

Neurobiology of Hearing
Salamanca, 21st May 2015

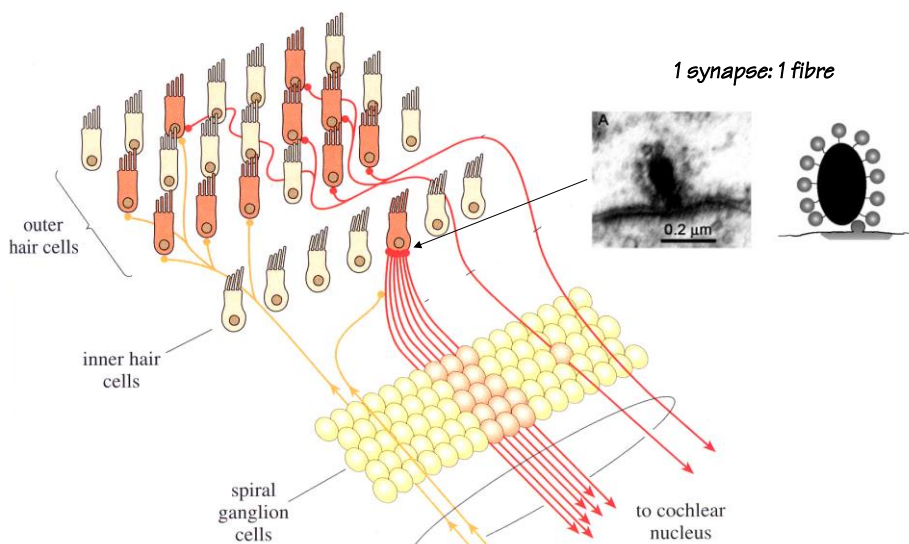
The cochlea: inner hair cells & the ribbon synapse

Jonathan Ashmore
Neuroscience, Physiology and Pharmacology
University College London

j.ashmore@ucl.ac.uk



Information transfer in the mammalian cochlea



Open University 'Signals'
Fuchs J Physiol 2005
Meyer et al, 2009

The cochlea must provide the central auditory system with :

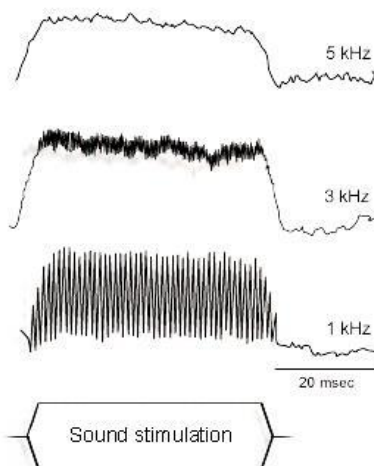
the frequency content of the sound;
achieved through mechanics and hair cell organisation

the relative phases between the time varying signals,
achieved by synaptic properties and multiple fibres?

the amplitudes of the constituent sound components.
achieved by synaptic properties and features of IHC?

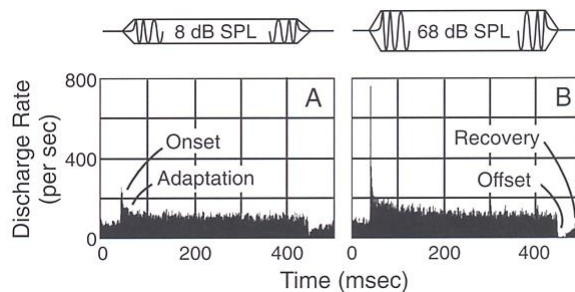
(.. and it must be correctly wired up!)

How does an inner hair cell respond to a 'high frequency' sound?



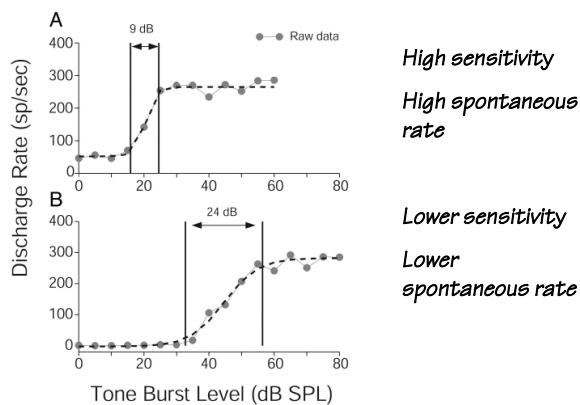
Russell & Sellick 1983

- IHC synapses*
- 1) preserve onset timing over a large range
 - 2) adapt rapidly
 - 3) are 'indefatigable'



Westerman and Smith, JASA 1987

The dynamic range problem: is intensity segmented?



Mouse: Taberner & Liberman 2005

Cat: Sachs and Abbas 1974

Liberman 1982

Cat: shows differential distribution of fibre types

The dynamic range problem:

- 1) *Sound intensity is encoded over > 80 dB range*
- 2) *Individual nerve fibres only cover 20-30dB*
- 3) *=> fibre population is 'stratified'*

A pre- or postsynaptic mechanism?

Synaptic technologies

Electrophysiology

Patch clamp

Extracellular recording from the nerve

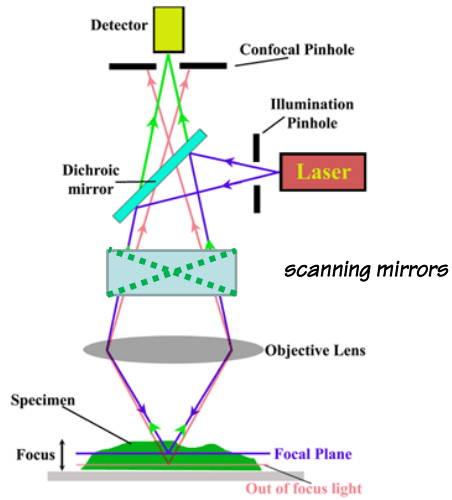
Imaging (CLSM, 2PCLSM, STED, TIRF etc)

With fluorescent tagged antibodies

With calcium indicators

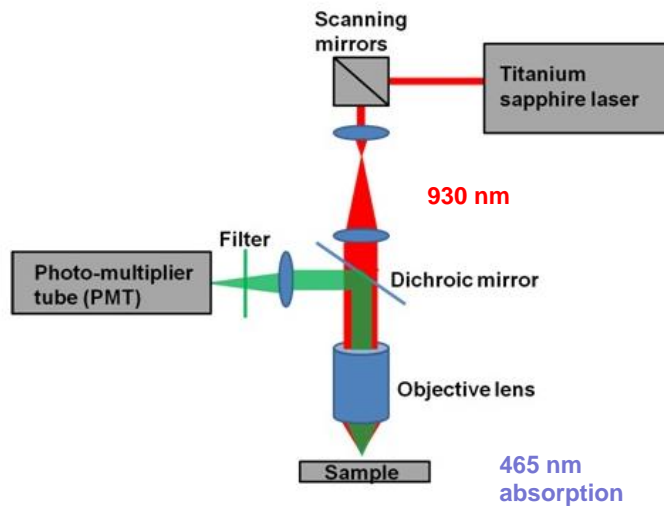
With membrane labels

Laser Scanning Confocal Microscopy



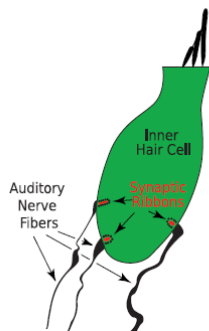
<http://www.uhasselt.be/UH/BIOMED>

2 photon laser scanning confocal microscopy

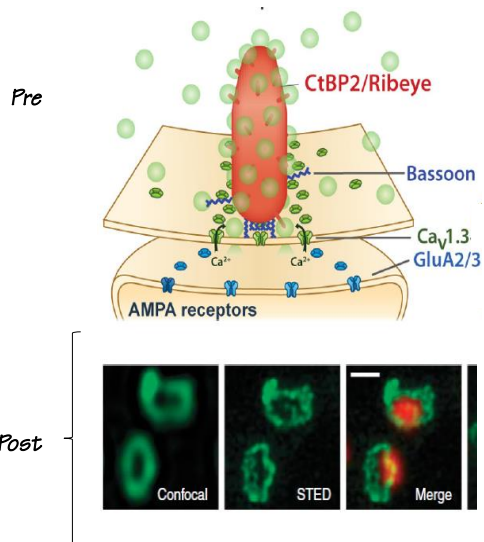


<http://biophotonics.illinois.edu/technology/mpm/>

The hair cell synapse

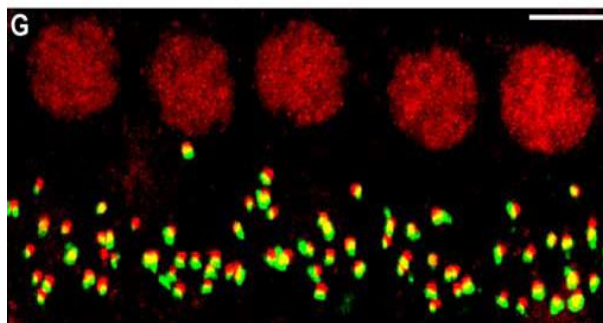


Baran et al., 2010



Zhing et al, J NSci 2013

Each inner hair cell is associated with multiple synaptic ribbons (10 - 30)

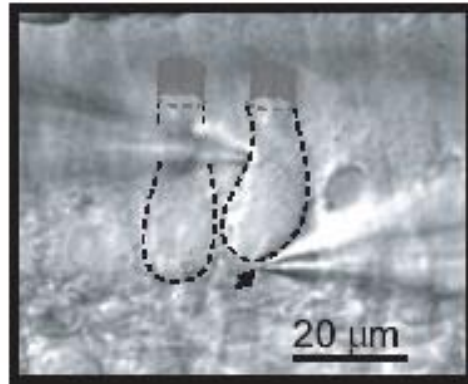


RIBEYE / CtBP2 – ribbon protein

GLUR2/3 – postsynaptic receptor

Brandt, Khimich & Moser, JNeurosci, 2005

Single and dual recording with patch clamp pipettes



High resolution but low return experiments

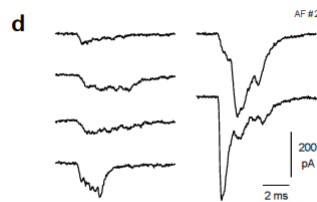
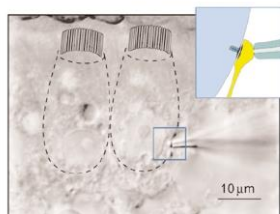
Measures a single ribbon at a time

May select subpopulation of boutons

Goutman et al, pnas 2007

Initial IHC synaptic release is multiquantal

(not like neuronal synapses)



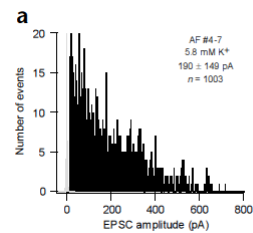
Glowatzki and Fuchs, Nat Neurosci, 2002

Total vesicles per ribbon ~200

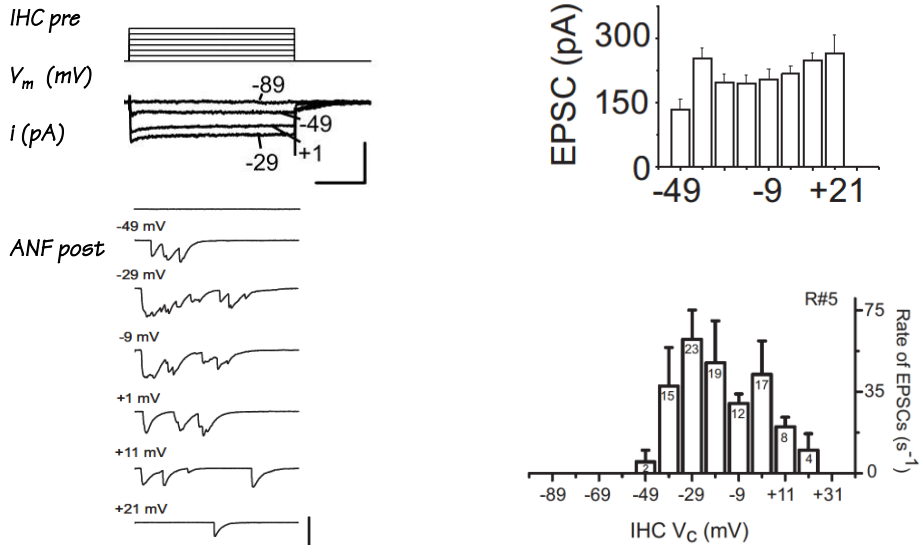
Initial release:

6-8 vesicles per ribbon (mammal)

2-3 vesicles per ribbon (frogs)



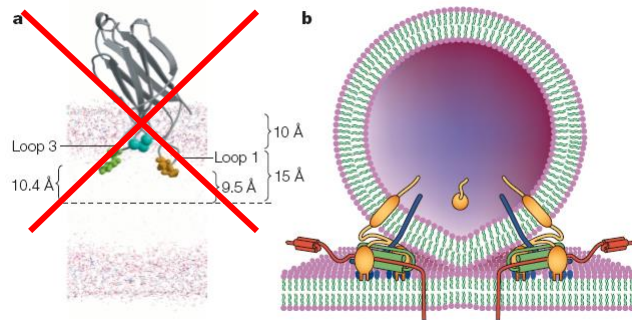
Steady release rate is controlled; initial release is multiquantal



Goutman & Glowatzki, pnas 2007

Calcium regulates vesicle fusion at synapses:

Normally, synaptotagmin (Syt) is the Ca^{++} dependent 'brake'



Syt 1 and 2 not present in IHCs: an alternative calcium sensor?

Otoferlin?

(Roux et al, 2006; Pangrsic et al, 2010)

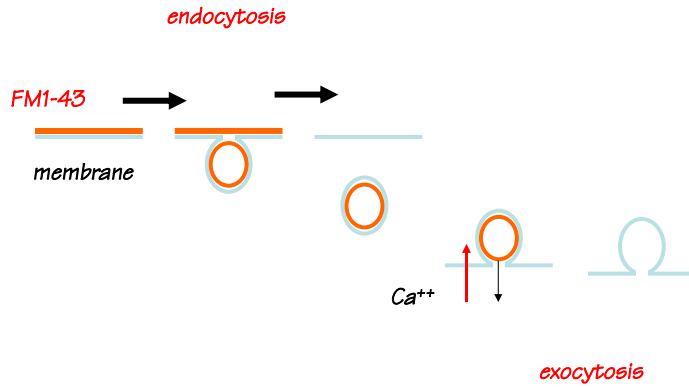
DFNB9: OTOF codes 6 Calcium binding domains

Syt 4?

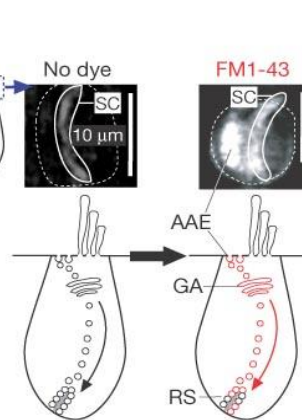
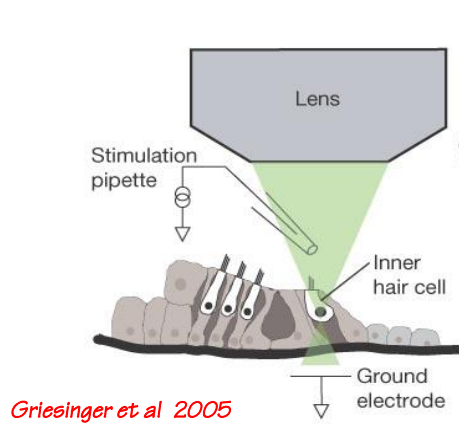
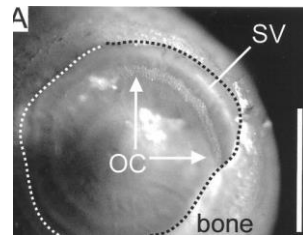
(Johnson et al, 2011)

Measuring vesicles seeing vesicles:

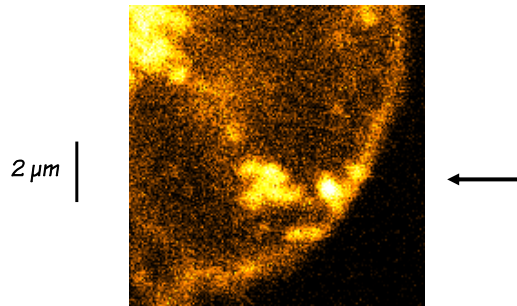
Use membrane labels: FM1-43 or vesicle tags e.g. pHluorins



Membrane dye FM1-43
endocytotic uptake
Guinea pig: Griesinger et al 2005



Fluorescent hotspots = ribbon release sites



Because:

Static

Correct position

Reasonable size (<1 μm diameter)

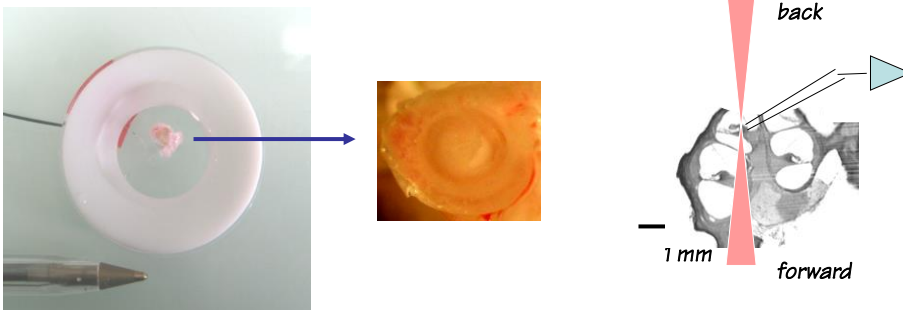
Fluorescence indicates each spot = about 300 vesicles

Can be destained with depolarising stimulus

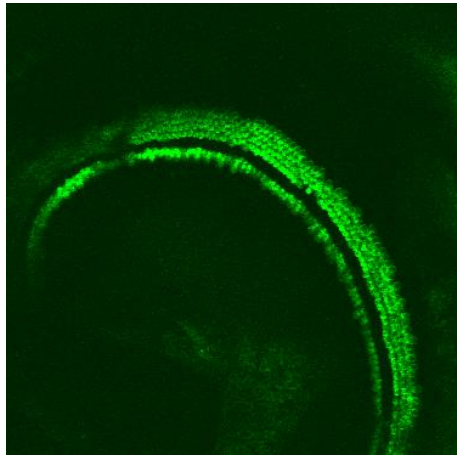
Destaining reduced by Ca channel blocker nifedipine

Spots recover fluorescence after destaining

The mouse organ of Corti in a dish: how to record from the hair cells



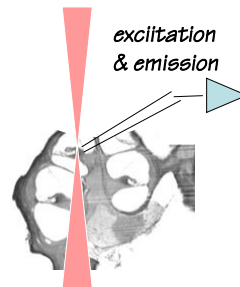
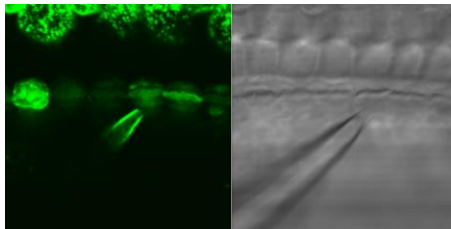
Mouse hair cells load with FM1-43 by an endocytosis pathway



200 μ M streptomycin (= transduction blocked): FM1-43 loads
 myo6 $-/-$ mice (= disrupted stereocilia): FM1-43 loads

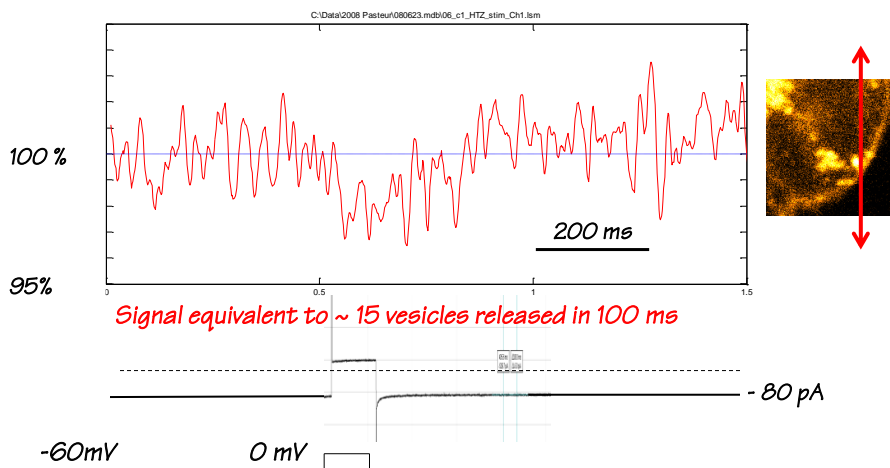
Whole cell recording under visual control through the bone

Excitation 840nm: back emission 500-650nm
 forward beam 840nm

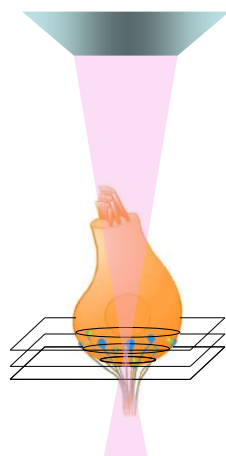


*substage
 detection*

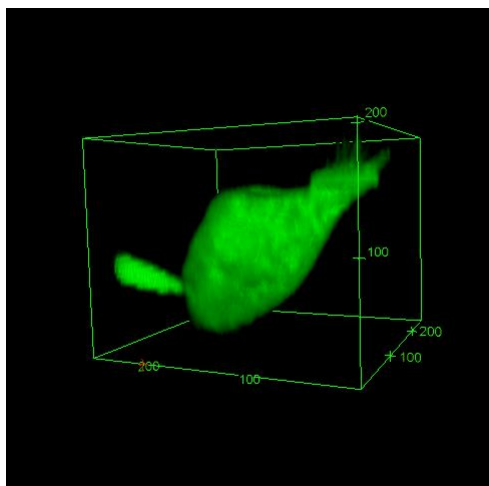
FM 1-43 hotspots destain under whole cell patch clamp

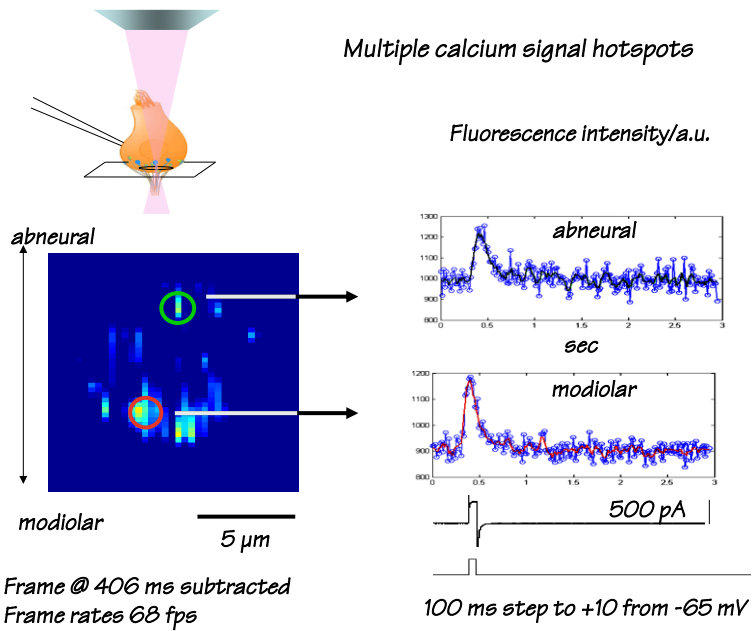


The signalling pathway: calcium signals in IHCs

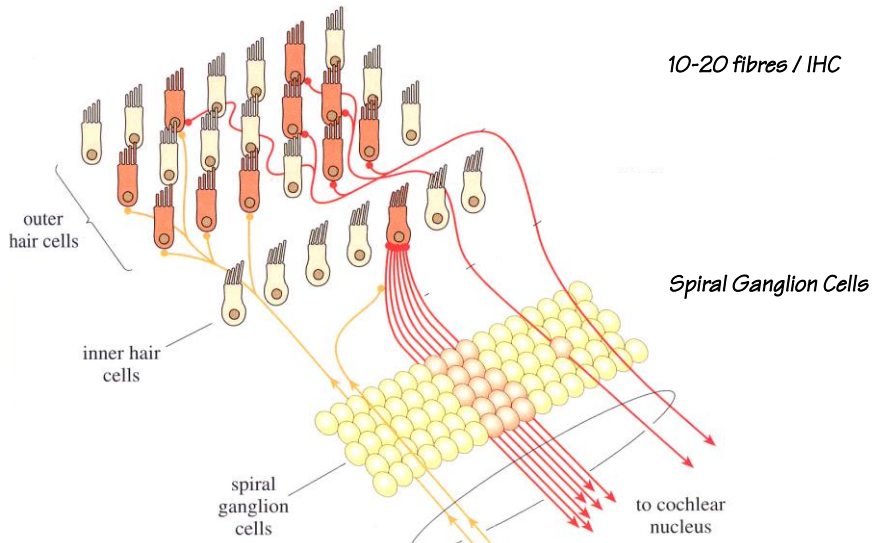


Pipette: 140 Cs 0.5 OGB-5N





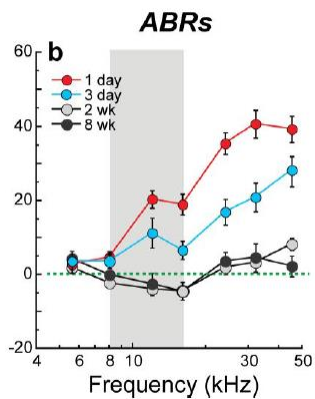
Information transfer in the mammalian cochlea



Open University 'Signals'
Fuchs J Physiol 2005
Meyer et al, 2009

Thresholds rise after noise exposure and then return to normal

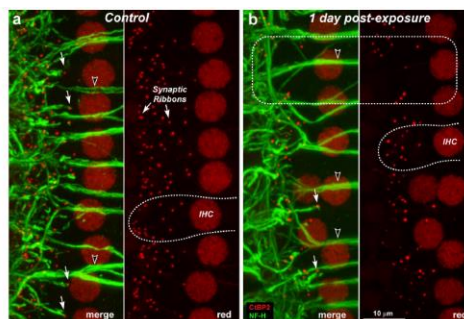
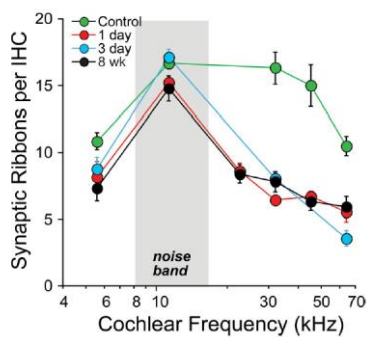
ABR = Auditory Brainstem Response
(non-invasive measure of whole auditory nerve activity)



Narrow band noise 106 dB

Kujawa & Liberman 2009

..but ribbons are lost



'Hidden hearing loss'

Kujawa & Liberman 2009

IHC synaptic transmission from the cochlea

Conclusions

- 1) *Multiple ribbon synapses per inner hair cell*
- 2) *Ribbon synapses are capable of rapid triggered release of neurotransmitter – mechanism unclear*
- 3) *Fibres may be organised to segment different sound level ranges. Cellular organisation and mechanism unclear*
- 4) *What regulates which synaptic sites survive?*