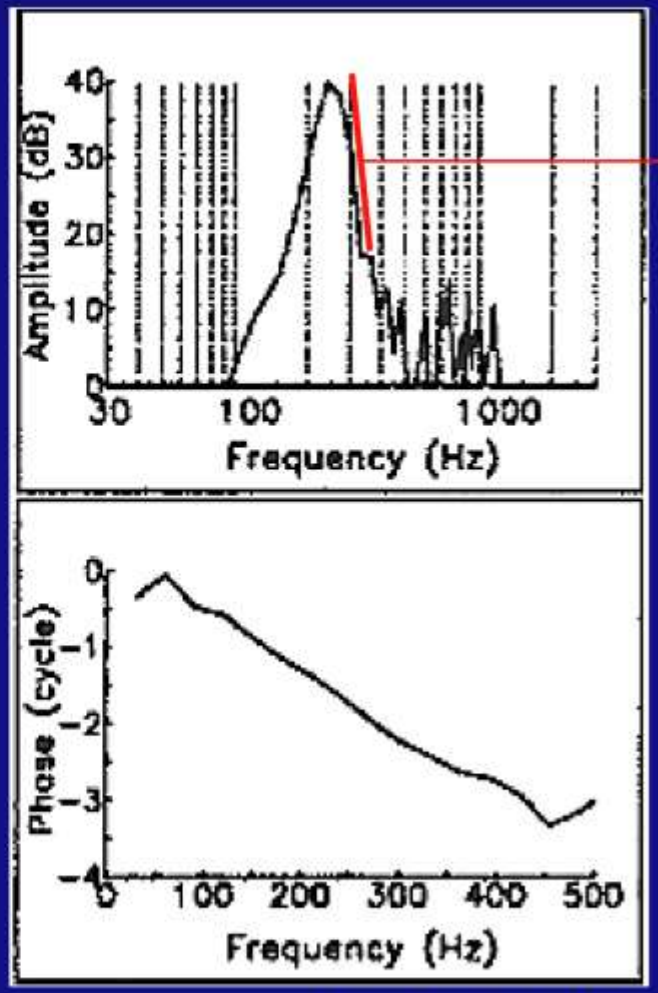
Divided
innervation
of hair cells
P=1
R<1

$$q = \left(\frac{\pi d^2 C P}{R} \right) \frac{Q}{C}$$

q: postsynaptic charge transfer for one vesicle
d: diameter of a synaptic vesicle
C: specific capacitance of biological membranes
P: probability that release occurs at an innervated active zone
R: innervation ratio of afferent fibers per hair cell
Q: postsynaptic charge transfer for an entire EPSC
C: increase in presynaptic membrane capacitance for an entire EPSC


Quiescent state
Primed state
Active state
Coöperative release
Compound release
Sequential release

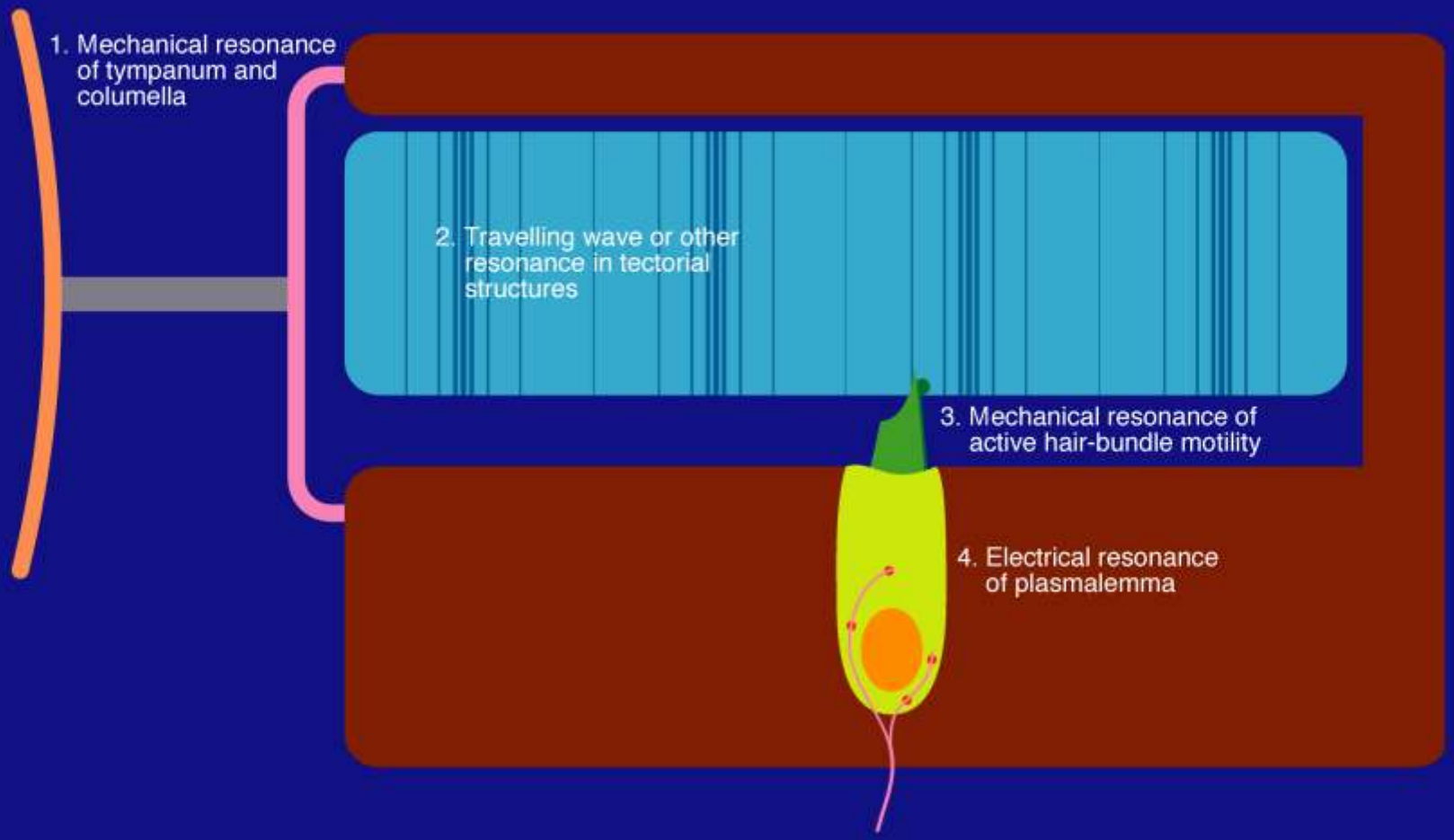
Tuning of an afferent nerve fiber
from the bullfrog's amphibian papilla

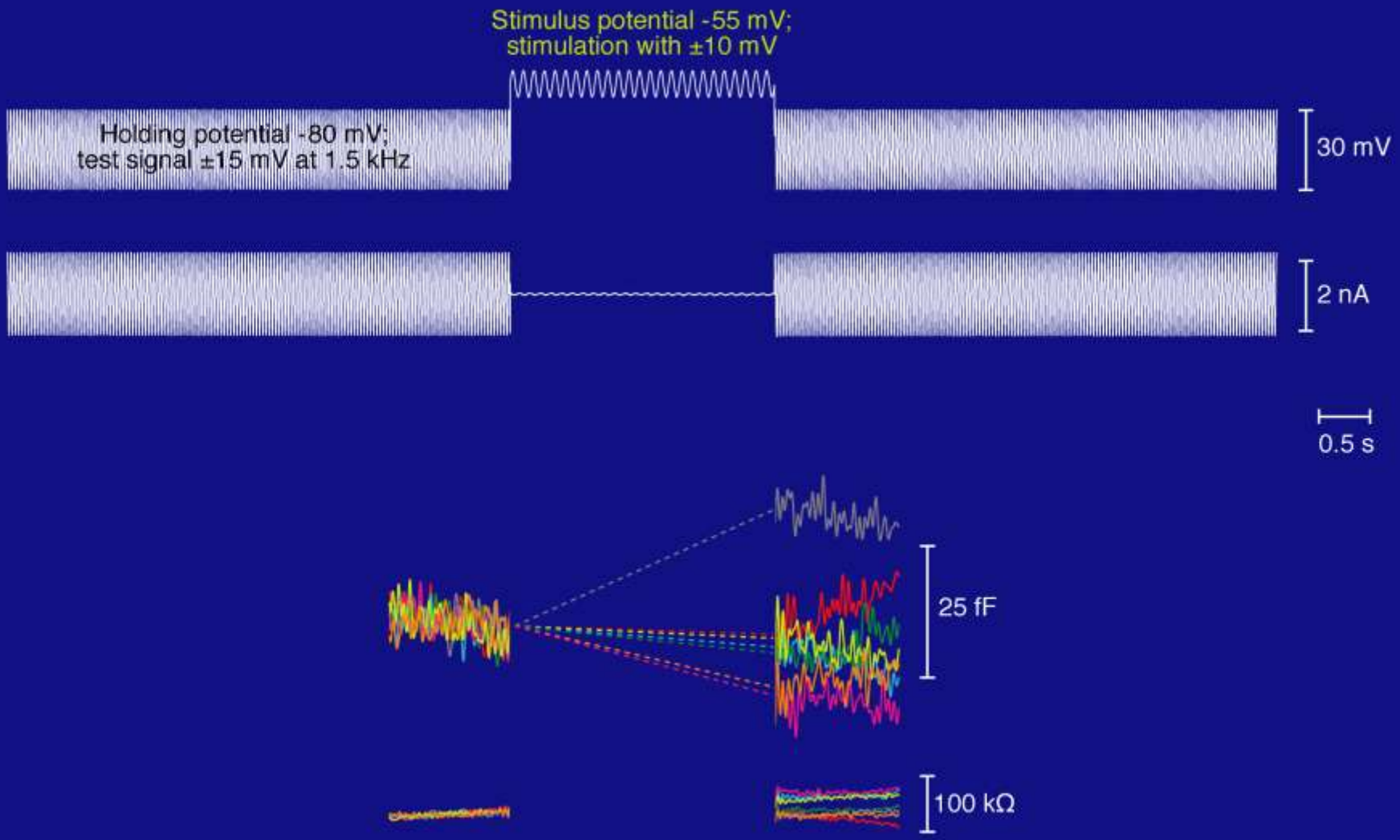


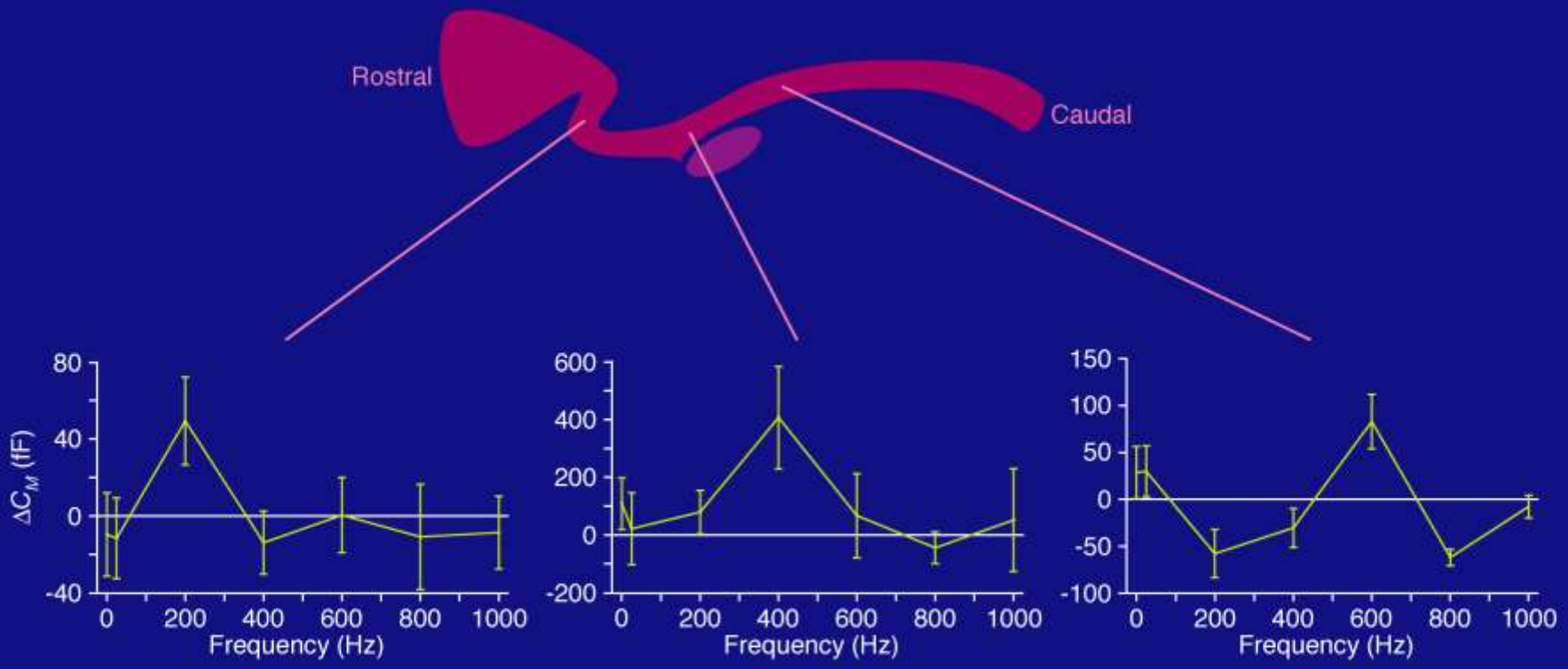
Slope 70-90 dB per octave
(12th- to 15th-order filter)

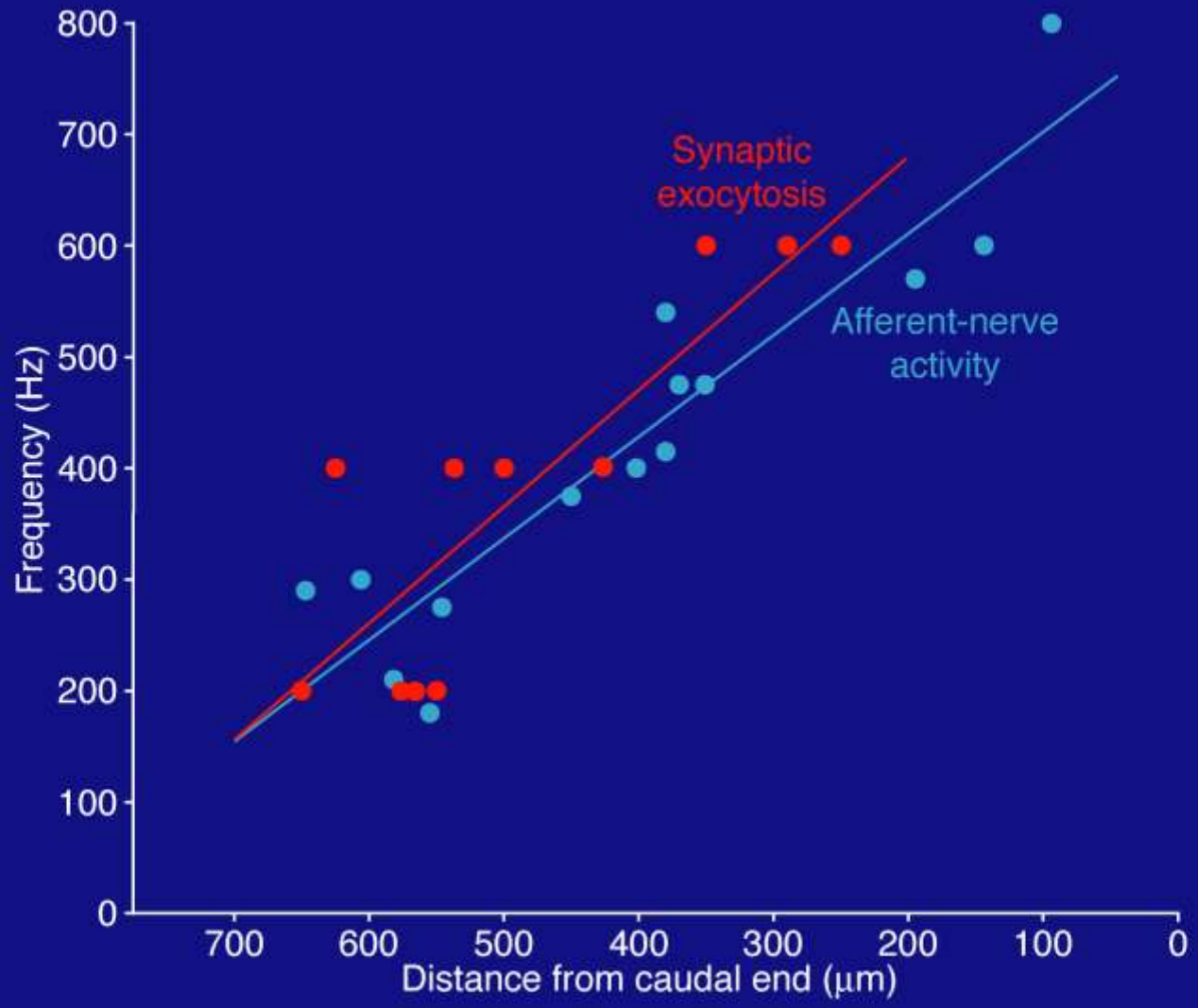
After Yu *et al.*, 1991

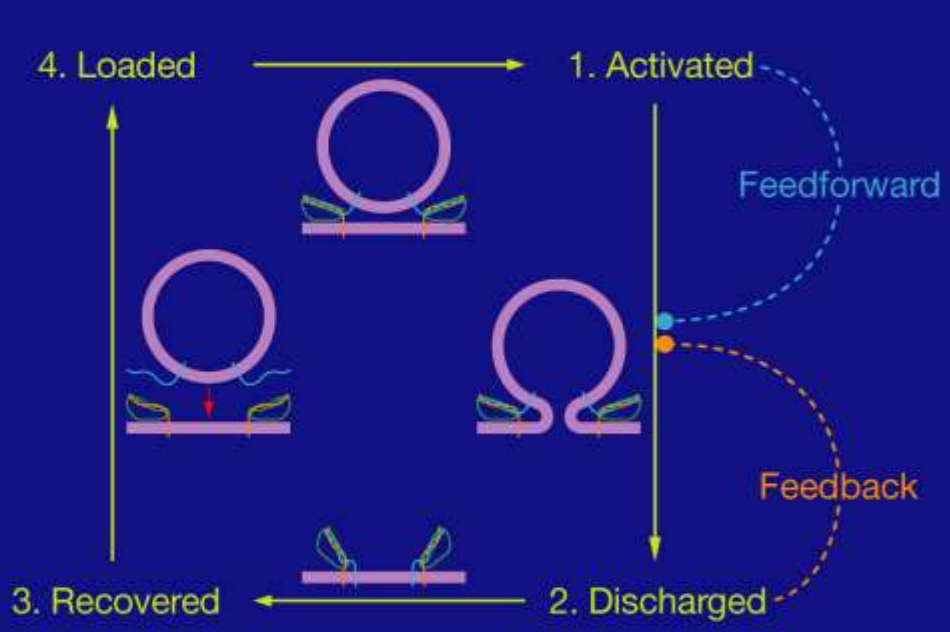
FREQUENCY – TUNING MECHANISMS IN THE AMPHIBIAN PAPILLA

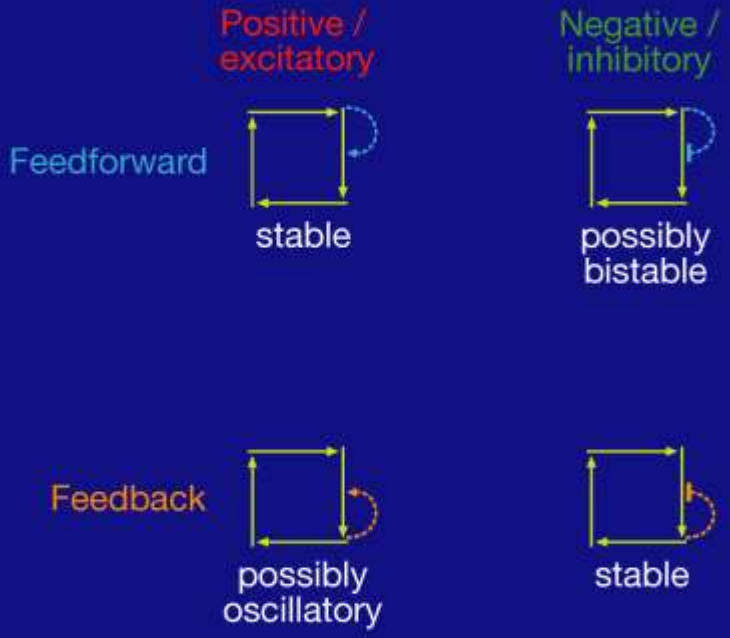


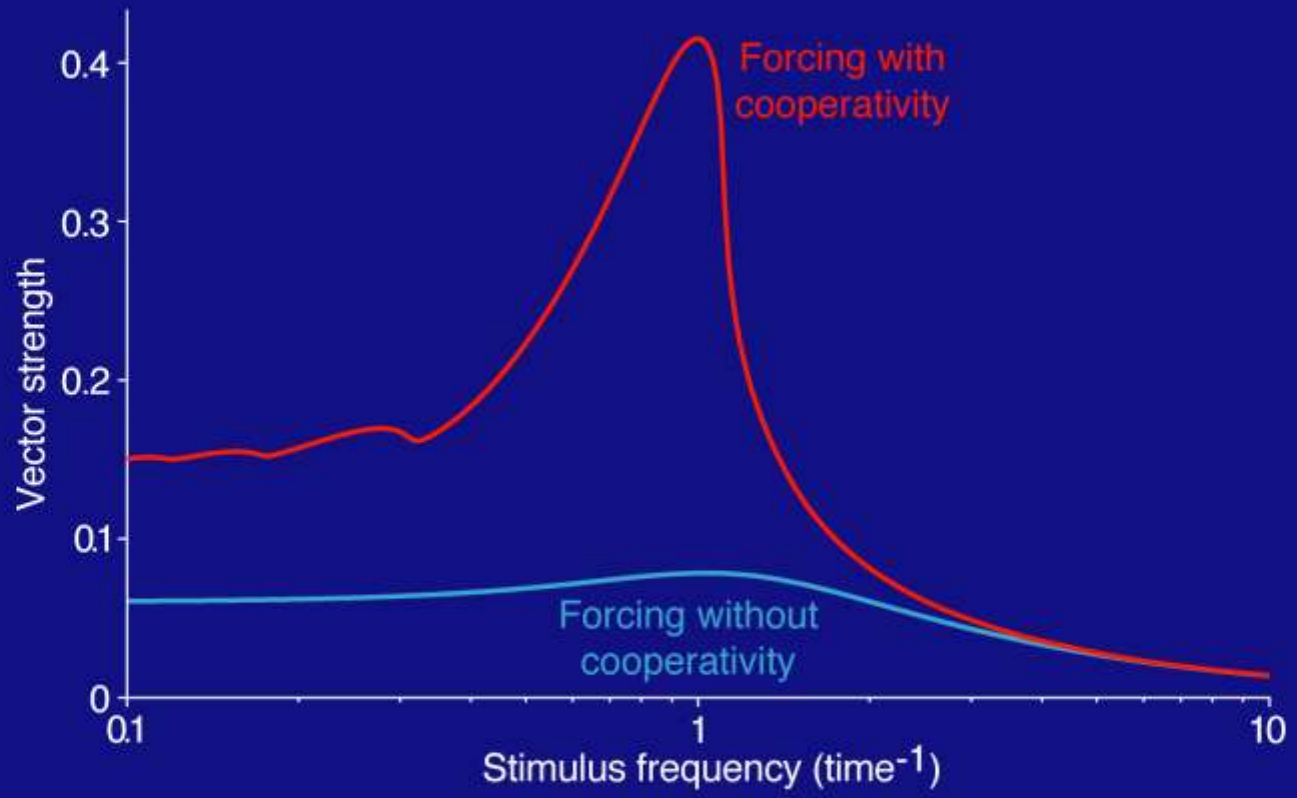

Stimulus potential -55 mV;
stimulation with ±10 mV
Holding potential -80 mV;
test signal ±15 mV at 1.5 kHz
30 mV
2 nA
0.5 s
25 fF
100 kΩ

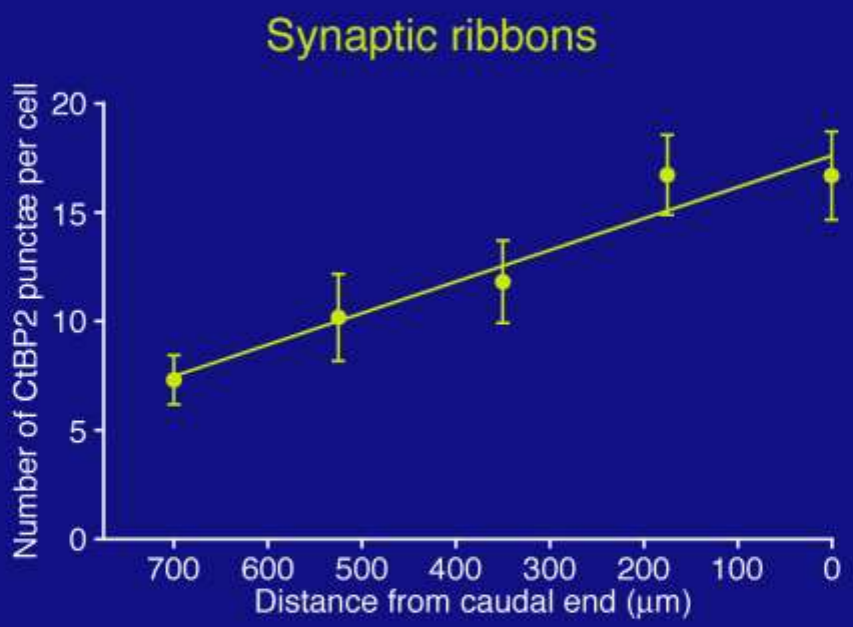

Rostral
Caudal
80
40
0
-40
ΔC_M (fF)
0
200
400
600
800
1000
Frequency (Hz)
600
400
200
0
-200
0
200
400
600
800
1000
Frequency (Hz)
150
100
50
0
-50
-100
0
200
400
600
800
1000
Frequency (Hz)

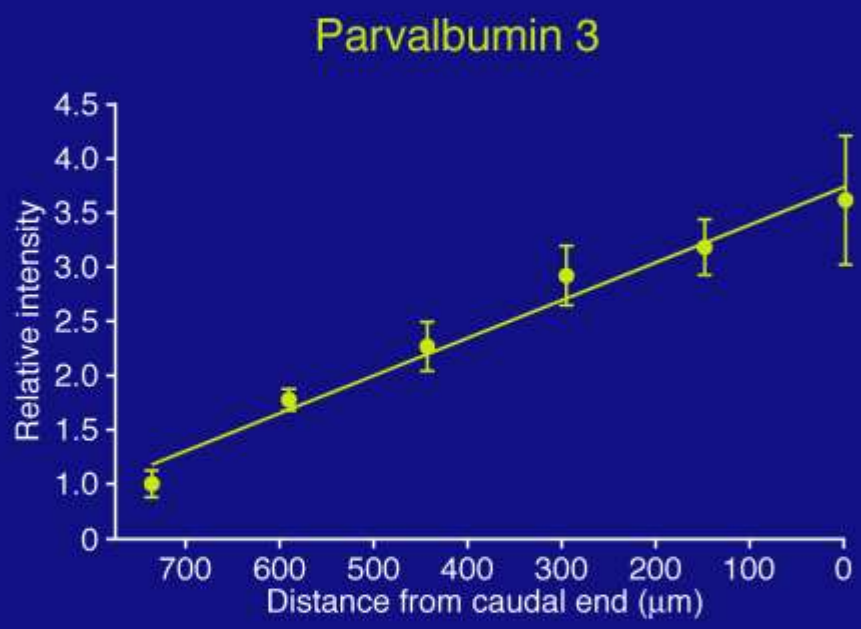

800
700
600
500
400
300
200
100
0
Frequency (Hz)
Synaptic
exocytosis
Afferent-nerve
activity
700
600
500
400
300
200
100
0
Distance from caudal end (μm)

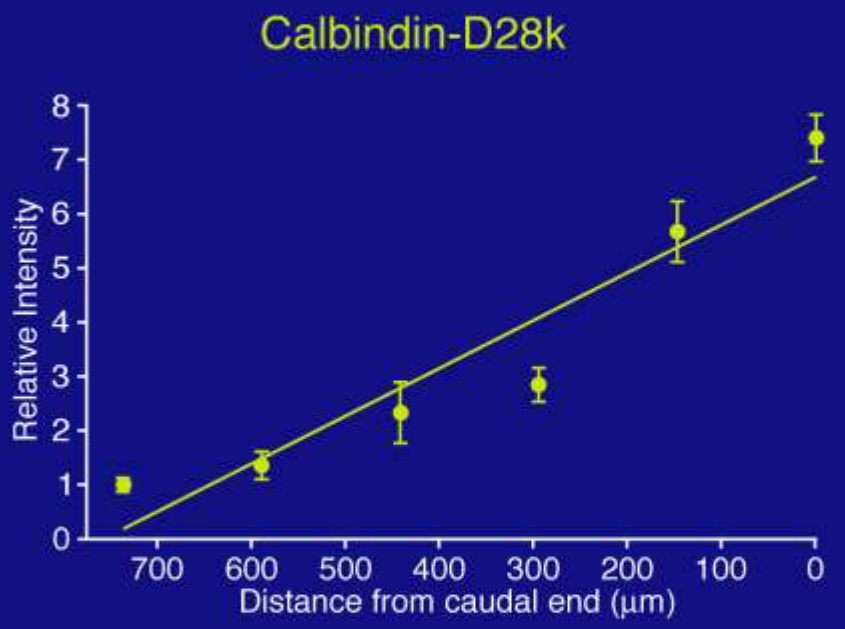

4. Loaded
1. Activated
Feedforward
Feedback
3. Recovered
2. Discharged


Positive / excitatory
Negative / inhibitory
Feedforward
stable
possibly bistable
Feedback
possibly oscillatory
stable

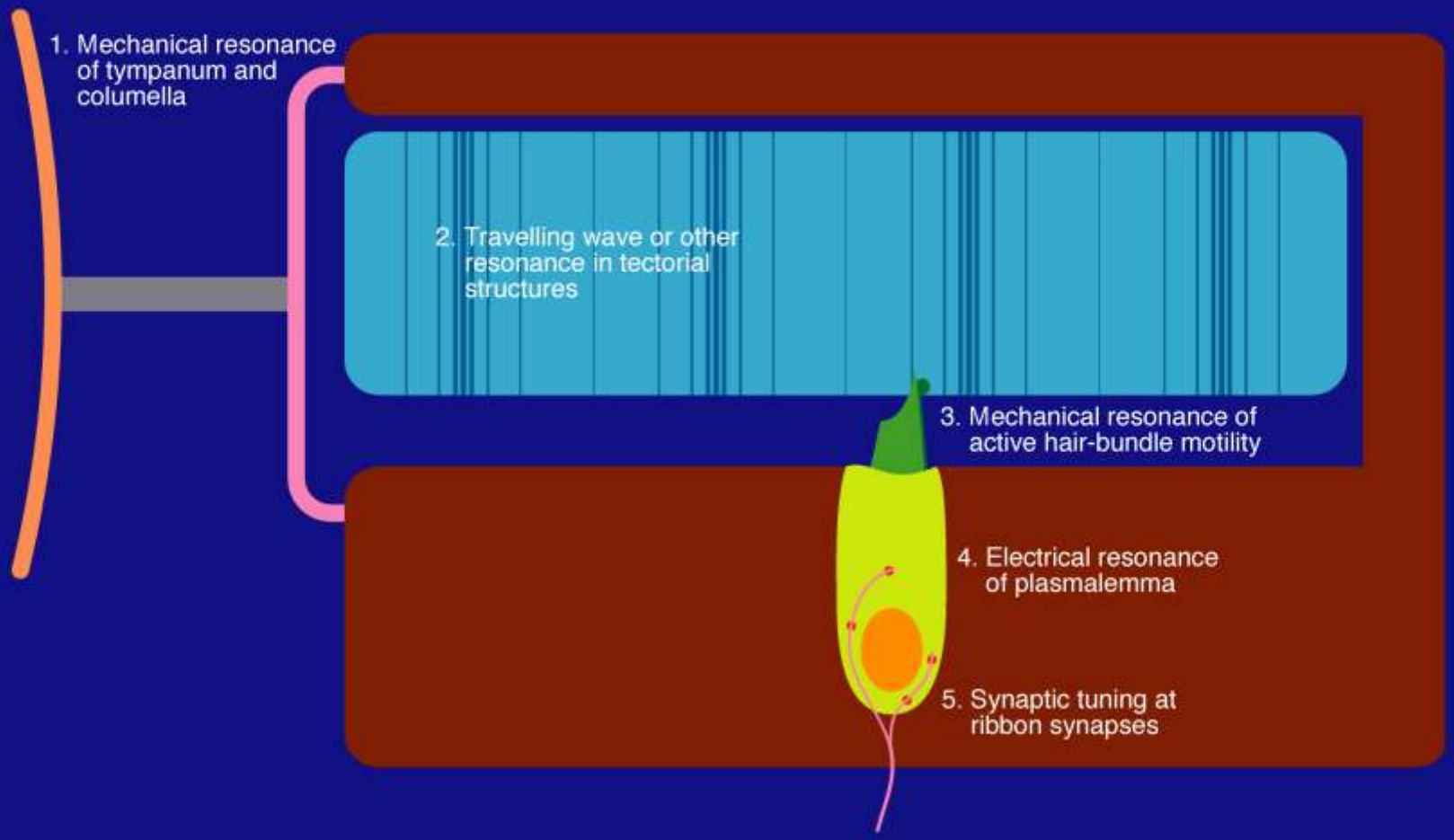

Forcing with
cooperativity
Forcing without
cooperativity
Vector strength
0.4
0.3
0.2
0.1
0
0.1
1
10
Stimulus frequency (time-1)


Synaptic ribbons
Number of CtBP2 punctæ per cell
20
15
10
5
0
700
600
500
400
300
200
100
0
Distance from caudal end (µm)

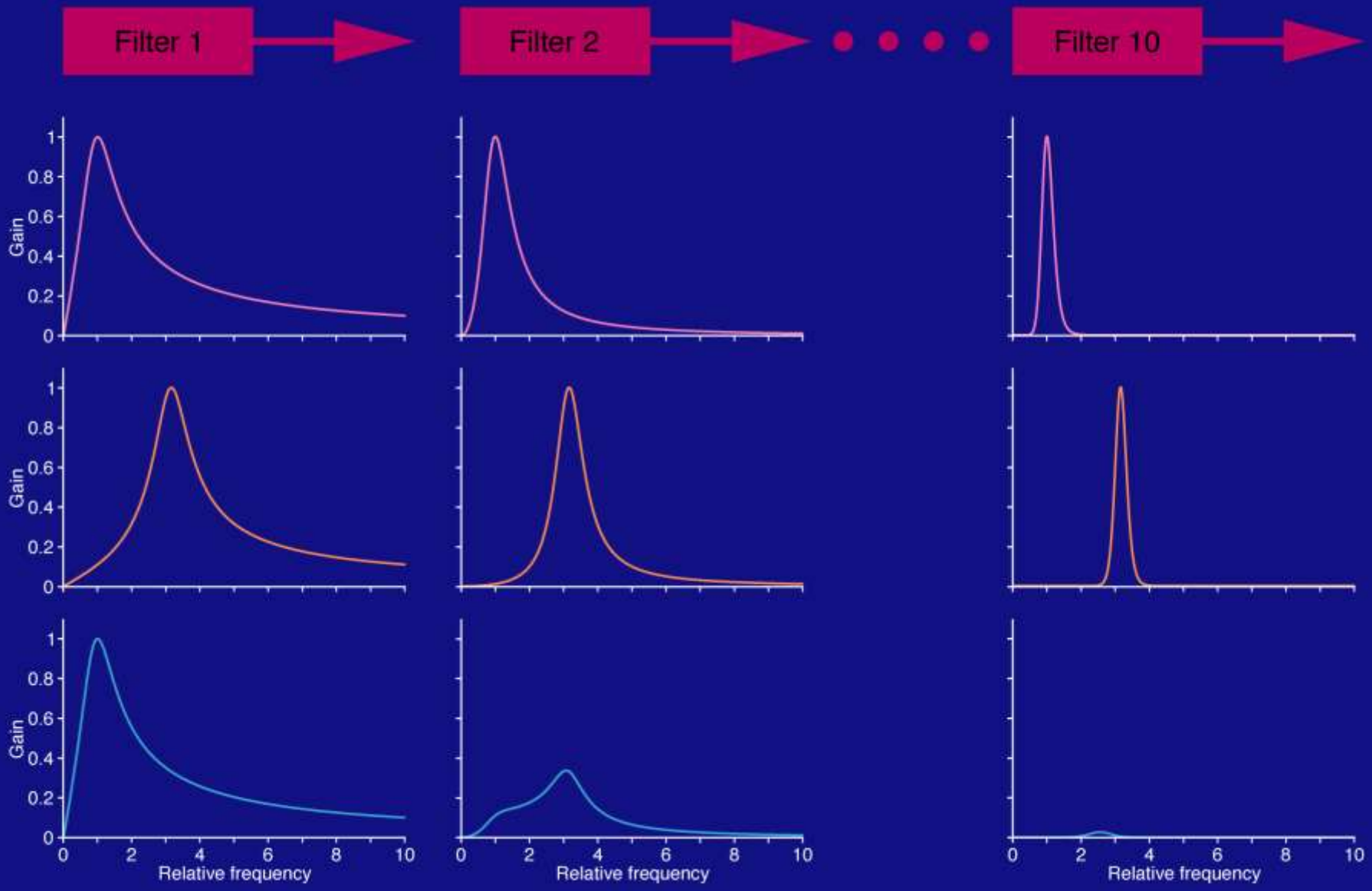

Parvalbumin 3
Relative intensity
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
700
600
500
400
300
200
100
0
Distance from caudal end (µm)

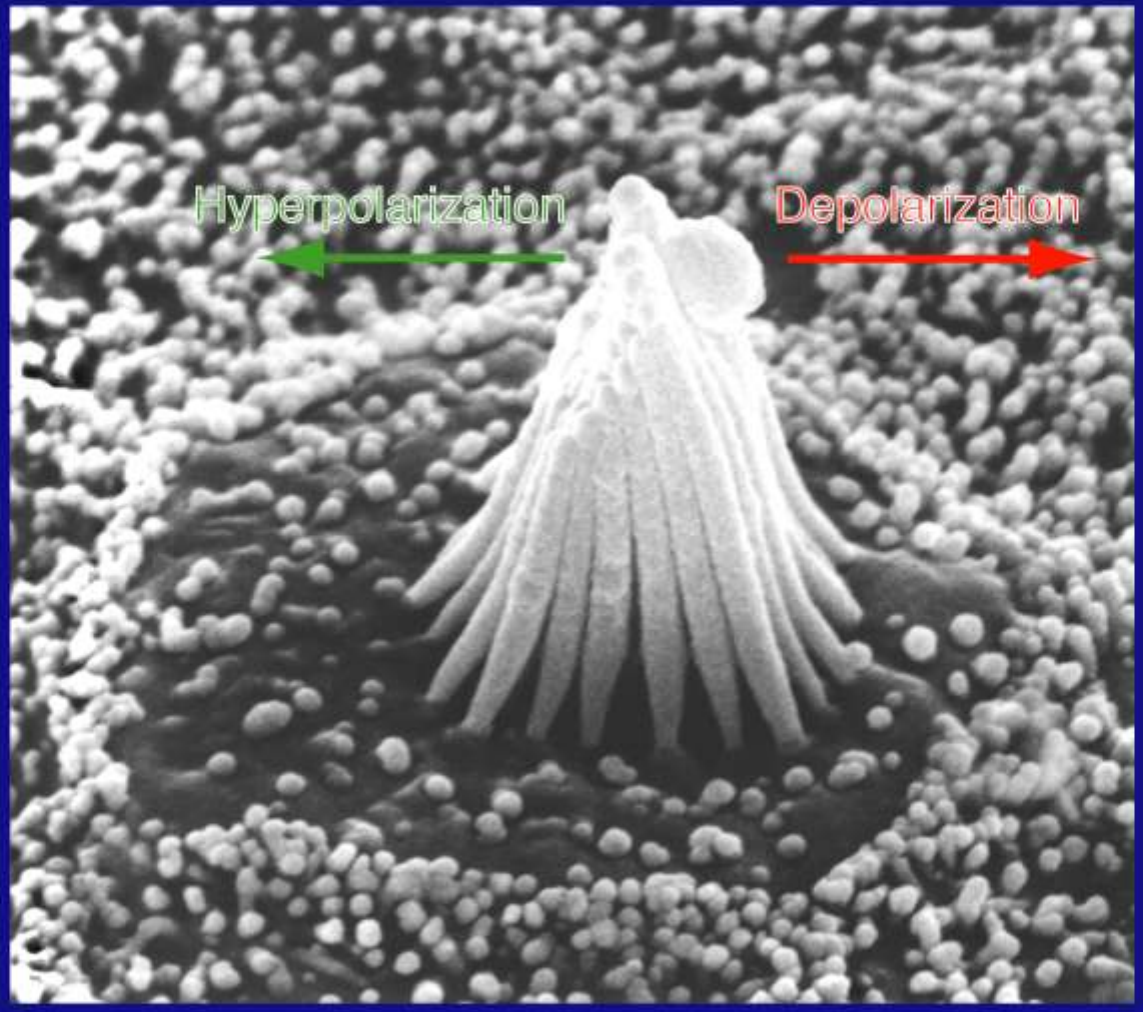

Calbindin-D28k
Relative Intensity
8
7
6
5
4
3
2
1
0
700
600
500
400
300
200
100
0
Distance from caudal end (µm)

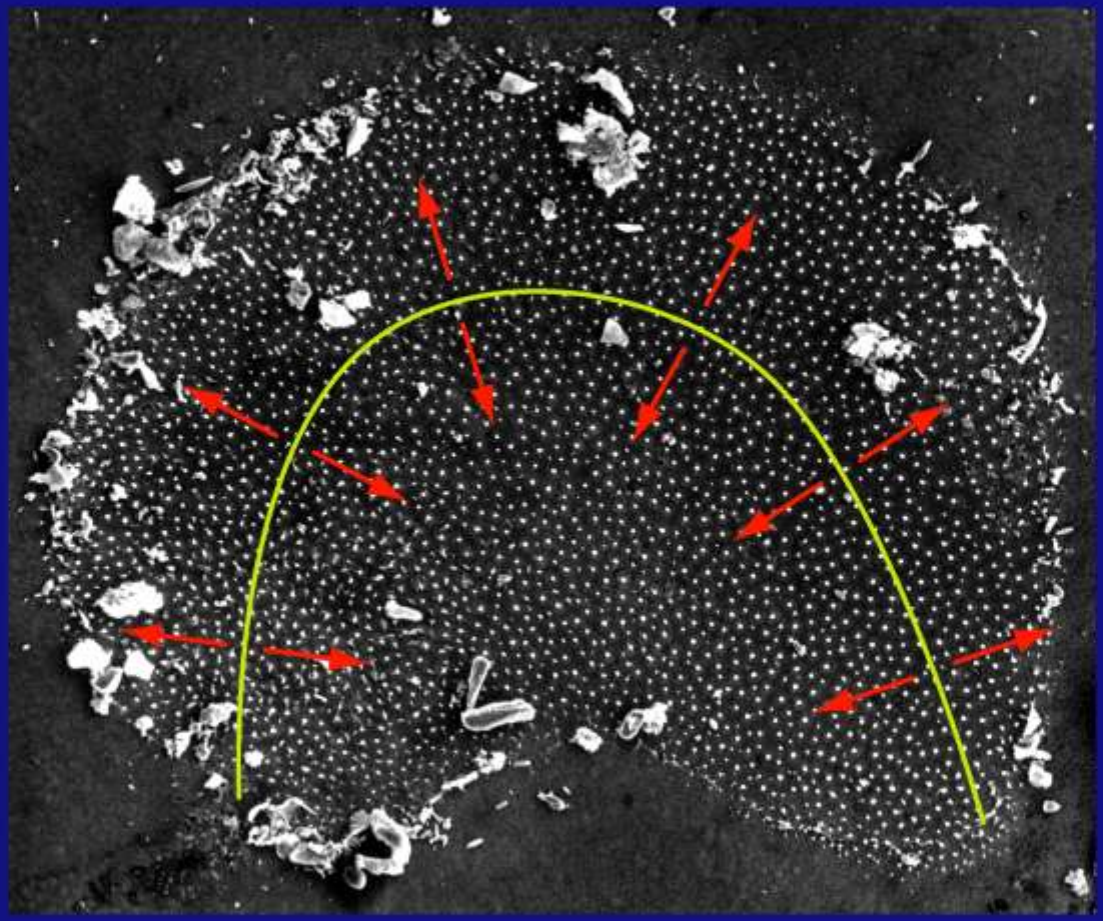
FREQUENCY – TUNING MECHANISMS IN THE AMPHIBIAN PAPILLA

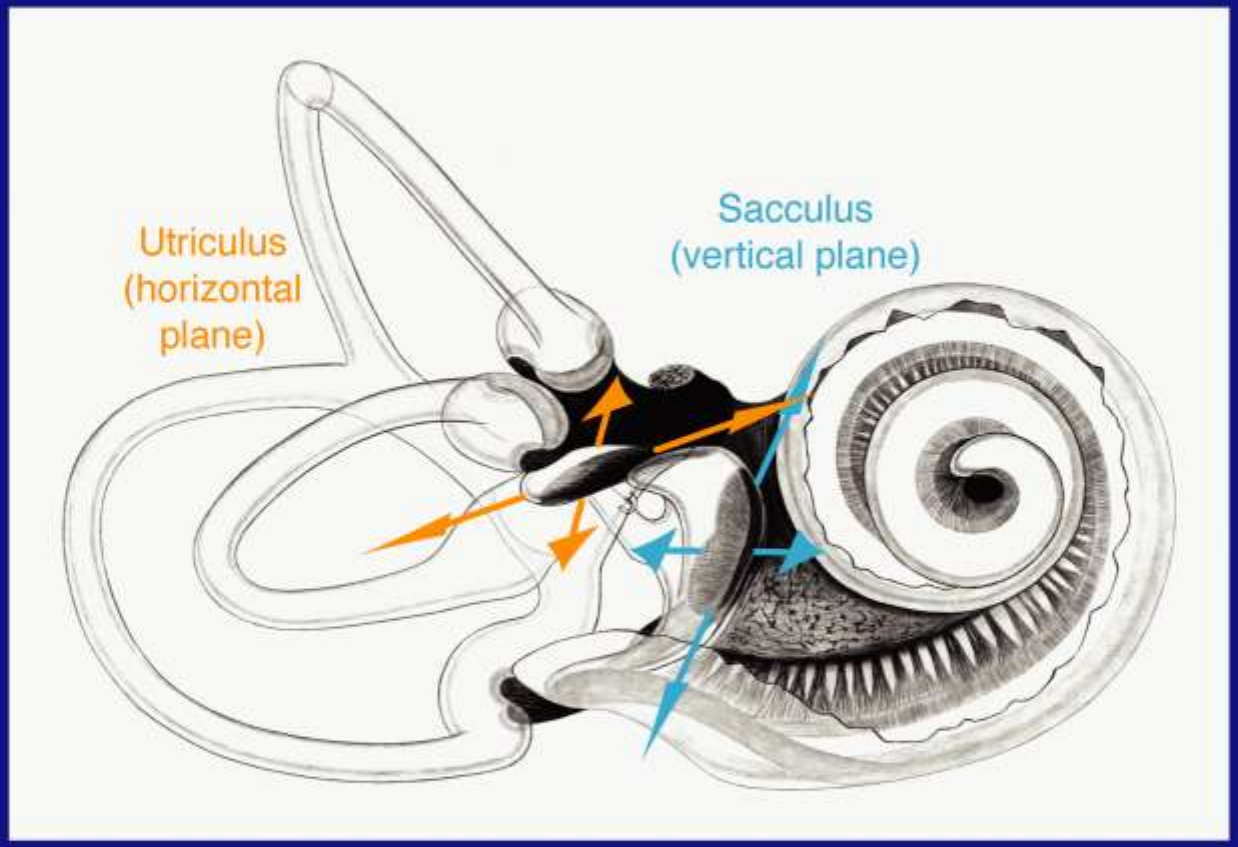


CASCADED FILTERS




Hyperpolarization
Depolarization



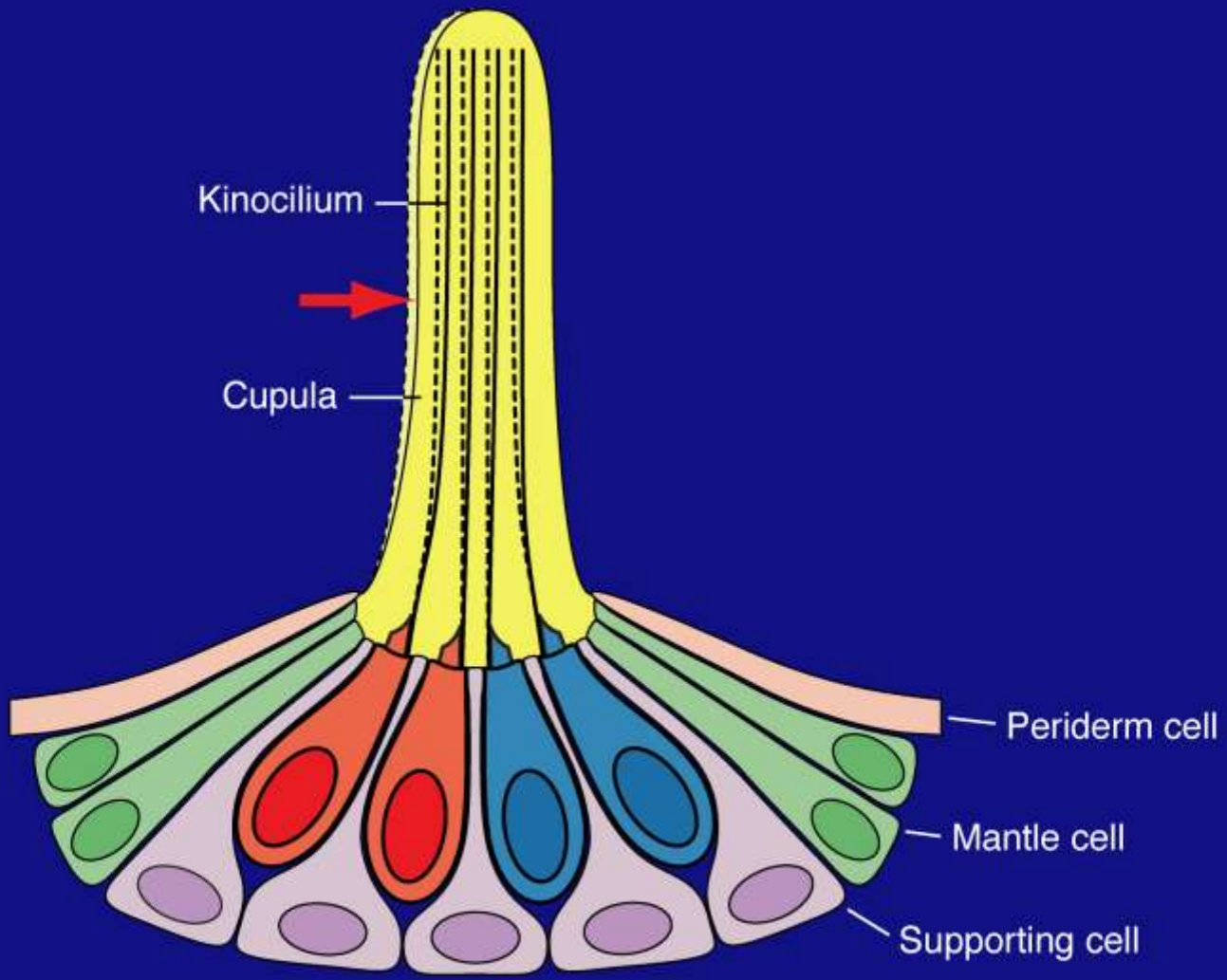


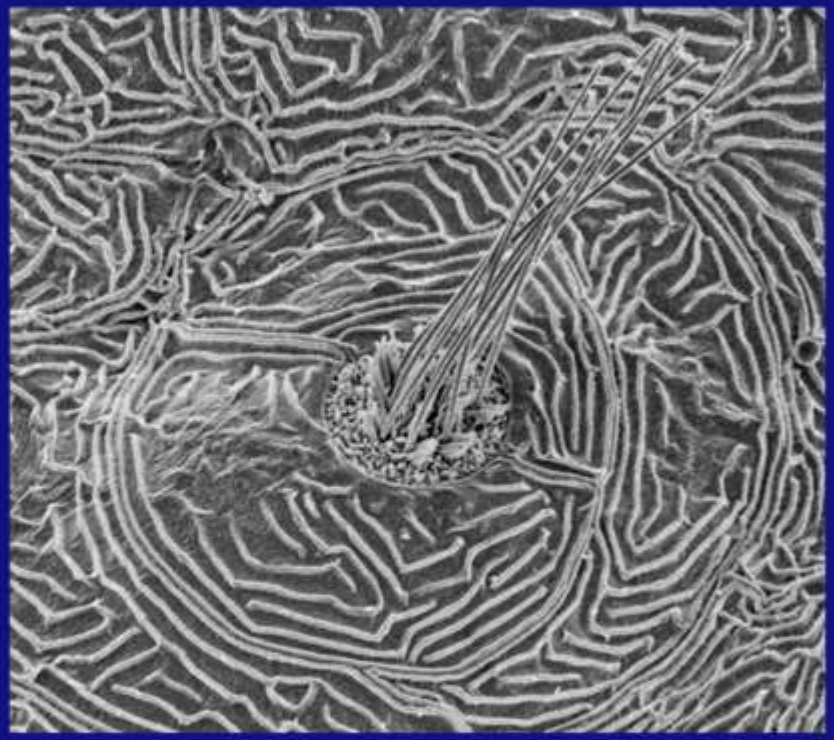
After Retzius, 1884

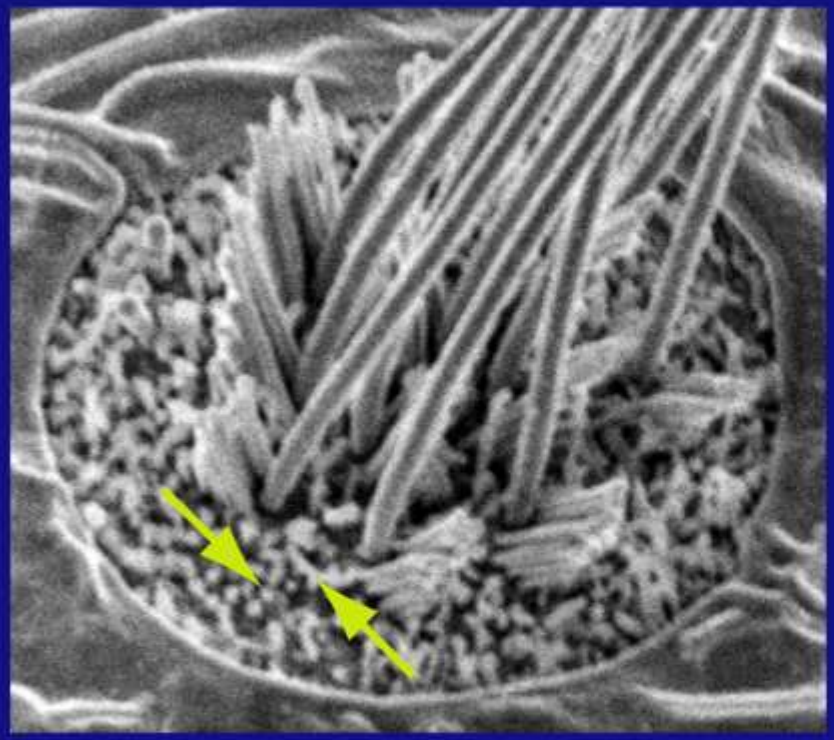
Lateral view

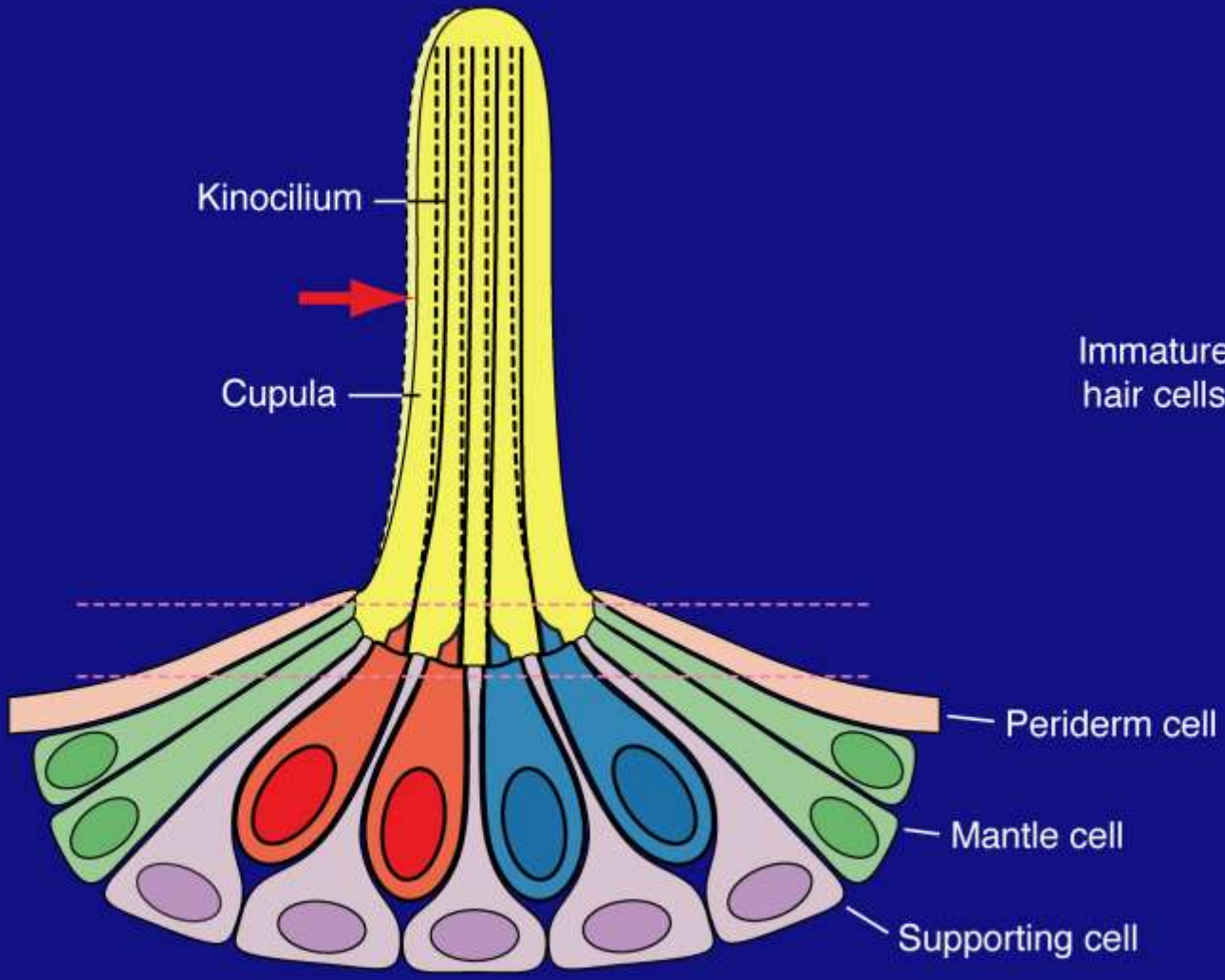
Posterior lateral line

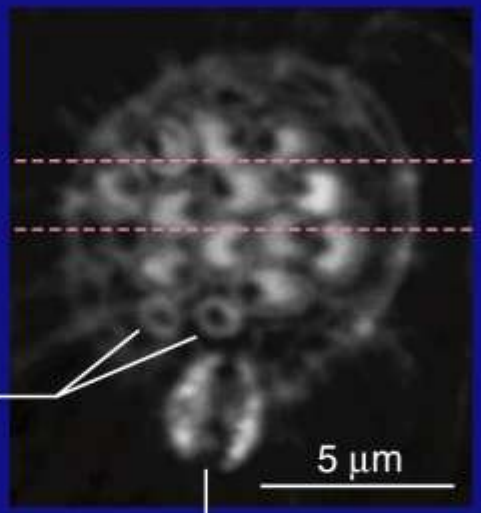
Ventral view

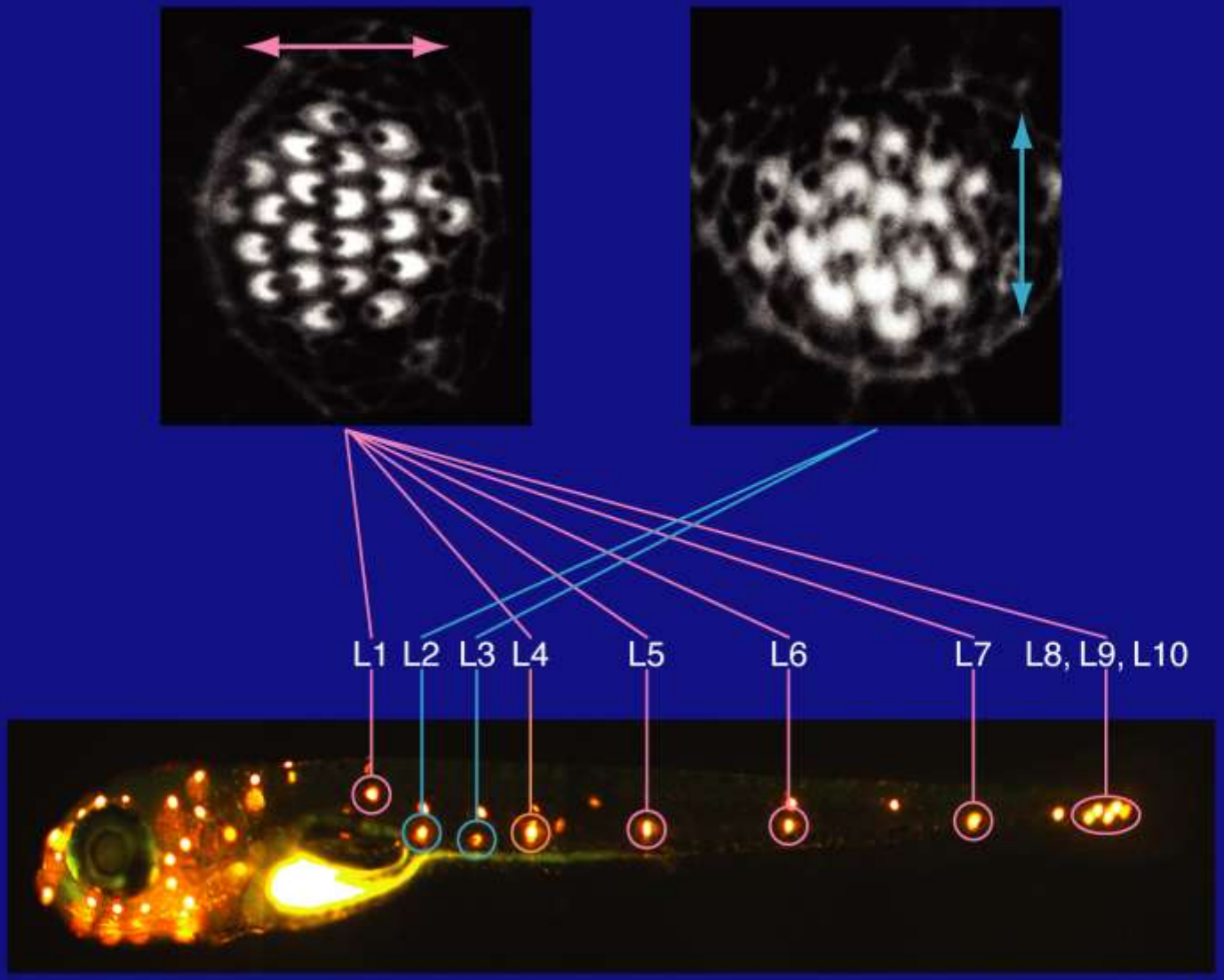

Kinocilium
Cupula
Periderm cell
Mantle cell
Supporting cell

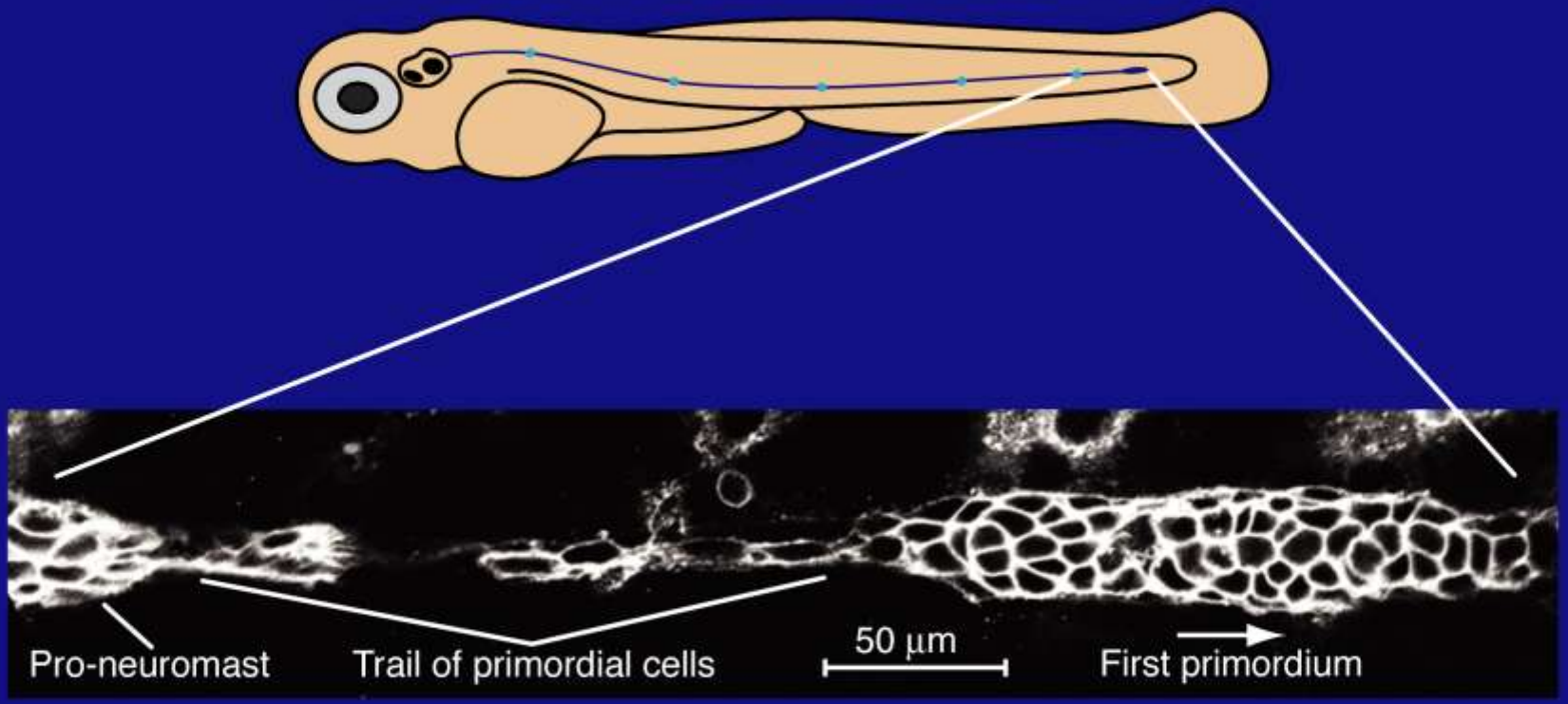


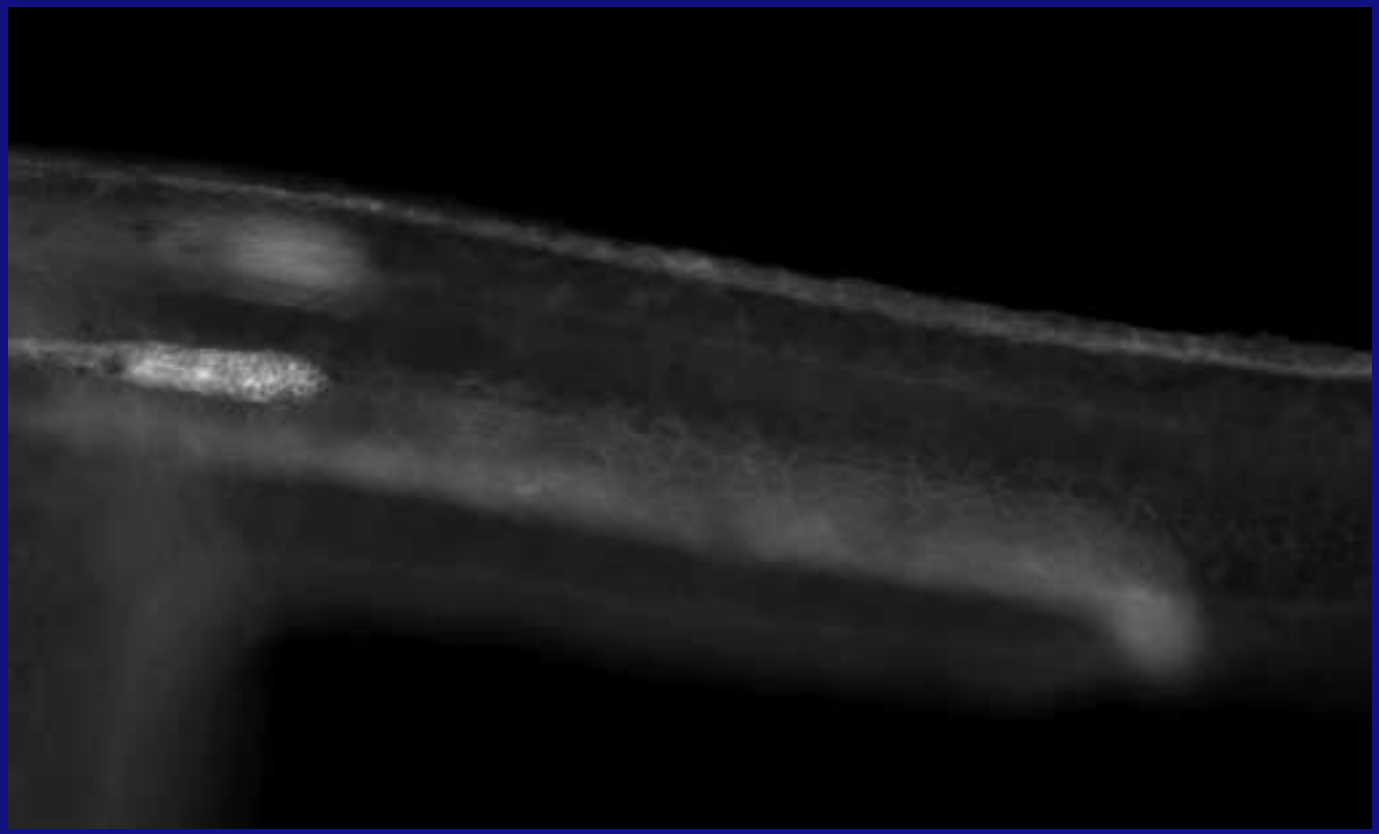


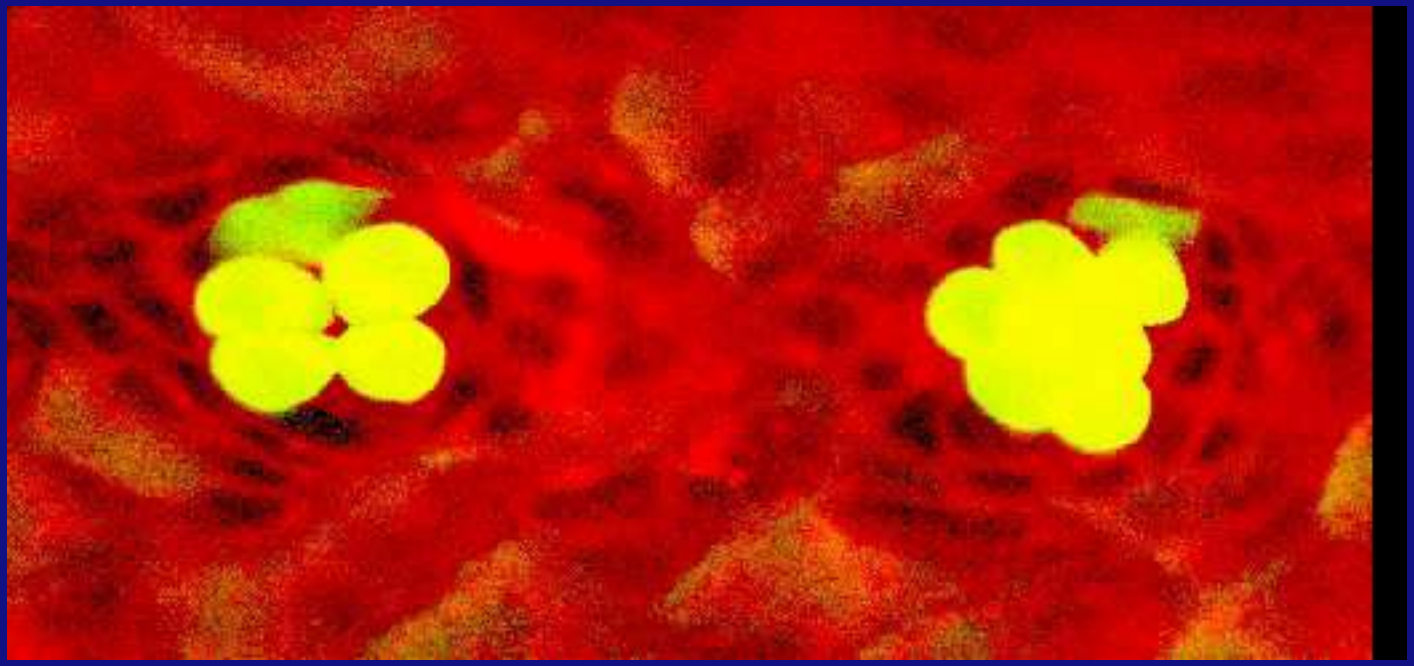

Kinocilium
Cupula
Periderm cell
Mantle cell
Supporting cell

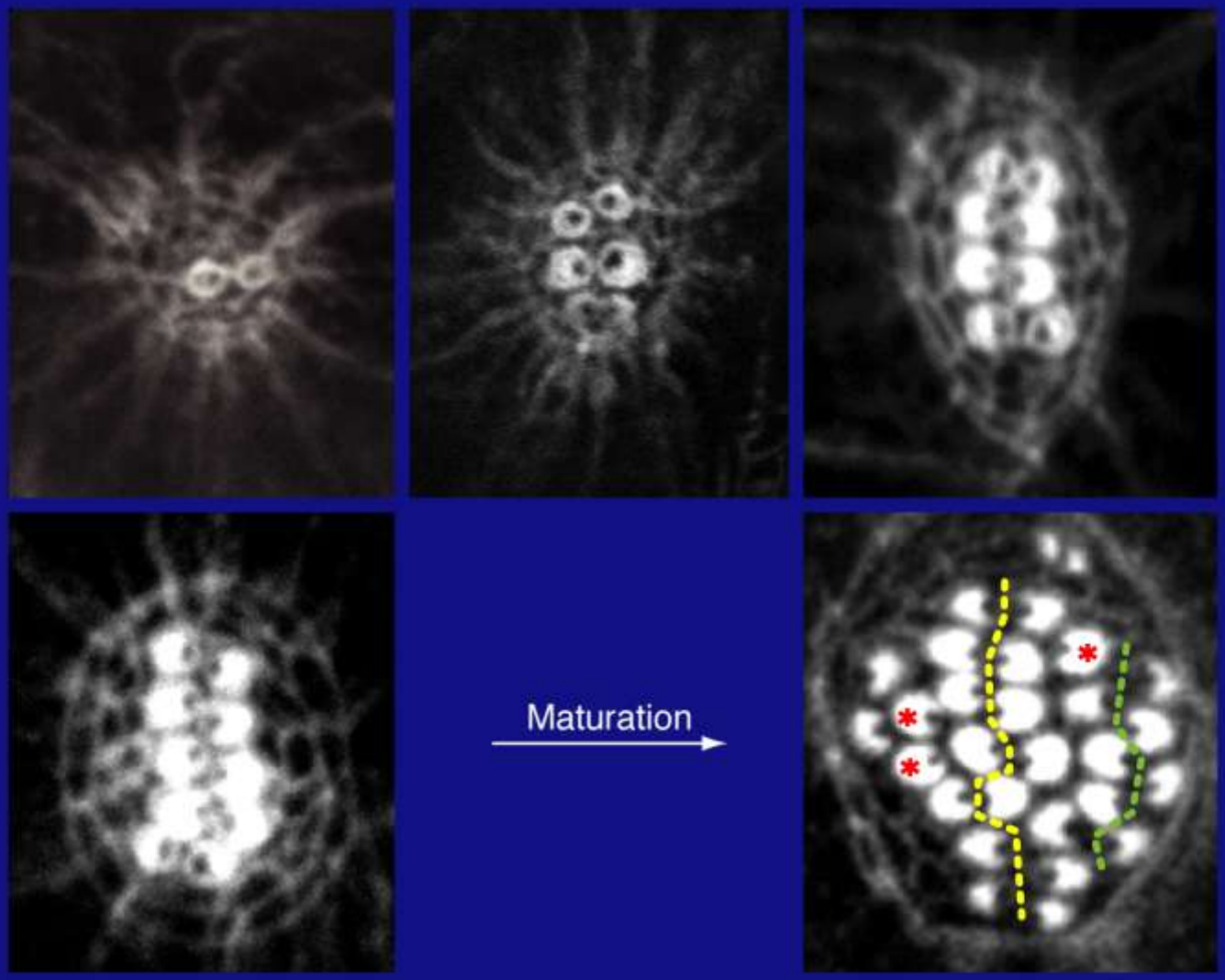

Immature
hair cells
5 µm
Postmitotic
progenitor

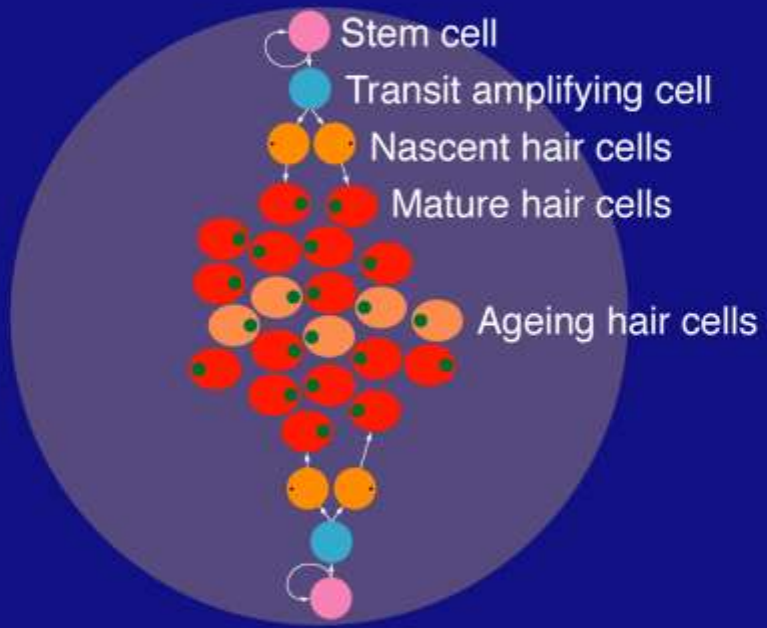

L1 L2 L3 L4 L5 L6 L7 L8, L9, L10

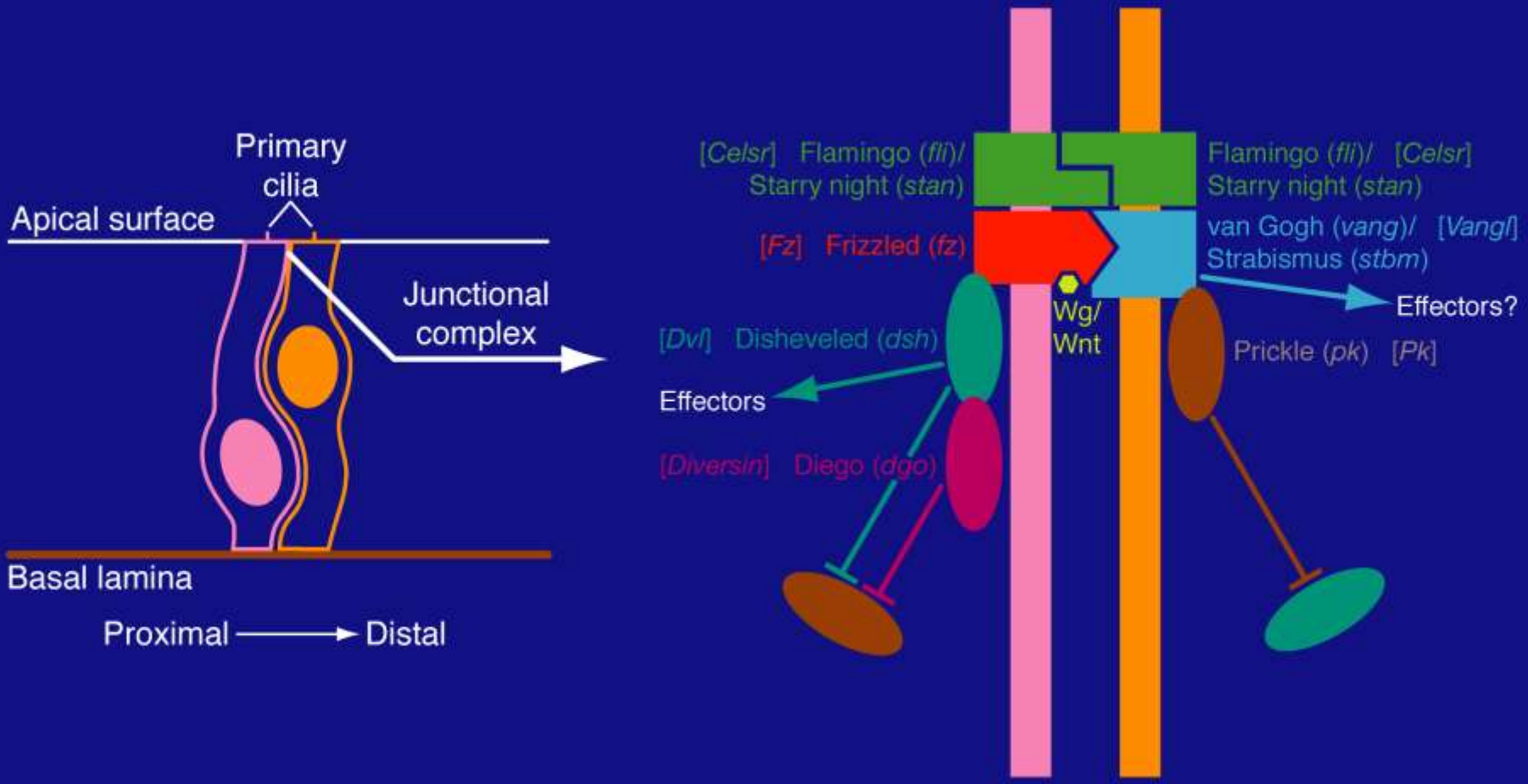

Pro-neuromast
Trail of primordial cells
50 µm
First primordium



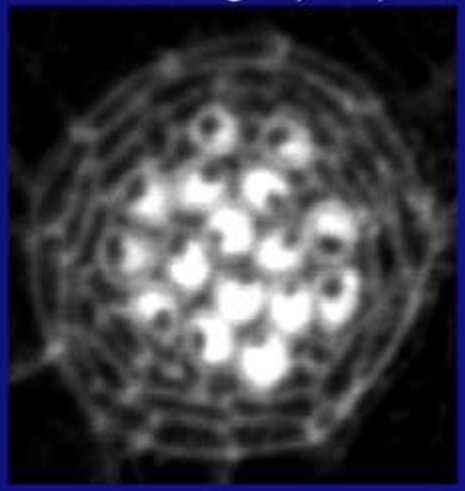



Maturation


Stem cell
Transit amplifying cell
Nascent hair cells
Mature hair cells
Ageing hair cells

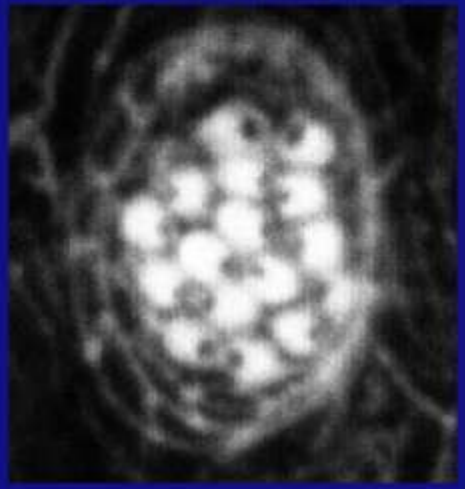

Primary cilia
Apical surface
Junctional complex
Basal lamina
Proximal
Distal
[Celsr] Flamingo (fli)/ Starry night (stan)
Flamingo (fli)/ [Celsr] Starry night (stan)
[Fz] Frizzled (fz)
van Gogh (vang)/ [Vangl] Strabismus (stbm)
Effectors?
Wg/ Wnt
[Dvl] Disheveled (dsh)
Prickle (pk) [Pk]
Effectors
[Diversin] Diego (dgo)

trilobite / vangl2 (stb1)

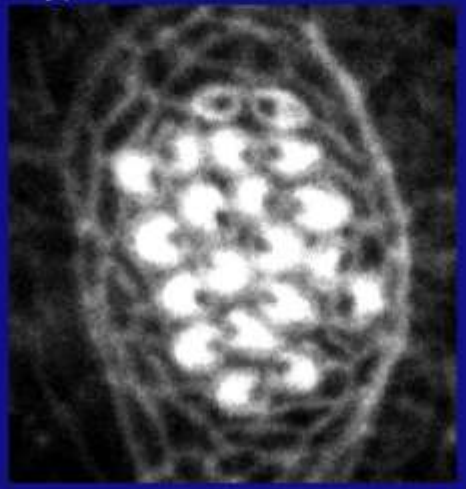


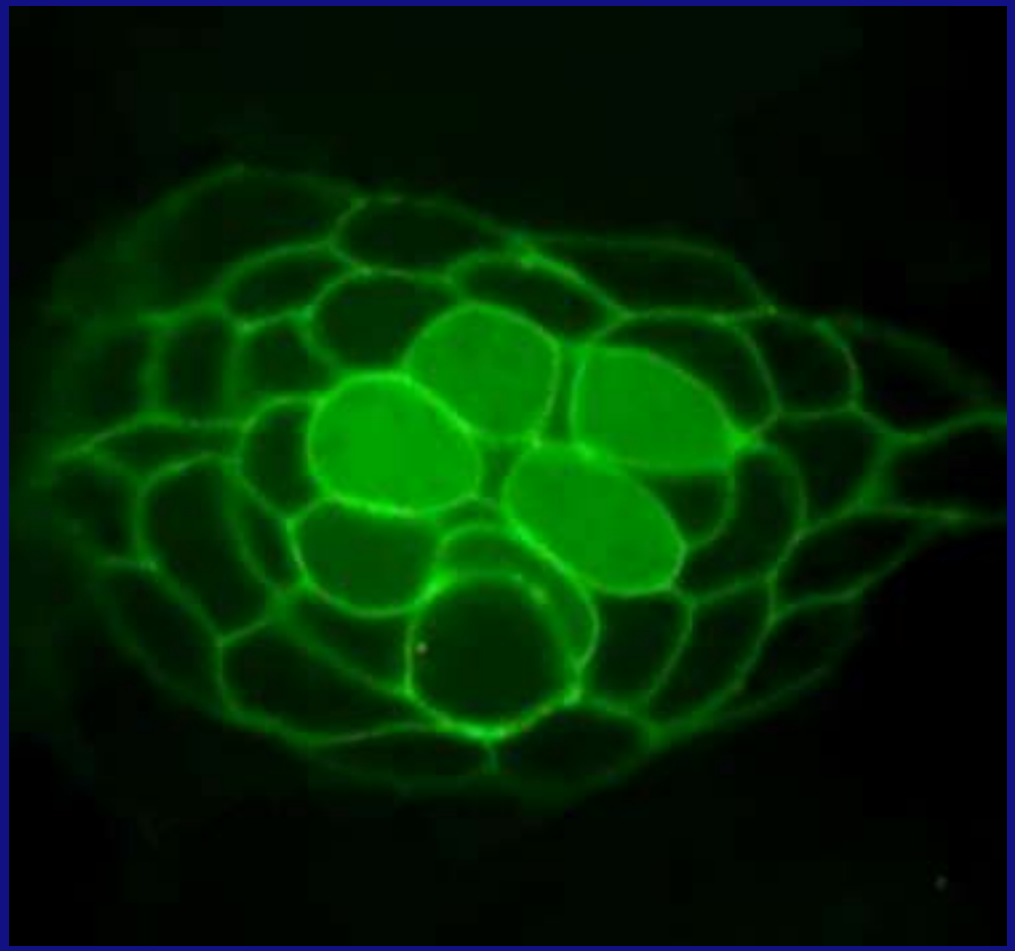
pipetail / wnt5a

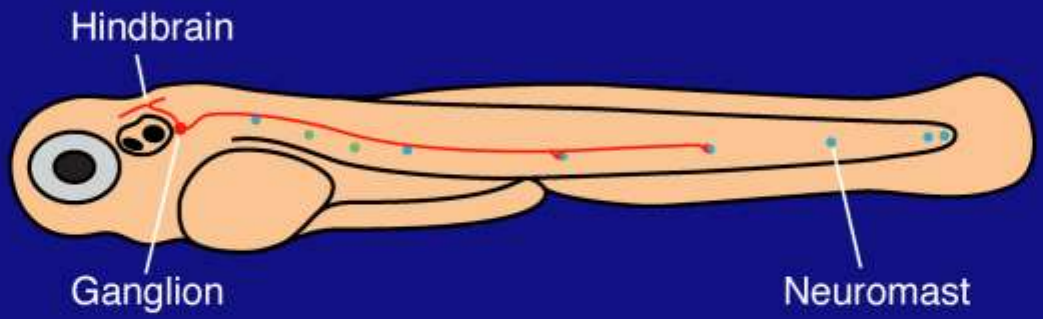
silverblick / wnt11

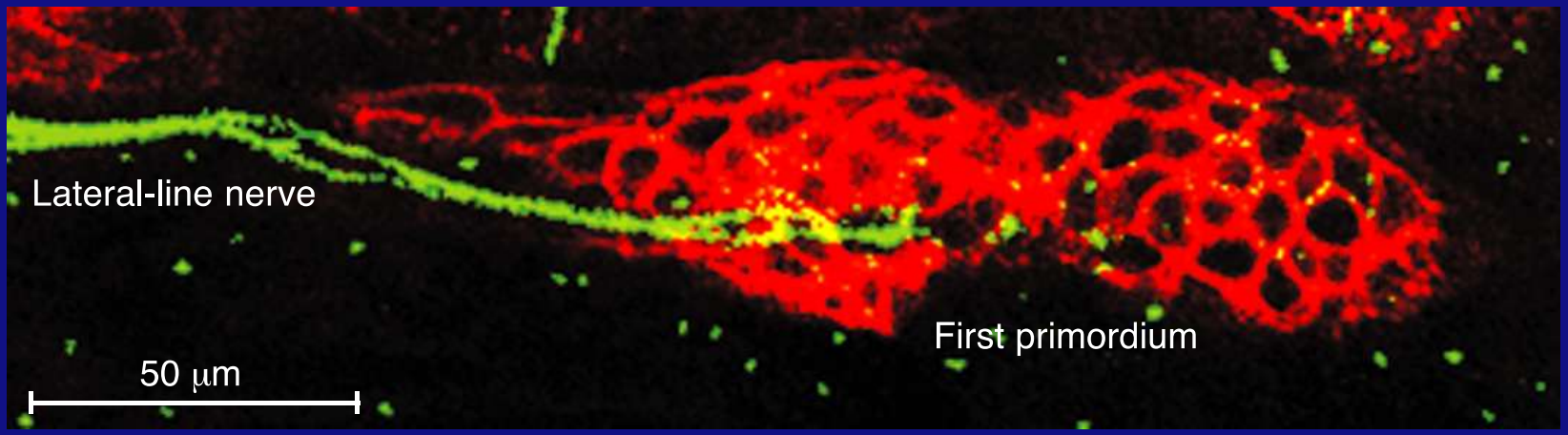


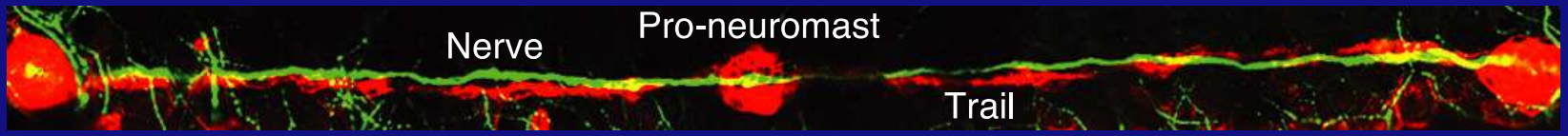
knypek

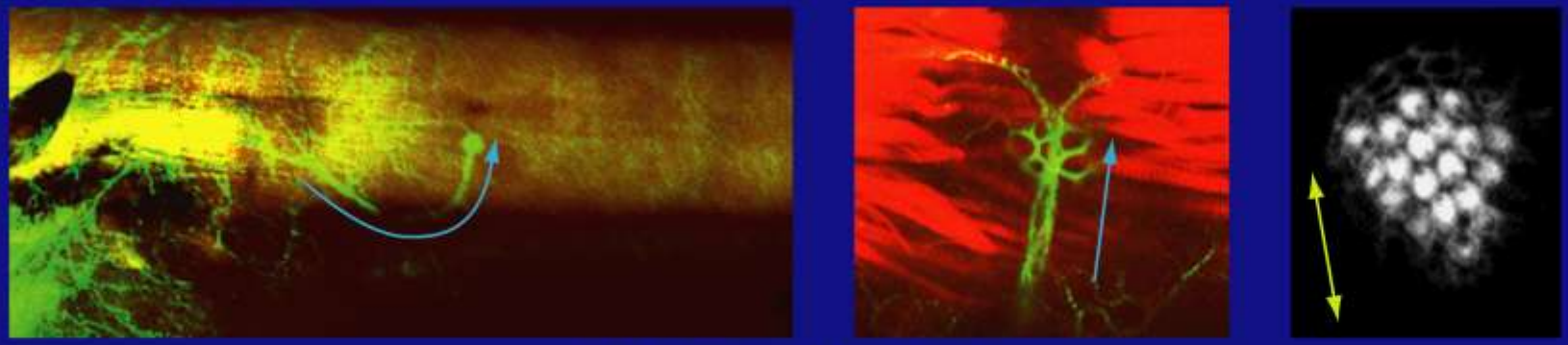





Hindbrain
Ganglion
Neuromast


Lateral-line nerve
First primordium
50 μm


Nerve
Pro-neuromast
Trail

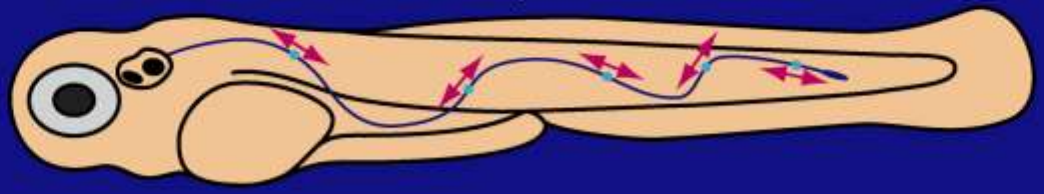


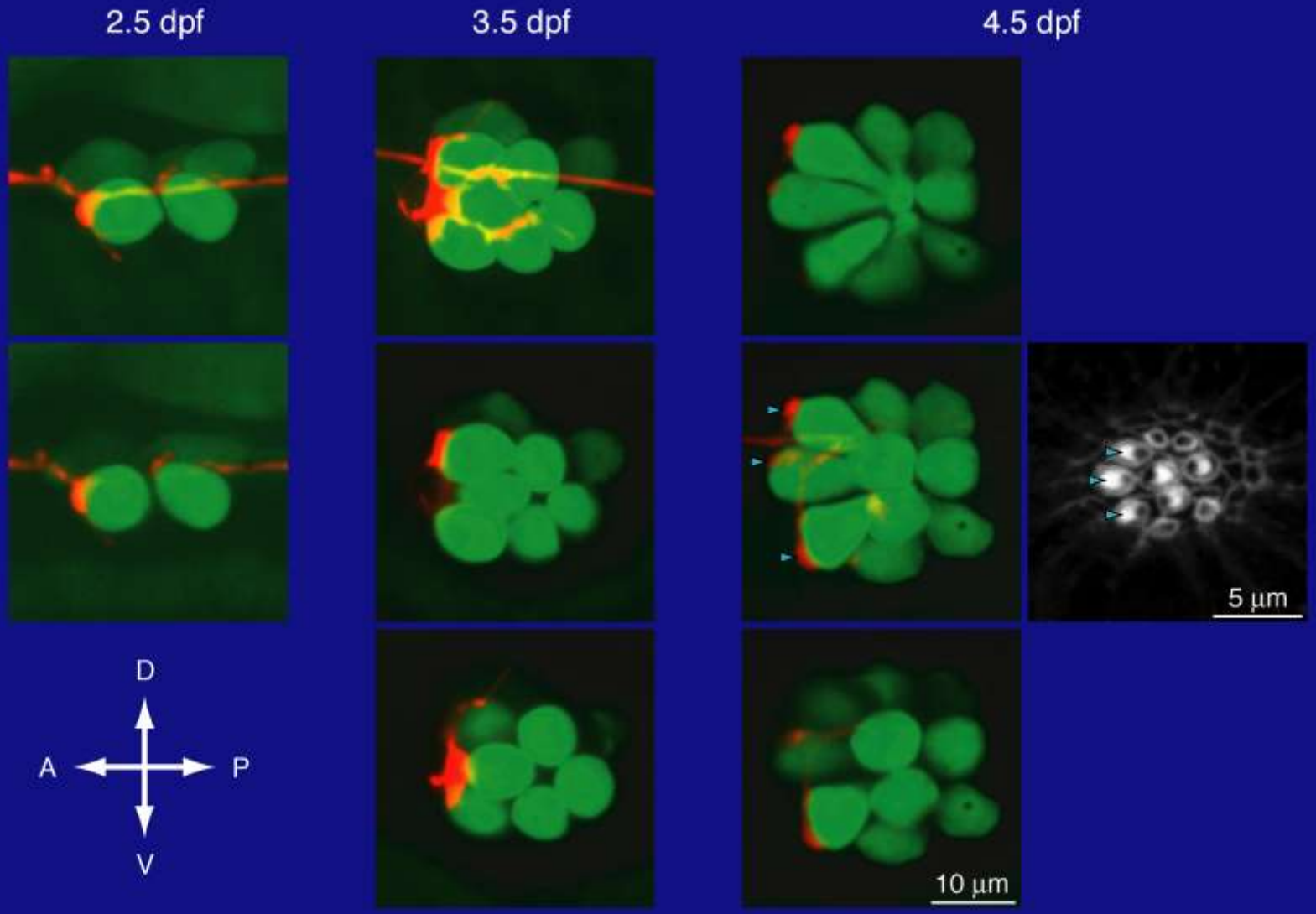
fused somites (*fss*) mutant

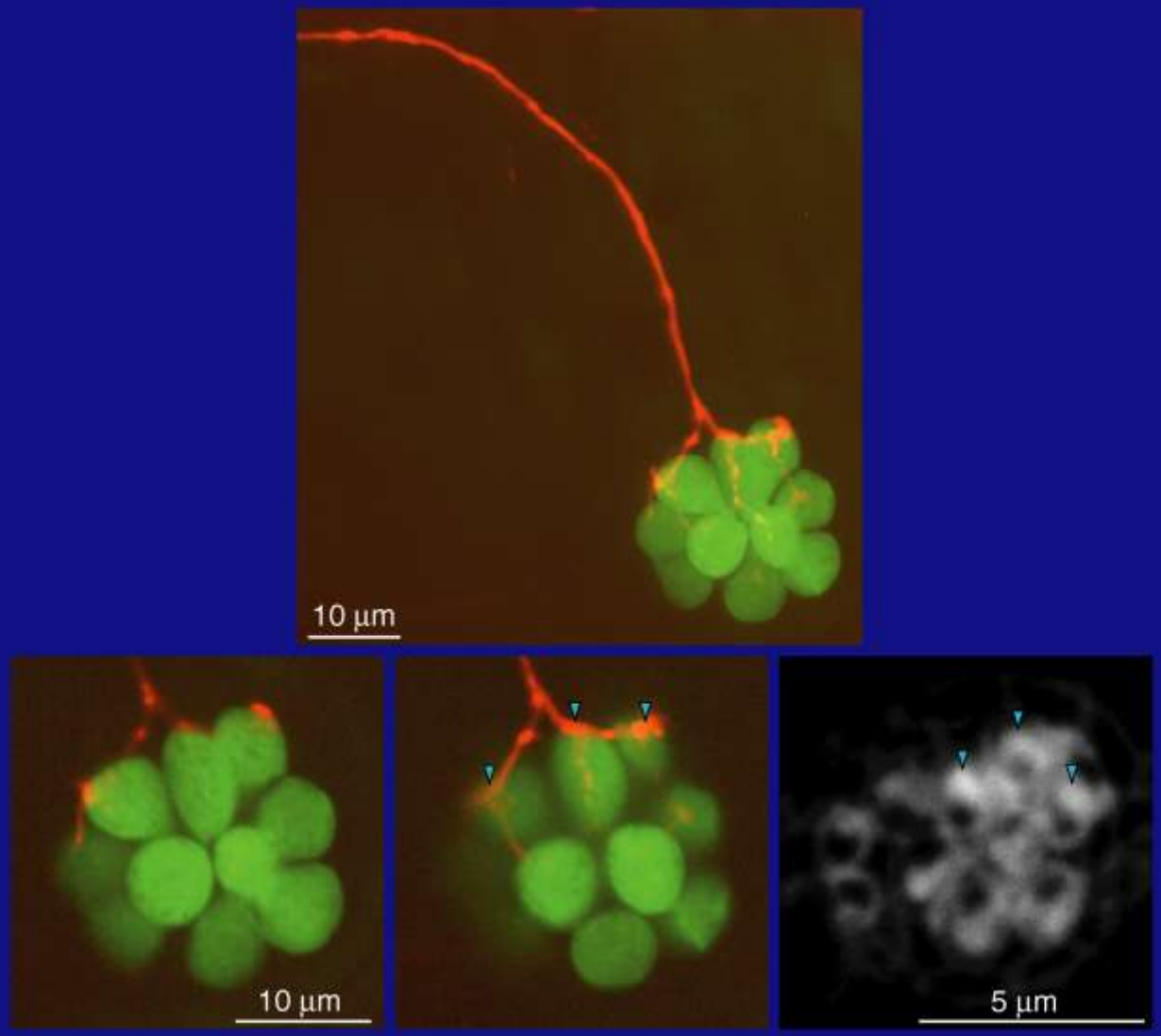
sdf1a morpholino and *sdf1b-GFP* plasmid

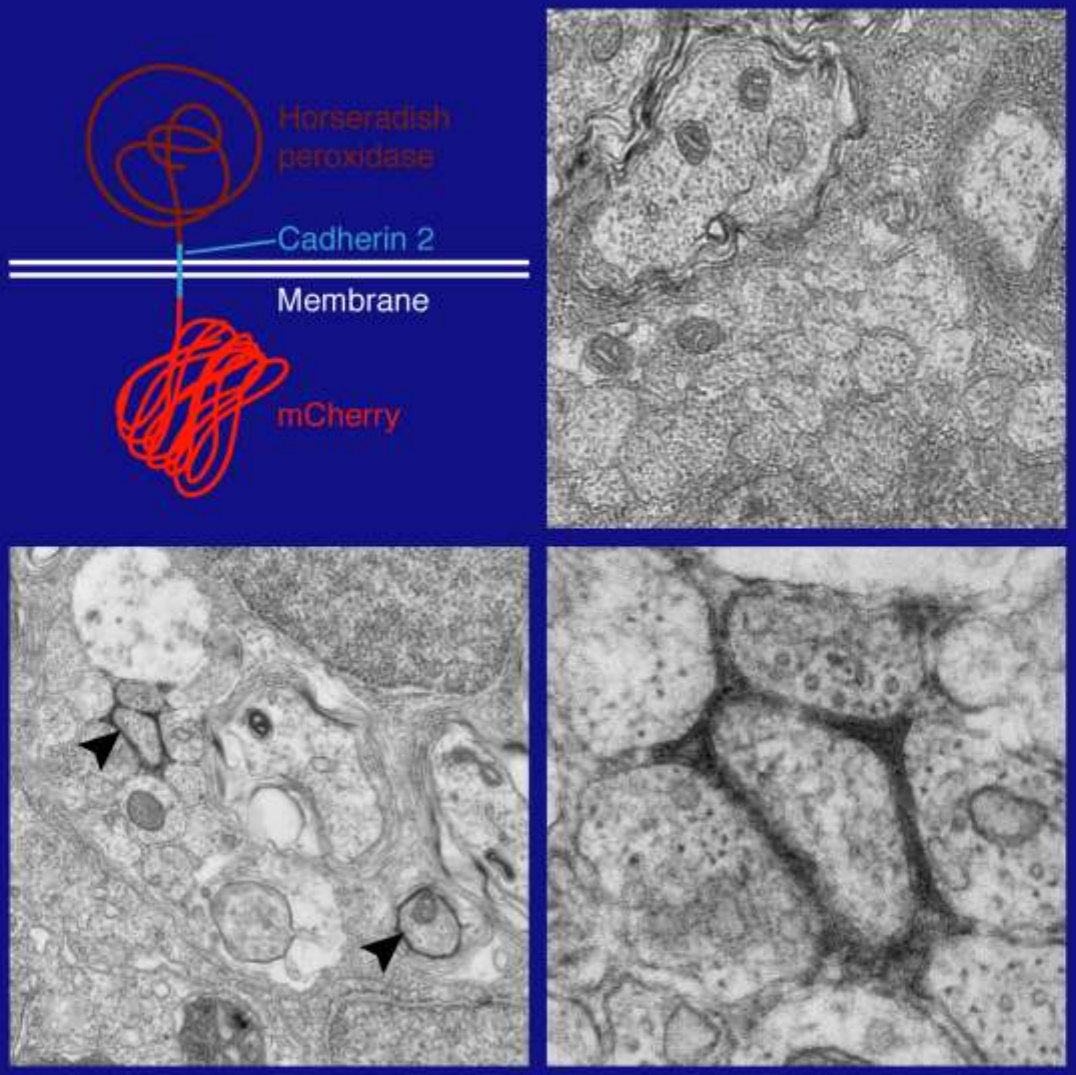
Wild-type larva

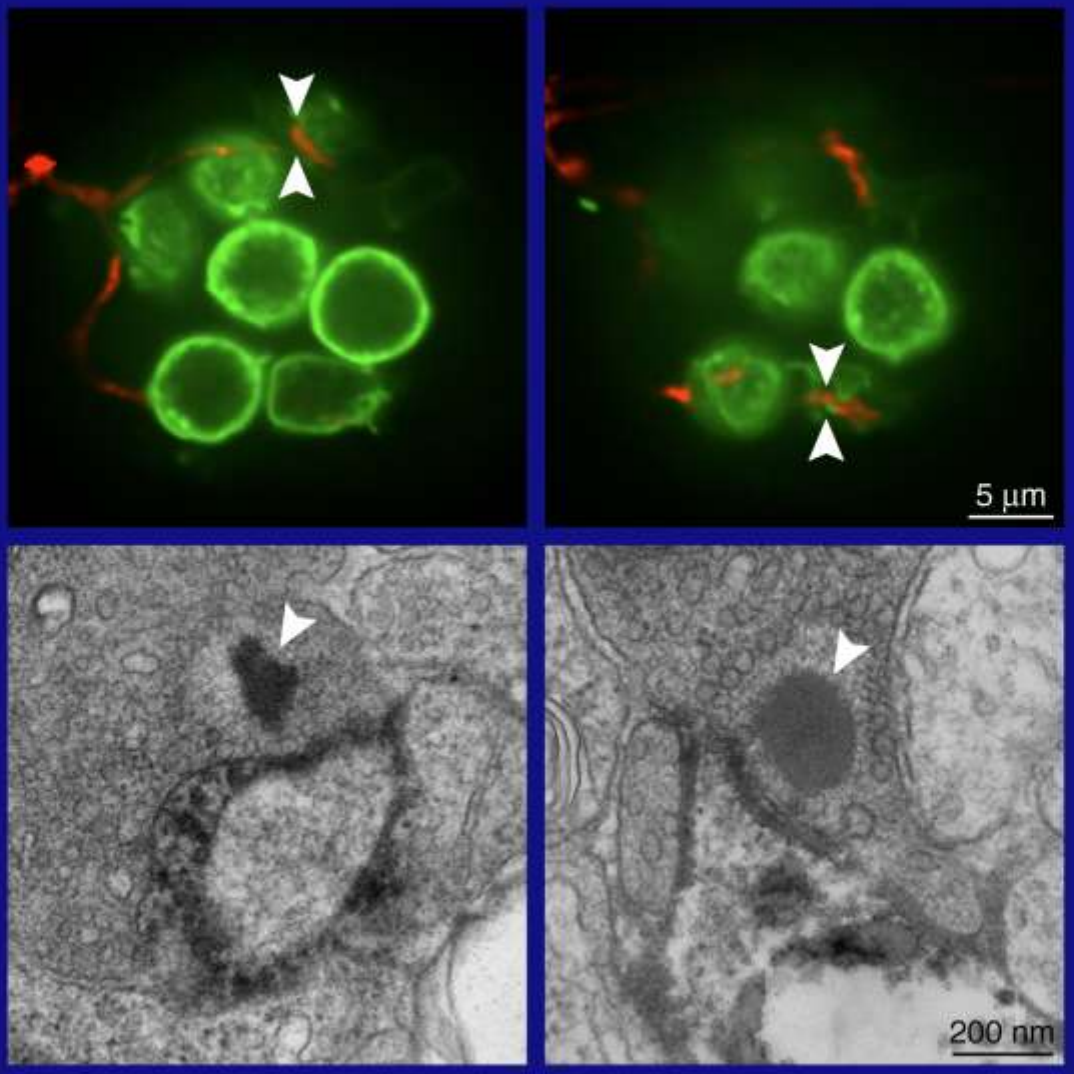
slow muscle omitted or *fused somites* mutant,
sdf1a morphant, *etc*.



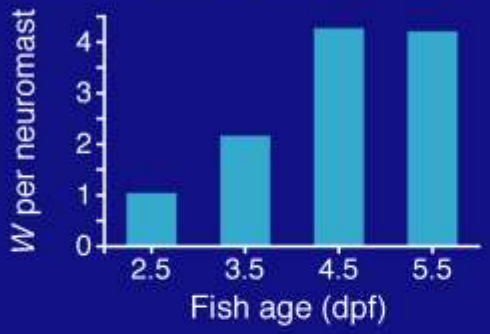

2.5 dpf
3.5 dpf
4.5 dpf
D
A
P
V
5 µm
10 µm


10 µm
10 µm
5 µm

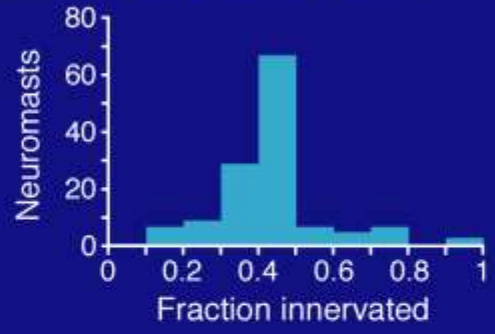

Horseradish
peroxidase
Cadherin 2
Membrane
mCherry


5 µm
200 nm

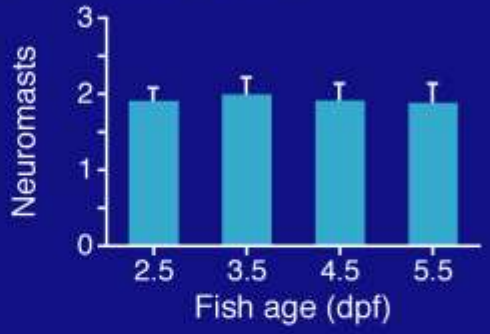
Statistical weight for biassed model of innervation



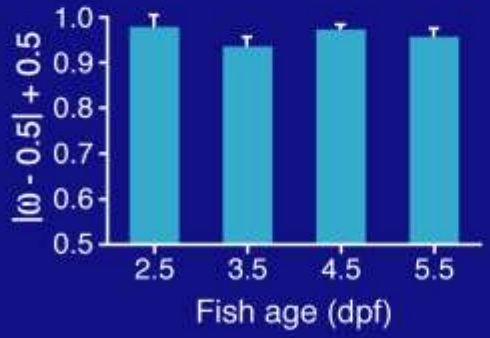
Fraction of hair cells innervated by labelled afferent fiber



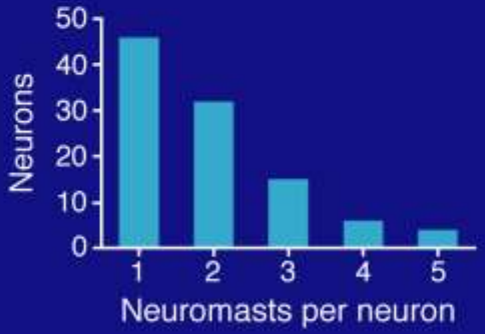
Neuromasts innervated per afferent fiber



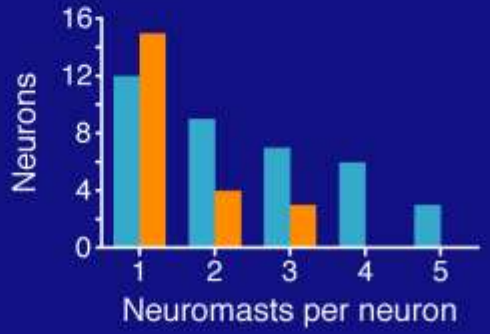
Degree of innervation bias

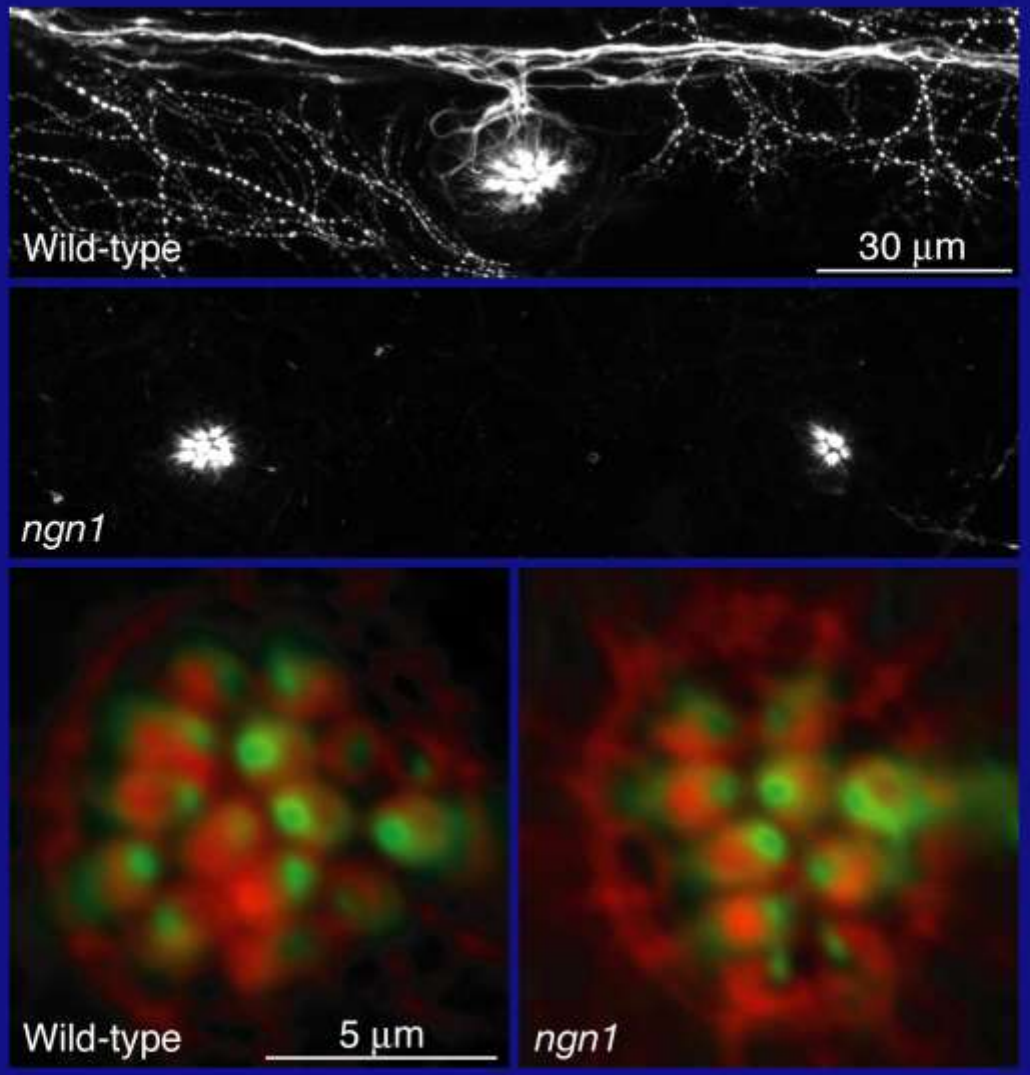


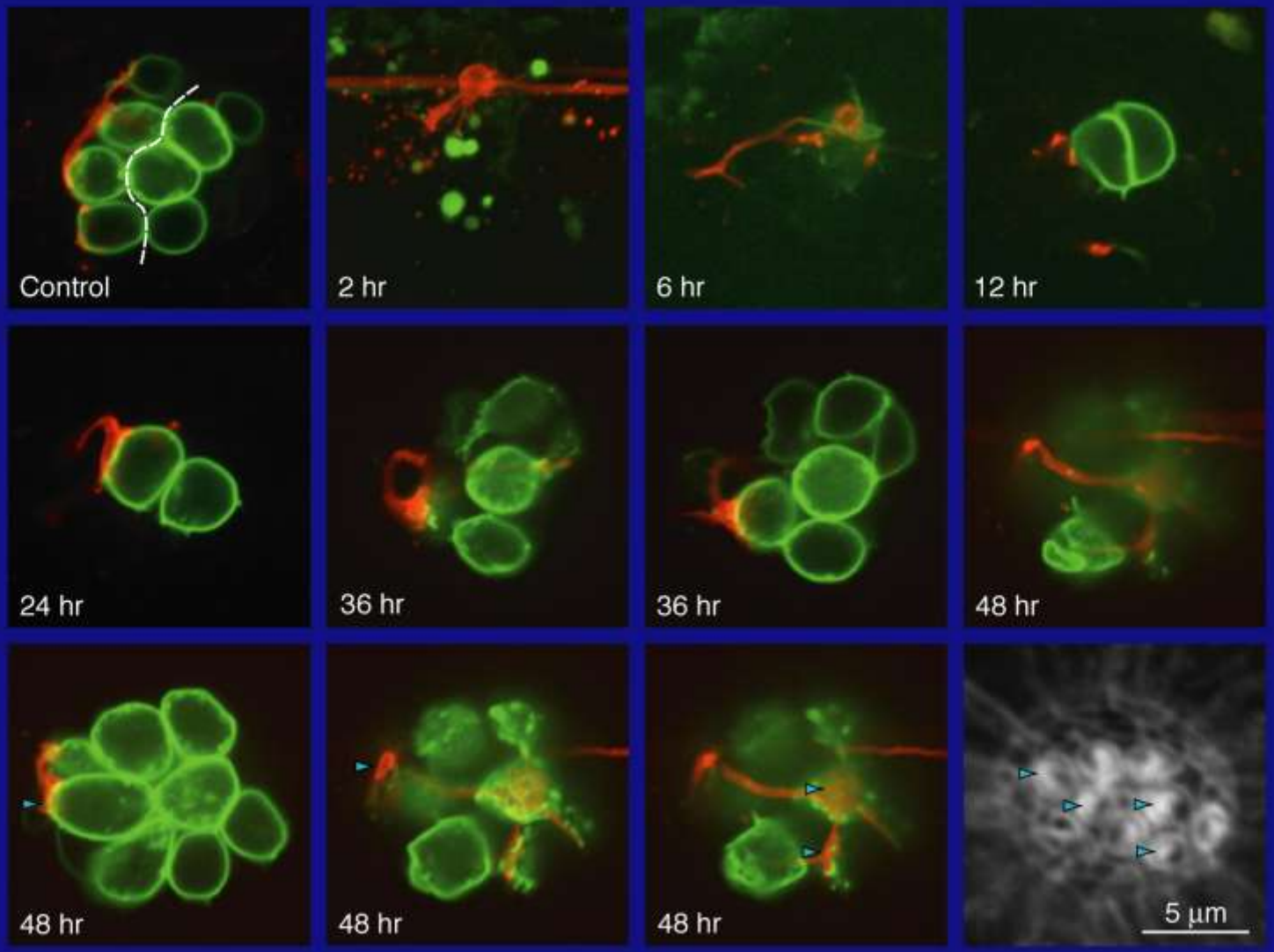
Receptive-field size of afferent fiber



Posteriorly (■) or anteriorly biased (■) afferent fibers



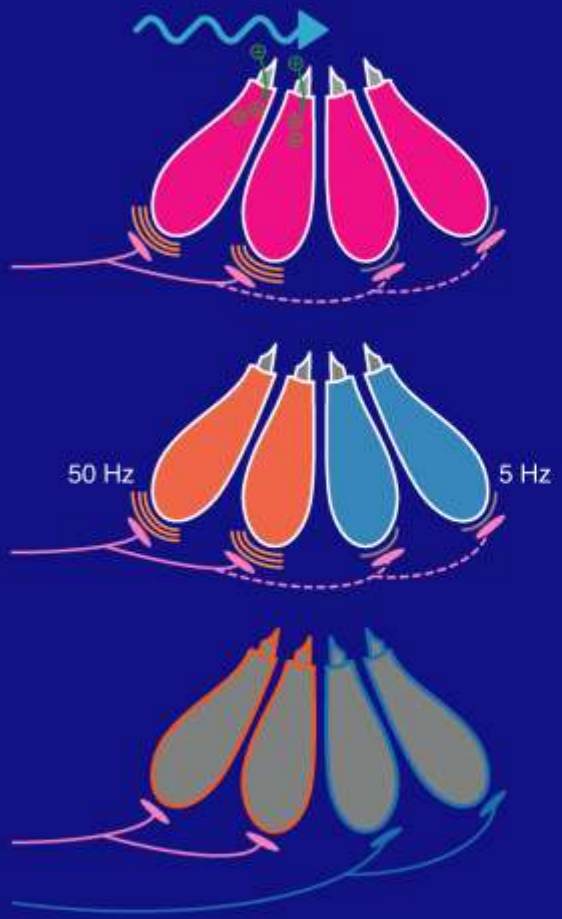

Wild-type
30 µm
ngn1
Wild-type
5 µm
ngn1


Control
2 hr
6 hr
12 hr
24 hr
36 hr
36 hr
48 hr
48 hr
48 hr
48 hr
5 µm

Activity dependence:
synchronous stimulation of
similarly oriented hair cells

Activity dependence:
distinct patterns of spontaneous
neurotransmitter release by
differently oriented hair cells

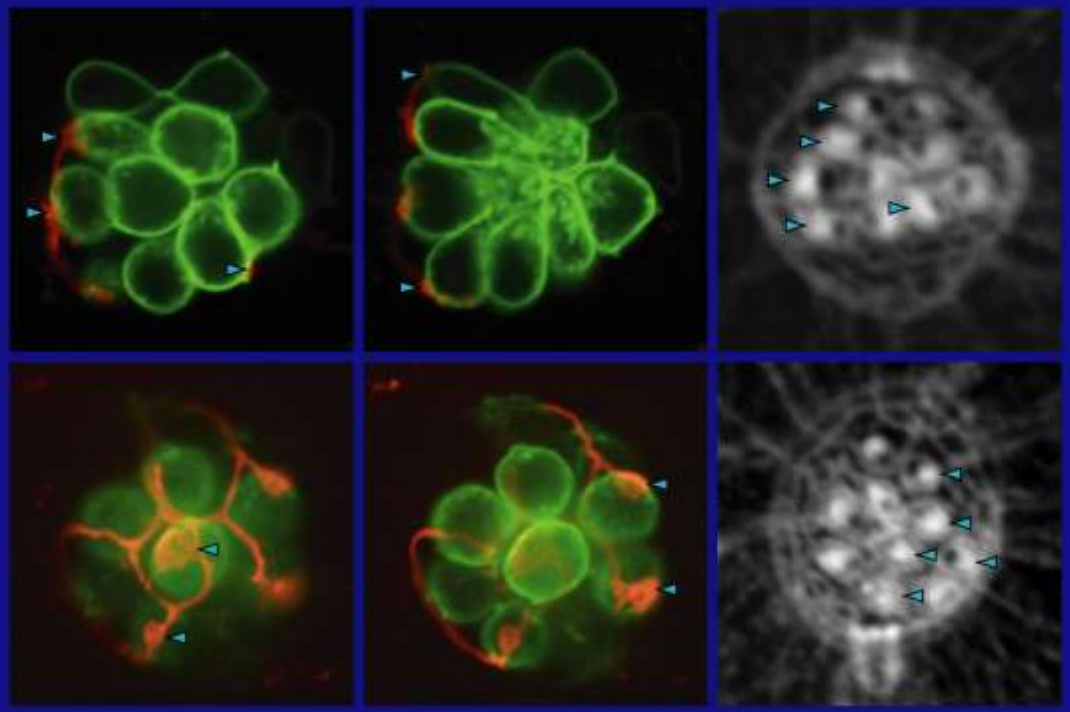
Chemical specification:
distinct molecular labels on
differently oriented hair cells



Mutations affecting mechanoelectrical transduction

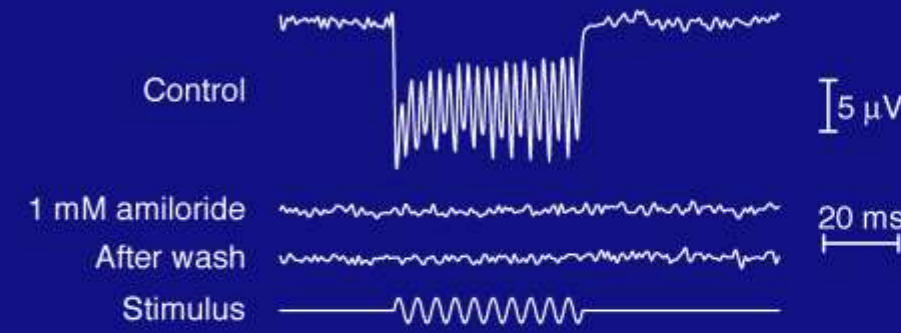
tmie
(transmembrane
inner ear protein
[Tmie])

orbiter / pcdh15a
(tip-link constituent
protocadherin 15a)

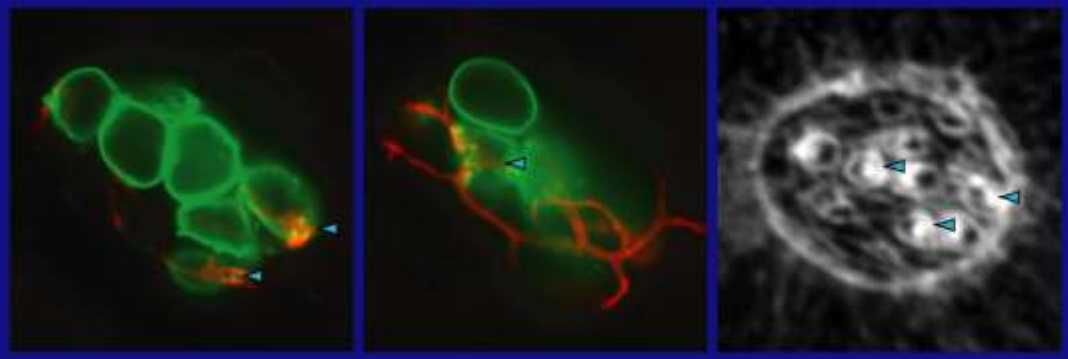


Exposure to amiloride, a blocker of mechanoelectrical transduction

Physiological effect of 1 mM amiloride for three days



1 mM amiloride for three days



Mutations affecting the release of afferent synaptic transmitter

gemini / cav1.3a
(L-type Ca^{2+} channel $Ca_V1.3$)

asteroid / vglut3
(vesicular glutamate transporter type 3)