

Peripheral Auditory System – Study Notes

Review papers

Fettiplace (2001), Clues to the cochlear amplifier from the turtle ear, Trends in Neurosciences, Vol 24, No 3.

- Cochlear amplification – sound-induced vibrations of the cochlear membranes boosted by energy supplied by the hair cells → necessary to overcome damping effects of cochlear fluids → central to explain cochlea's high sensitivity and its high Q.
- *OAE might be due to active bundle movements – common to all vertebrates.*
- (Brad's comment): Fast adaptation is the entry of Ca^{2+} → binds to a site near or on the transduction channel → causes transduction channel to close (unknown mechanism). $\tau \sim \text{ms}$. (Article) – fast adaptation probably requires a direct interaction between Ca^{2+} and the mechanotransducer channel to modulate the probability of opening.
- (Brad's comment): Slow adaptation – involves Myosin movement along actin fibers to adjust resting tension. Tension $\uparrow$, motor will then slip down the filament slowly. Tension $\downarrow$, motor will climb up. $\tau \sim 10\text{ms}$
- Deflection of bundle → Ca^{2+} to enter, binds channels intracellularly → return bundle back towards original resting position → Ca^{2+} entry $\downarrow$ → channels open again → negative feedback (bundle generating force to oppose its initial deflection) → can produce oscillations tune to specific frequency.
- In turtle hair – adaptation and active bundle movements are faster in hair cells tuned to higher frequencies (tonotopically mapped)
- Why mammals have added extra somatic motility → could be bundle-based mechanism found in lower vertebrates cannot deliver sufficient force to overcome the viscous damping at higher frequencies used by mammals → OHC gives larger movement booster.

Nobili (1998), How well do we understand the cochlea?, TINS Vol 21 No 4 pp159-167

- BM motion – due to exponentially graded membrane stiffness → effect is different at opposite sides → response being smaller at stiffer (more basal) side, so the semi wavelength of the oscillation decreases from base to apex → Sinusoidal input → monotonically increasing in phase delay → characteristic shrinking of traveling wavelength at a frequency-dependent critical point.
- IHC and OHC both convert displacement of their stereocilia, caused by the relative motion of the reticular lamina and the tectorial membrane, into transducer current that produces a modulation of the cell receptor potential.
- OHC motility is a fast motor (unlike other motile cells that depends on ATP hydrolysis) and is capable to generate forces at 20kHz.
- 2 functions of OHC amplifier saturation properties:
 - Equalization – perception of sufficiently different sound frequency-components largely independent of intensity.
 - Suppression of one tone by an adjacent one ~ lateral inhibition typical of neuronal structures → frequency masking.
- Activation of efferent pathways (through the release of Ach) operate on 2 time scales:
 - Rapid action ($\sim 10\text{s ms}$) → modulating nerve responses to transient acoustic stimulation
 - Slow action ($\sim 10\text{s s}$) → protect ear from acoustic overstimulation.

- Reptiles and amphibia use electrical tuning (same mechanical stimulus delivered to each hair cell, but the receptor cell itself extracts frequency information)

Synaptic Transmission

Fuchs (1996)

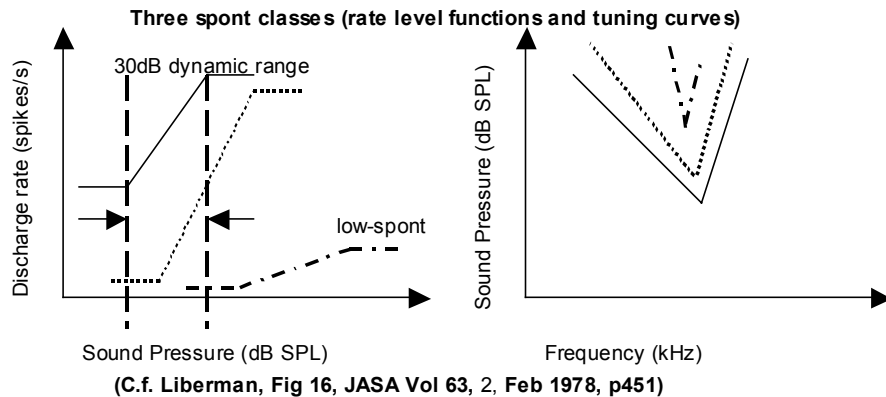
- Depolarizing receptor potentials in mechanosensory IHC open voltage-gated calcium channels (VGCC) in basolateral membrane → evoke transmitter release without generating Action Potential (AP).
- HCs subject to feedback inhibition by efferent, i.e., HC → presynaptic onto afferent and postsynaptic targets of efferent.
- Mechanical input → deflects towards tallest stereocilia → opening of non-selective cation channels → depolarization → ↑ opening probability of VGCCs in basolateral membrane.
- Increased influx of Ca^{2+} accelerates vesicle fusion and release of chemical transmitters, BUT transmitter release in HCs continues at rest and is modulated up and down during mechanotransduction (unlike other neuromuscular junction).
- Transmitter release from HC appears to depend on dihydropyridines (DHP)-sensitive, or neuronal L-type, channels, in contrast to the varieties of non-DHP-sensitive channels supporting rapid transmitter release from neurons.
- Site of transmitter release in HCs is characterized by a thickening of plasma membrane and an osmiophilic 'dense body' surrounded by synaptic vesicles.
- Co-localization of calcium-activated potassium channels and the active zone → comparably rapid kinetics channel activation and vesicle fusion.
- Afferent fibers with different spontaneous and saturated firing rates contact morphologically and topologically distinct active zones of IHCs. (Hypothesis: active zone structure and VGCC number are somehow related)
- Efferent inhibition of HC has cholinergic pharmacology but is blocked by unusual antagonists such as curare, atropine and strychnine.
- Hypothesis: Ach receptor is a ligand-gated cation channel through which Ca^{2+} enters to activate nearby K^+ channels → initial action of Ach is a small depolarization then ultimate hyperpolarization.
- Compelling evidence that $\alpha 9$ is a component of the hair cell Ach receptor.
- Want to know whether Ca^{2+} flux through $\alpha 9$ -containing receptors sufficient to activate Ach-sensitive K^+ channels; are $\alpha 9$ -containing receptors co-localized with Ca-activated K^+ channels analogous to VGCs and BK channels.
- HCs appear to have 2 physiologically distinct classes of Ca-activated K^+ channels (SK, efferent, BK, afferent) → cell's Ca buffering prevents 'crosstalk'.

Auditory Nerve Response (Daryush)

Lieberman, Auditory-nerve response from cats raised in a low-noise chamber, JASA 1977.

- Type I : Type II fibers = 95% : 5%
- Auditory nerve fibers (ANF) are not homogeneous → fiber thresholds vary by 60dB → one CF region, spontaneous discharge rates vary from 0 to over 100 spikes/s.
- Three functional groups: high/mid/low-spont rate fibers: 60% : 25% : 15%

- Controlled experiment: raised cats in low noise chambers to ensure different spont rates of ANF are not from accumulated ear damage.
- Low-spont units showed a large range of saturation rates, i.e., heterogeneous (compared to mid/high-spont fibers – homogeneous in threshold and saturation rates)
- Among low-spont units, highest thresholds → lowest discharge rates at saturation, vice versa. NB low-spont fibers could be used to encode information not encoded by other fibers, particularly at high sound pressure levels.
- Since each auditory nerve fiber has a limited dynamic range (~30 dB), the existence of different fiber populations with different sensitivity ranges could be useful in stimulus coding over a wide range of sound pressure levels.



Sachs and Young, Encoding of steady-state vowels in the auditory nerve: Representation in terms of discharge rate, JASA, 1979

- The smeared response at high sound levels may be due to
 - Broadening of the tuning curve. At high SPL, many fibers fire, including fibers not in the CF region corresponding to the stimulus frequency.
 - Saturation. The discharge rates of fibers do not increase ad infinitum as stimulus level increases. Each fiber has a characteristic maximum discharge rate.
 - Two-tone rate suppression. Vowels can be viewed as a two-tone stimulus at F1 and F2. The response of a CF nerve fiber at F2 is diminished in the presence of a frequency component at F1.
- (Liberman's Lecture 2) Flow chart view: Sharp Tuning and Sensitivity (Tectorial membrane, Basilar membrane and Outer Hair Cell feedback amplification). Synchrony Limits due to IHC Voltage Compartment, IHC presynaptic apparatus, Auditory nerve. Saturation due to IHC Presynaptic apparatus and Auditory Nerve.

Transduction by Hair Cells (Prakash)

Hudspeth, Corey (1977), "Sensitivity, Polarity and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli", PNAS, 74(6) 2407-22411.

- It was known that receptor potentials arose in hair cells in response to sound stimuli, but transduction mechanism could not be directly observed → Experiment to measure intracellular potential *in vitro*. (Saccule of bull frog)
- Haircell was bathed on both apical and basolateral membranes in a saline medium similar to perilymph → contrasts with *in vivo* situation.

Corey, Judspeth (1979), "Ionic basis of the receptor potential in a vertebrate hair cell", *Nature* **281** 675-677

- Objective: test the permeability of displacement-gated channels to different ions.
- Need to separate effect of voltage-gated channels and the mechanically-gated channels. → Use Voltage-clamp to perform I-V characteristic.
- *Patch Clamping Method (Ref. Cell Biology, Fig 11-31, p644)*
 - Extremely tight seal between micropipette and membrane → current can enter or leave micropipette only by passing through channels in the patch of membrane covering its tip.
 - "Clamp" – membrane potential is clamped at a set value while recording ionic current through individual channels.
 - Recordings of current through channels can be made with patch still attached to rest of the cell, or detached → Advantage of detached patch – easily alter the composition of solution on either side of membrane. (detached patch can also be produced with opposite orientation, i.e. cytoplasmic surface of membrane faces inside of pipette)
- Data indicates sharp increase in membrane conductance at voltages above -50mV (outward rectification) due to voltage-gated K^+ channels open.
- 2nd experiment: single-cell, clamping membrane potential to different +ve and -ve values, measure transmembrane current in response to maximum mechanical stimulation → data suggests channels are ion-independent.
- 3rd experiment: test specific ions by measuring microphonic current → different ionic solution was used on apical side of epithelium measuring saturating mechanical stimuli → results indicated all monovalent ions and Ca^{2+} , were about equally permeable to mechanically-gated channels.

Hacohen, Assad, Smith, Corey (1989), "Regulation of Tension on Hair-Cell Transduction Channels: Displacement and Calcium Dependence", *J. Neurosci.* **9**(11)

- Model suggests tension in tip links between stereocilia regulates open/closed state of ion channel. 3 objectives:
 - Examine slow change of membrane conductance in response to prolonged mechanical stimuli (adaptation)
 - Relationship between adaptation rate and relaxation and re-tensioning mechanisms.
 - Dependence of extracellular Ca^{2+}
- $I(x)$ curve – I is the transepithelial current in a voltage clamp, measured for a series of brief mechanical stimuli (x).
- $I(x)$ curve shifts towards higher x as adaptation time increases (based on transduction model → slackening of tip-links), but $I(x)$ curves remain parallel → suggests stiffness (spring constant) of links is unchanged, but the length of links changes.
- Initial slope of tension changes → adaptation rate R , dependent on adaptation step size x .
- $R(X)$ curve is affected only for $+x$ → Ca^{2+} enter cell and promote relaxation process, but leave retensioning mechanism unaffected (Ca^{2+} specific)
- Intracellular measurements and trans-epithelial measurements are equivalent because flow of a particular ion into cell across apical membrane must be balanced by a corresponding flow out of the cell basolaterally to maintain intracellular potential. This is ensured by active ion pumps.

- Microphonic measurements → measure transepithelial voltage V_E (which is 0 if there is only one fluid) and I_R ($V = I_R R_a + I_R R_b$). For a fixed V_E , changes in I_R correspond changes in membrane conductance.
- (Brad's comments): Rate at which cell adapts to a decrease in gating tension is relative constant → motor slowly walks up actin filament, limiting rate is the phosphorylation and hydrolysis of ATP, which is not affected by Ca^{2+} , tension or anything else.
- Slippage / Relaxation process is dependent on the amount of tension, usually much faster than climbing. (not sure why) (in general, phosphatase is needed to break ADP-myosin bond and relieve linkage of myosin and actin. Calmodulin and phosphatase compete with each other, level of Ca^{2+} inside cell determine the priority??)

Santos-Sacchi, "Cochlear Physiology" – Chapter 18, pp357-391

Hair Cell Physiology (Transduction by Hair Cells)

- Hair Cells – Specialized epithelial cells, the plasma membrane of hair cells is polarized into an apical portion and a basolateral portion.
- Tight junctions – tightly join the apical circumference of hair cells to adjacent supporting cells, thereby preventing the intermixing of ions and molecules of apical and basal compartments (endolymph and perilymph). Apical → hair cell's transduction channels.
- Hair Bundle (stereocilia) deflected toward the tallest stereocilia row → depolarization → increase in neurotransmitter release and excitation of afferent nerve fiber. (Opposite direction → hyperpolarization → decrease in transmitter release → inhibition of fiber activity.)
- Even in the unperturbed position, a standing inward current exists through a small proportion of mechanically activated channels (~10-25%) which are currently believed to be located within each stereocilium.
- Relation between degree of bundle deflection and receptor potential magnitude is neither linear nor symmetric – depolarizing → More effective than equal but opposite displacement. (Sigmoidal and shifted from its midpoint → symmetrical sinusoidal deflection → produce both AC and superimposed depolarizing steady state DC changes in membrane potential) Hence asymmetric and rectifies [due to LPF characteristics, due to membrane time constant] (especially over kHz). NB, if it were symmetrical, voltage response at high frequencies would be attenuated to an imperceptible level by membrane characteristics.
- (Daryush/KC): Receptor potential at different stimulus level and frequencies:
 - Low level, low frequency: no rectification, output follows input.
 - Low level, high frequency: DC offset due to forward transduction limit.
 - High level, low frequency: output follows input with rectification (saturation on hyperpolarization side)
 - High level, high frequency: DC offset with rectification (small anyway)
- (Brad): Maximum synchrony rate → refractory period of nerve. [Maximum firing rate → Rate of vesicle recycling (which includes re-uptake among other things)]
- Molecular basis of hair bundle's response polarities → tip links → believe to provide tension required to open transduction channels during deflection. Hyperpolarizing → tip link tension slackens and channels tend to close.
- Destruction of tip links by enzymatic or chemical (Ca^{2+} chelators) treatments can abolish mechanical transduction. Aminoglycoside blocks stereocilia transduction channels →

compliance (originally in bell shaped, max occur when half transduction channels open) changes are abolished. Regeneration of broken tip links reestablishes transduction.

- Hair bundle, when statically deflected, induced receptor current decays with a time constant (about 20% of initial value) → adaptation → shifts sigmoidal displacement-response function along the displacement axis in the direction of the deflection.
- If adaptation did not occur, then during a static deflection, superimposed deflections would produce little response → can be viewed as a functional return of the bundle towards its resting position, where bundle deflections are most efficient in transducing mechanical stimuli.
- Adaptation is lacking in the absence of extracellular Ca^{2+} . $\text{Ca}^{2+}\uparrow \rightarrow \text{adaptation} \uparrow$
- Adaptation may be related to direct actions of Ca^{2+} on the transduction channels or on a mechanism responsible for tip link tension control → molecular motor (myosin I) maintains a resting tension on tip link by constantly attempting to move the tip link's upper insertional plaque up the length of stereocilium.
- Deflection → tip links initially further tensed → open transduction channels to permit Ca^{2+} influx → Ca^{2+} are believed to cause molecular motor to slip during its climb up stereocilium → slacken link's tension → allowing channel's gate to close under constant bundle deflection.
- Reestablishment of resting tip link tension follows, cause the motor renews its climb as Ca^{2+} influx is halted, ion is buffered intracellularly, actively pumped out of the cell, or both. With reestablishment of resting tip link tension → hair cell prepared to efficiently signal small mechanical perturbations superimposed on the initial static one. (Adaptation occurs to a greater extent in excitatory direction, but static deflection in inhibitory direction also evoke adaptation → motor attempt to reinstate resting tip link tension by climbing)

Afferent Synapses (Anton)

Roberts et al (1990), Colocalization of ion channels involved in frequency selectivity and synaptic Transmission of Presynaptic Active Zones of Hair Cells, J. Neuroscience 10 (11): 3664-3684

- Background: Knew that voltage sensitive Ca^{2+} channels and the Ca -sensitive K^+ channels together could produce an electrical resonance due to opposing action of slower K^+ channel response.
- Background: voltage-sensitive Ca^{2+} channels ~ classic L-type Ca^{2+} channels, but activated at lower voltages (20-50mV), and activation and deactivation times were faster.
- Experimental approach (Grass frog saccule hair cells):
 - Electrophysiology → number of each type of channels present in HC; number of synaptic sites
 - Microscopy → count number of synaptic sites (cross check with electrophysiology results)
 - Freeze Fracture analysis → count number of channels at each synaptic site.
- Ca^{2+} triggers release of chemical synaptic transmitter, where vesicles are tightly clustered at ribbon synapses.
- Ability to see Ca^{2+} current and K^+ current separately → electrical resonance.
- Finding in paper: From electrophysiological experiments, estimated each cell has 1800 ± 400 Ca^{2+} voltage sensitive channels (per cell with K^+ channels blocked)

- 550 ± 70 Ca^{2+} -activated K^+ channels (buffered Ca^{2+} situation) and 820 ± 80 Ca^{2+} sensitive K^+ channels (using Ca^{2+} current) $\rightarrow$ showing Ca^{2+} current can open all cell's Ca^{2+} sensitive K^+ currents.
- Estimated clustered at about 20 synaptic sites (<5% of estimated basolateral surface area), i.e. 90Ca^{2+} channels and 40Ca^{2+} activated K^+ channels per site. (Freeze fracture results agree with above). These presynaptic sites lie toward the basal side of the basolateral membrane.
- These 2 types of channels must be clustered together, because an increase in Ca^{2+} buffering mechanisms (BAPTA) cannot stop the K^+ channels from opening $\rightarrow$ Ca^{2+} -sensitive K^+ channels must be $\sim 150\text{nm}$ from Ca^{2+} voltage sensitive channels, otherwise Ca^{2+} would be taken up by BAPTA.
- Ribbon synapse $\rightarrow$ single release of quanta (5000 molecules) is capable of generating Action Potential.
- Expect AMPA (ligand-gated glutamate receptor operating ionic channel) to be dominant because of its fast dynamics (cycle of opening and closing) [in contrast to metabotropic gates, which has slow dynamics]
- Miniature excitatory post synaptic potential (epsp) shows quantal characteristics due to quantal release of vesicles from ribbon synapse.
- Unlike gap junctions (direct electrical connections), nervous system is connected electro-chemically (chemically mediated)
- (Brad's comment): To ensure no cross-talk, HC's have a very high concentration of cytoplasmic calcium chelators, such as calbindin-D28k and parvalbumin-beta, which bind up the free calcium quickly. By providing this "buffer" $\rightarrow$ keep endogenous calcium in the cell to a very low background level, and prevent localized currents of calcium from spreading past the immediate site.
- (Brad's comment) Distance/colocalization. By localizing calcium channels close to where they exert their effect (either on the MET, BK, or SK channels) a localized increase in calcium will be able to reach the channels before the buffer can take them up.

Flowatzki (2002), *Transmitter release at the hair cell ribbon synapse*, *Nat Neurosci*, 5(2): 147-54 (Hui)

- Background: Each of 10-30 afferent fibers contacting IHCs is served by a single ribbon synapse $\rightarrow$ each synapse can have spont rates up to 100 Hz and evoked rates up to 300 Hz, with phase locking up to 5kHz.
- 3 types of glutamate receptors (NMDA, AMPA, kainite) are possible ion channels on the postsynaptic neuron $\rightarrow$ AMPA and kainite are fast (which allows fast excitatory postsynaptic currents EPSCs) $\rightarrow$ AMPA mediates EPSCs in HC afferent fibers, NMDA does not.
- Membrane capacitance measurement $\rightarrow$ high rate of vesicle fusion and single vesicle release $\rightarrow$ AP in postsynaptic cell.
- Want to find out whether ribbon synapse is quantized and how it's varied with cell's membrane potential.
- Experiment: Used whole-cell tight-seal methods to record EPSCs from afferent fibers. (Apply CNQX – antagonist to AMPA/kainite receptors and cyclothiazide – selectively relaxes adaptation of AMPA receptors) to see what glutamate receptors are present.
- EPSCs are mediated by AMPA receptors without any NMDA receptors, but still need to test the presence of kainite.

- EPSCs can be fit with a double exponential, one with a fast τ (994-18ms) and the other a slow τ (65-250ms) → not equivalent to neuromuscular junction with single Poisson process.
- Amplitude distribution of EPSCs is invariant with receptor potential.
- Take home: Ribbon synapses have coordinated multivesicular release. Number of vesicles released determines the EPSC amplitude.
- Amplitude distributions EPSCs do not change with receptor potential in the spontaneous condition or the evoked condition, only frequency of EPSCs is changed.

“Modifiable Synapses for Nondeclarative Memory” – Chapter 2, pp 30-34

Neural Signals

- External plasma membrane of the cell maintains at rest an electrical difference of about 65mV; this is the resting potential. It results from an unequal distribution of Na^+ , K^+ , and other ions across the nerve cell membrane such that the inside of the cell membrane is -ve charged in relation to the outside. (Outside of membrane is arbitrarily defined as 0V).
- An increase in membrane potential (-65 to -75mV) is called hyperpolarization (inhibitory); a reduction (-65mV to -50mV) is called depolarization (excitatory).
- Action Potential (AP) is a depolarizing electrical signal that travels from the dendrites and cell body of the neuron, along the entire length of the axon, to its presynaptic terminals. It is a rapid and transient, all-or-none electrical signal with an amplitude of 100-120mV and a duration at any one spot from 1-10ms. Amplitude remains constant throughout axon.
- Signal at the synapse – synaptic potential, is graded and modifiable.
- 3 components to a synapse: a presynaptic terminal, a postsynaptic target cell and a small space in between, separating 2 neurons – synaptic cleft (20nm).
- As AP reaches the presynaptic terminal, the electrical signal leads to the release of a simple chemical substance called a “chemical synaptic transmitter” or “neurotransmitter”.
- Common neurotransmitters – amino acids or their derivatives such as glutamate, gamma-aminobutyric acid (GABA), acetylcholine, epinephrine, norepinephrine, serotonin, dopamine.
- Hormonal action, a gland cell releases a chemical messenger (a hormone) into the bloodstream to signal a distant tissue.
- Synaptic transmitters typically operate over a much shorter distance and a single synaptic transmitter (unlike hormone) can produce a variety of different responses in the target cell.
- AP invades the presynaptic terminals, opens up membrane channels for Ca^{2+} that cause a large and rapid increase in Ca^{2+} into the presynaptic terminals → release of chemical transmitter. (Ca^{2+} entry causes vesicle fusion and transmitter release... transmitter molecules bind to excitatory receptors, receptor channels open and Na^+ enters the postsynaptic cell.)
- Synaptic transmitters are released not as single molecules but as one or more packets of fixed size, each packet containing about 5000 molecules → “quanta”

Basolateral Membrane

- Several types of channels exist in the basolateral membrane.
- Depolarization of the membrane opens Ca^{2+} channels → Ca^{2+} enters the cell. Intracellular Ca^{2+} bind to a site on Ca^{2+} -activated- K^+ channels → open and permit outward flow of K^+ ions → probability of Ca^{2+} -activated- K^+ channels increased as membrane potential is depolarized → result in a preferential increase in total membrane conductance on depolarization-

rectification → cell will repolarize toward the resting potential as K^+ ↑ forces the membrane potential toward the equilibrium potential of K^+ → Ca^{2+} channels close on this repolarization.

Hair Cell to 8th Nerve Afferent

- 95% afferent innervation to IHC.
- Characteristics (eg voltage dependence, Ca dependence, kinetics, conductance) of both presynaptic and postsynaptic ionic channels will control rate and pattern of information transfer from the periphery.
- AC receptor potentials up to ~4kHz drive phase locked responses in 8th nerve fibers.
- Depolarization of hair cell membrane → ↑ conductance of Ca^{2+} → net increase of Ca^{2+} (favorable in chemical gradient, NB extracellular is perilymph), → promote fusion of intracellular membranous vesicles containing neurotransmitter → release neurotransmitter into the extracellular space between hair cell and nerve terminal → neurotransmitter molecules bind receptors located on postsynaptic membrane of nerve fiber [Na^+ enters nerve ending via glutamate receptor] → result in depolarization → AP if depolarization is sufficient.
- (Liberman's Lecture 2) – After IHC loss, neural response area shows:
 - no response to sound
 - no spontaneous activity
- IHC loss will NOT show loudness recruitment – response of signal is carried by “wrong” fibers.
- IHC loss eliminates spontaneous discharge in auditory nerve fibers → silent band produces an abnormal response gradient in quiet → Tinnitus

OHC

- Observations that support OHC as cochlear amplifier: tuning properties of 8th nerve fibers can be altered by selective destruction of OHCs; tuning properties of IHCs can be altered by 1) stimulation of the crossed efferents that innervate OHCs, 2) injecting of currents into the scala media, 3) transient asphyxia, 4) acoustic overstimulation → sustained depolarization of OHCs → modify mechanical input to IHCs → reduced receptor potential amplitudes.
- (Liberman's Lecture 2) After OHC loss, the neural response area shows:
 - Decrease in sensitivity of 40dB
 - Loss of sharp tuning
 - No change in maximum discharge rate; no change in discharge rate at high levels; no change in spontaneous discharge rate.
 - Audiogram shows a “notch” – eg “4kHz cookie bite audiogram”.
 - Loudness recruitment: NB at high level, response in both ears are the same, but at low level, no response in damaged ear. (c.f. IHC, no loudness recruitment)

(OHC motility is deliberately omitted in this summary. Please refer to other more updated notes.)

(Cell-to-cell coupling in the organ of corti is also omitted in this summary.)

Efferent Synapse

- 2 targets for efferent: 1. Hair cells; 2. afferent nerves and endings
- Major divisions:

- MOC – cell bodies medial to olivary complex, myelinated fibers, path near base of 4th ventricle, most cross midline, innervate OHC
- LOC – cell bodies lateral to olive, unmyelinated axons, most ipsilateral, mostly innervate type I afferents. ?? Functions ??
- Usually inhibition → functionally hyperpolarizing hair cells.
- Acetylcholine (ACh) as efferent transmitters.
- OHC – ionotropic, cholinergic channels (α -9, α -10 ACh nicotinic receptor subunits) – mostly sensitive to Ca^{2+} → inhibition (via excitation)

Vetter *et al* (1999) [Yoko's presentation]

- α -9 subunit plays a role in developing normal synaptic connections between efferent fibers and hair cells.
- Complete functional deafferentation, in the sense that classic effects of electrically shocking the OC bundle are completely absent in the knockout mouse.
- α -9 subunit is required for the classic suppressive effects of the olivocochlear efferent pathway on cochlear responses.

Flock *et al* (1975) Summary [Should Reread]:

- Intracellular recordings were made on hair cells in lateral line canal organ of the burbot.
- Ipsps were recorded from hair cells when efferent fibers were excited. Ipsps were abolished when fish was injected with Flaxedil (known to block efferent synapses)
- Ipsps → ↓ resistance of hair cell membrane and ↑ intracellular receptor potential.
- Spontaneous and mechanically evoked Epsps recorded intracellularly from post-synaptic afferent nerve terminals were reduced in amplitude for the duration of ipsp.

Efferent Control for Inner ear

- Hypotheses for Middle Ear Muscles Function (act as a HPF)
 - Stapedius (controlled by VII facial nerve) – 1) Extend Dynamic Range (gain control); 2) Protect inner ear from acoustic injury; 3) Control masking from continuous Background noise [Bell's palsy → paralysis of VII cranial → more vulnerable to acoustic injury]
 - Tensor Tympani (malleus) → Aid in middle-ear aeration → force air to Eustacian tube
- Efferent from LOC – Unmyelinated; Afferent Excitation / suppression (not sure of the function)
- Efferent from MOC – Myelinated; Cochlear Suppression
- LOC (→IHC) 90% Ipsilateral vs MOC (→OHC) 66% Contralateral
- OC Effects (using Crossed OC bundle stimulation): CAP suppression → Shifts CAP amplitude to the right (CAP vs Tone pip level) significantly in middle compressive region (30-70dB SPL) (-ve feedback) For higher tone pip level, cochlear amplifier already saturated.
- Biggest at high frequencies (more terminals in basal end) and at CF.
- Suppression in BM motion, again biggest at low sound pressure
- Effects on IHC – DC dip during COCB stimulation, more dramatic at lower level; on OHC – reduction of ac amplitude during hyperpolarization (dc offset).

- Suppression in IHCs, level shift when efferents stimulated. High / mid / low-spont fibers also have similar level shifts respectively. Mid-spont has an added plateau depression.
- DPOAE: stimulation – ipsilateral f_1 , f_2 , contralateral noise → when contra noise on, DPOAE is lowered, when OCB cut, no depression of DPOAE → without mechanical motor to drive non-linear feedback. NB 100ms latency time for –ve feedback.
- OHC motility decreases with hyperpolarization ← non-linearity
- Hypothesis: 2 stage effect: 1) Ach activated Ca^{2+} entry, 2) Ca^{2+} -activated K^+ out of OHC (therefore slight depolarization before hyperpolarization)
- α -9 knockout → Cochlear function normal (normal CAP and DPOAE), but it is functionally de-efferented, i.e. no effect on MOC stimulation (no level shift for CAP during COCB stim)
- Hypotheses for MOC Function:
 - Extend dynamic range through gain control
 - Mediate selective attention: auditory vs visual; high vs low frequency
 - Control masking from background noise (have time to turn on efferent for constant background) [*An Introduction to the Physiology of Hearing, Chapter 8 The Centrifugal Pathways: pp243-244* “With no OC stim, background noise flattened the tone intensity function, re Fig8.6, with COCB stim, partially reversed the flattening, so that tone could now produce a greater relative change in firing rates” → Winslow and Sachs suggests “Effect was to lower the firing rate at low tone levels (cause the noise drove fiber less strongly) and increase tone-induced rate at high tone levels (cause less adaptation of firing to the continuous background noise) → rate-intensity function steepened]
 - Shape normal cochlear development (more on lateral system)
 - Protect inner ear from acoustic injury
- Unilateral De-efferentation → bigger threshold shift in high frequency after acoustic trauma.
- MOC Reflex strength: weak, intermediate, strong → predicts vulnerability??

Murugasu (1996), The Effect of Efferent Stimulation on Basilar Membrane Displacement in the Basal Turn of the Guinea Pig Cochlea, Journ of Nerusci, 16(1):325-332

- OCB stimulation → loss of sensitivity around CF of the fiber, but little or no effect on sensitivity of low-frequency tail. (Observed in suppression of receptor potential in IHC and afferent nerve activity), but want to directly measure it on BM.
- BM displacement resembles neural tuning curves, max sensitivity occurs at CF, below CF, high threshold tail.
- OCB stimulation causes a reversible 18dB decrease in gain at CF (no effect for tones much higher or lower)
- Max decrease in gain occurs at low sound level intensities (at high intensities, very little decrease in gain)
- Q of tuning curve and phase of traveling wave do not change in response to efferent stimulation.
- Strychnine reversibly blocks α 9 channels → BM displacement does not change in the presence of efferent stimulation, but loss of gain after wash out.
- Theory 1: Efferent alters stiffness of cochlear partition (OHC somatomotility has been shown to alter stiffness of cochlear partition).

- Counter argument: if model is correct, partition would essentially take on characteristics of a more basal (stiffer) partition → decrease in sensitivity of low-frequency tail (require more energy to cause the same amount of motion); Increase in the rate of change of phase; decrease in Q ← Did not observe these in experiment.
- Theory 2: Efferent reduces driving voltage to cochlear amplifier
 - Counter: driving voltage is a buildup of +ve charge inside cell contributing to depolarization. Modeling basolateral cell membrane as a R||C circuit → LPF for efferent effects ← But efferent can modulate high-frequency gain.
- Theory 3: Efferent alters operating point of forward transduction
 - Counter: If BM is displaced, ciliary bundles will shift into a new position (stretched or relaxed) and will cause a shift in operating point ← Does not account for adaptation (Adaptation occurs in ms, efferent effects occur over 10ms) (Also actual lengthening of HCs due to efferent hyperpolarization is less than noise threshold of BM vibration)
- Theory 4: Efferent alters operating point of reverse transduction
 - Reasonable -- OHC hyperpolarized, operating point of reverse transduction shift towards a region of decreased gain → total change in length of hair cell ↓ (small efferent induced hyperpolarization can cause a significant decrease in somatic length change).

Maison et al (2000), Predicting Vulnerability to Acoustic Injury with a Noninvasive Assay of Olivocochlear Reflex Strength, Journ of Neurosci, 20(12):4701 – 4707.

- Large variation in how well ear can be protected from acoustic trauma.
- Permanent threshold shift has a strong negative correlation with strength of MOC reflex. → raises possibility of noninvasive measurement of efferent reflex using DPOAE
- Methods: To determine MOC strength, use difference of max and min post-onset adaptation.
- Results: mice with a strong MOC reflex had least permanent threshold shift (PTS), vice versa.
- From prior studies we know that:
 - Reflex strength is likely mediated by MOC component of efferent system based on results from $\alpha 9$ knock mice and *in vivo* blockade of $\alpha 9$ receptors.
 - Effects of MOC activation include elevation of cochlear thresholds and decrease in BM motion (effect is maximal at low sound pressures)
- Contradicting results from studies prior to this that argues for a LOC mediated effect rather than MOC mediated effect:
 - Mice lacking $\alpha 9$ appear to be no more vulnerable to PTS than wt controls (though used infamous mouse strain that is very resistant)
 - Completely de-efferented guinea pigs are more vulnerable to PTS, while midline lesions (eliminating 66% MOC feedback, 10% LOC) do not make guinea pigs more susceptible.
- Conclusion: acoustic trauma is still poorly understood.

Santos-Sacchi, "Cochlear Physiology" – Chapter 18, pp357-391

Bioelectric potentials

- $[K^+]$ and organic anions is high intracellularly and $[Na^+]$ and $[Cl^-]$ is high extracellularly. Maintenance of Na^+ and K^+ concentrations is assisted by a metabolically active pump that translocates K^+ into the cell and Na^+ out of it.

- K^+ rides on the chemical concentration gradient, diffuse from high to low concentration (out of cell). However, as K^+ moves across the membrane, +ve potential set up relative to the inside. Electromotive force counteracts the outward diffusion.
- Nernst potential is the electric potential that balances the chemical potential caused by the concentration gradient.
- $V_n = \frac{RT}{z_n F} \ln\left(\frac{c_n^{out}}{c_n^{in}}\right)$; z_n = valence of ion; F = Faraday's constant
- For multiple ion species, the resting potential is a weighted sum of the Nernst potential for each ionic species.
- Weighting is by the conductances of the membrane $V_{rest} = \sum_n \frac{G_n}{G_m} V_n$; $G_m = \sum_n G_n$
- Therefore, in general, since K^+ is more permeable at rest than other ions, Nernst equation simplifies to that of K^+ .
- Voltage clamp technique – delivering currents through an intracellular electrode to maintain a desired voltage within a cell, is used to isolate and study cellular mechanisms (eg, ionic channel gating) that are dependent on changes in transmembrane voltage.
- Cell membranes typically have a fixed capacitance of $1\mu F/cm^2$.

An Introduction to the Physiology of Hearing – Chapter 4 The Auditory Nerve

Anatomy

- 30,000 auditory nerve fibers in man. About 20 fibers innervate each inner hair cell, 6 fibers innervate each outer hair cell.
- Each fiber to IHCs connects with one and only one hair cell, OHC branch and innervate about 10 hair cells.
- (In cat) 95% of cells connect with IHCs have bipolar cell bodies in spiral ganglion, myelinated cell bodies and axons. (Type I)
- Type II cells – connect with OHCs are monopolar and not myelinated.

Joe Adams – Control of cochlear fluid volumes

- Flux of K^+ into HC as mechanically stimulated must be matched by equal flux of ions into the endolymph from the source (stria vascularis) [exact mechanisms not known]
- If 2 processes don't match → osmotic gradients → fluid volumes change.
- ↑ Endolymph volume is apparent by distention of Reissner's membrane (endolymphatic hydrops, caused by endolymph becoming hypertonic wrt perilymph) (e.g. Meniere's disease)
- ↓ Endolymph volume → collapse of Reissner's membrane → caused by endolymph becoming hypotonic wrt perilymph. (Can cause by stria vascularis not functioning properly due to genetic defects)
- NB Endolymph is NOT secreted by stria vascularis. Stria secretes K^+ ions and perhaps other ions or compounds into the endolymph. If volume of endolymph changes, due to response of fluid to osmotic gradients.

Physiology

- (Liberman and Kiang 1978) Many fibers show random spontaneous activity → bimodal distribution of spontaneous discharge rates. Quarter discharge at below 20Hz, most discharge at 0.5Hz or less. The other group has a mean of 60-80 discharges/s, max 120/s.

Response to tones:

- In absence of other stimuli, tones are always excitatory, never inhibitory.
- Tone bursts → sharp onset response → drops rapidly over the first 10-20 ms → more and more slowly over the next several minutes.
- At low frequencies, <1kHz, tuning curves are symmetric, high frequencies, curves become increasingly asymmetric, with steep high-frequency slopes (sensitive tip) and less steep, broadly tuned, low-frequency tail.
- Fiber firing rate vs intensity shows a sigmoidal shape, saturating at each frequency at an intensity some 20-50dB above the threshold at that frequency, i.e. dynamic range.
- The max rate for frequencies below CF commonly occurs at higher rates, and max rate at frequencies above the CF commonly occurs at lower rates than the CF itself.
- Phase-locking and mean firing rates can be thought of as being related to different aspects of IHC function: phase-locking → ac, mean firing rate → dc.
- Tonal stimulation → fibers fire preferentially during one part of cycle of stimulating waveform if stimulus < 4-5kHz. Fibers are excited by deflection of BM in only one direction.
- Fibers with CF <4-5kHz, clicks preferentially evoke responses at certain intervals after the stimulus. A histogram of Action Potentials made wrt time after stimulus → fibers are activated by half cycles of a decaying oscillation of mechanical resonance on BM. Frequency of oscillation = CF of fiber.

Read Page 95-99, re de Boer's reverse correlation to find out the impulse response of a nerve fiber, reversed in time (using a broadband noise stimulus)

Two-tone suppression

- The presence of one stimulus can affect the "responsiveness" of nerve fibers to other stimuli.
- The dip in activity at the beginning of the suppressing tone looks like the transient suppression seen at the end of an excitatory stimulus, and the activity at the end looks like the onset burst seen at the beginning of an excitatory stimulus.
- Believed that interference (suppression) occurs right at the mechanical stage, and depends on the nonlinearity of the mechanics (can be demonstrated in BM mechanics and in the IHCs)
- Masking – "Line busy" effect → if one stimulus has pre-empted the firing of a fiber, superimposed stimuli will not be able to provoke an increment in firing.
- If ear is stimulated with 2 tones simultaneously, combination tones may be heard → not physically present in the stimulus (Cubic distortion tone $2f_1 - f_2$, NB all other cubic combinations are above the primaries, could have been masked or filtered out hypothetically)
- Amplitude of cubic distortion tone is strongly dependent on frequency separation of the primaries.
- Cubic distortion tone is heard for stimuli near threshold → not merely an "overloading" type of distortion, must be regarded as part of normal operation of the auditory system.
- As measured by cancellation method, relative amplitude of distortion tone with respect to primaries is almost independent of the amplitude of the primaries (if they're varied in level together).

Endolymphatic Potential (Joe Adams)

- EP – +ve standing potential (70-100mV) measured from scala media referenced to ground.
- No EP, No K^+ current (like open circuit)
- Stria marginal cells consume energy in production of potential – ATP fuels Na^+-K^+ -ATPase: $2K^+$ into marginal, $3Na^+$ out to intrastria space (minuscule volume) per ATP hydrolyzed.
- K^+ taken basolaterally by marginal cells → apically into scala media (perilymphatic space) → supply to hair cells for receptor currents.
- Na^+ are supplied by $Na,K,2Cl$ -cotransporter (neutral charge) in the basolateral membrane of marginal cells. (Combined action of ATPase and co-transporter = $(2+3)K^+$ / ATP consumed.
- Marginal cells → unusual, +ve resting potential slightly higher than endolymphatic potential (83mV) → K^+ expelled apically is not against electrochemical gradient.
- View K^+ as current → source of EP identified by V drop across resistance → Stria Intermediate cells (Wangemann)
- Intermediate cells depolarize ← low extracellular K^+ → K^+ into intrastria space via Kir4.1 (K^+ Inward Rectifying). Marginal cells take up K^+ → keeps K^+ ↓ in intrastria space → K^+ current ↑.
- Continuous supply of K^+ for Intermediate cells from connections via gap junctions to Type 2 fibrocytes of spiral ligament (also use $Na-K-2Cl$ co-transporters).
- Type 2 cells take K^+ from perilymphatic space – some diffuse expelled from HC. Most K^+ expelled by OHC. Kir 4.1 channel carries K^+ through 1 big intracellular space, connected by gap junction (Deiters / Hensen / Claudius / root)
- EP → +ve biasing voltage across apical membrane of HC + -ve resting potential of HC → Extra-large electrochemical gradient for K^+ to move from scala media into HC's.
- High doses of Furosemide blocks $Na,K,2Cl$ co-transporter results in transient elimination of EP → transiently eliminated tips of tuning curves (70-80dB loss in sensitivity at CF), tails of tuning curves is depressed only by 20dB caused by EP loss.
- From electromicroscopy looking at OHCs, lack of vesicles in the afferent fiber endings and efferent terminals have lots of vesicles. (Pre-synaptic, ribbon synapse)

	2 PUMP MODEL (ONE ON APICAL, ONE ON BASOLATERAL)	SINGLE PUMP MODEL	2 CELL MODEL
K^+ source	Pump in marginal cell (apical membrane)	Passive diffusion in marginal cells (apical membrane)	Passive diffusion (apical) in marginal cell
EP generation	As above	Passive diffusion of Na^+ at basolateral membrane marginal cell	In stria (passive in apical, pump in basolateral)
$E_{\text{marginal cell (ref endolymph)}}$	-ve	+ve	+ve
Intrastria space	Not important	Not important	Low K^+ , high potential

Endolymphatic Potential (Sasha)

- The EP arises from resistance to a K^+ current that begins and ends in the scale media.
 - (1) Mechanical movement of the basilar membrane leads to the deflection of the stereocilia on outer hair cells.
 - (2) Deflection of the stereocilia in the positive direction (toward the tallest stereocilium) causes transduction channels open and cations flow into the OHCs. K^+ is the most highly concentrated cation in the scale media (endolymphatic space) therefore it is principally K^+ that flows through the transduction channels into the OHCs.
 - (3) K^+ is expelled through the basolateral end of the OHCs and is likely taken up by the Deiters cells via the K^+ channel Kir4.1 and KCl cotransporter KCC4¹.
 - (4) K^+ passes through the supporting cells (Deiters, Hensen, Claudius, and root cells) via a series of gap junctions and is then expelled into the perilymph.
 - (5) Type II fibrocytes take up K^+ from the perilymph via the $Na^+-K^+-ATPase$ pump and the $Na,K,2 Cl$ co-transporter.
 - (6) K^+ passes through a series of gap junctions connecting Type II fibrocytes to Type I fibrocytes and finally enters the basal cells of the stria vascularis.
 - (7) K^+ flows from the basal cells into the intermediate cells via gap junctions.
 - (8) K^+ is expelled into the intrastrial space via the specialized K^+ channel Kir4.1. The large voltage drop responsible for the EP can be localized to the stria-facing membrane of the intermediate cells.
 - (9) Marginal cells of the stria vascularis continually take up K^+ from the intrastrial space via the $Na^+-K^+-ATPase$ pump and the $Na,K,2 Cl$ co-transporter. Such an energy-consuming process ensures that the K^+ concentration in the intrastrial space is kept low so that K^+ will expel out of the intermediate cells.
 - (10) A slightly positive resting potential of the marginal cells relative to the endolymphatic space allows for passive diffusion of K^+ across the apical membrane of the marginal cells. The K^+ loop is completed.
- EP is thought to be the battery source for active processes → exquisitely tuned tips of high frequency tuning curves in mammals.

Salt (1987), Mechanisms of Endocochlear Potential Generation by Stria Vascularis, Laryngoscope: 97, pp 984-991

- At that time, EP was accepted to be generated by electrogenic transport of K^+ across marginal cells in stria vascularis.
- Method: A broken-tipped double-barreled electrode advancing through stria at a rate of $44\mu m/min$, measuring the electrical potential and $[K^+]$ along the way.
- Results: 3 areas of high $[K^+]$ but low potential (Fig 1). Authors assume areas correspond to basal cells, followed by intrastrial space (high electrical potential, low $[K^+]$). About $200\mu m$ into stria – endolymphatic space (high electrical potential, high $[K^+]$). Separate recording around $200\mu m$ → marginal cell (high electrical potential, and even higher $[K^+]$ than endolymph).
- Electrical potential was recorded during forward and reverse advancement of electrode as a control to make sure stria is not damaged by broken tipped electrode.

¹ Hair cells die in knockout mice engineered to lack KCC4 or Kir4.1. However, note that other theories of K^+ recycling in the cochlea (e.g. Wangemann, 2002) posit that K^+ is expelled basolaterally by the OHCs into the perilymph, where it then diffuses across the perilymphatic space towards the Type II fibrocytes. Such theories ignore KCC4 and Kir4.1 in the Deiters cells.

- Major finding (take home): With low $[K^+]$ in intrastrial space (and a large +ve potential) → no need for electrogenic transport for K^+ across basal cells into intrastrial space.
- Marginal cells must actively take up K^+ from intrastrial space. ($Na^+-K^+-ATPase$ has been localized to marginal cells)
- +ve potential from marginal cells to endolymph → K^+ can passively diffuse across apical membrane of marginal cells into endolymph (without electrogenic transport)
- Flaw: failure to account for intermediate cells.

Takeuchi (2000), Mechanism Generating Endocochlear Potential: Role Played by Intermediate Cells in Stria Vascularis

- By selectively blocking K^+ channels and recording both intermediate cell and endolymphatic potentials, authors find a strong correlation that support their hypothesis that EP is dependent on voltage jump across intermediate cells.
- Major finding: changes effected in the large voltage drop across the intrastrial-facing membrane of the intermediate cells by application of K^+ channel blockers → correlates with changes in EP (Provided $[K^+]$ on intrastrial space is at least 3.6mM)
- Conclusion: EP generates at membrane m2. (Re: Fig 8)

Resting Potentials in Cochlea

- Endolymphatic potential (EP) ~80mV near the base of cochlea, slightly smaller in higher turns.
- Source is thought to be the stria vascularis – provides electrical driving force for the movement of +ve ions through stereocilia transduction channels.
- Supporting cells resting potentials ~ -70 -- -100mV

Stimulus-related Potentials

- Field-potential recordings are averages of responses from many hair cells distributed along the length of the basilar membrane.
- Acoustic responses = AC (cochlear microphonic – frequency is the same as the stimulus) + DC (summing potential – displaces EP in a negative direction for the duration of stimulus).
- Measures of CM tuning are not as sharp as BM tunings → disparity due to remote recording technique, as the response is from 1000s of hair cells whose electrical signals are summed.
- AC receptor potentials measured in apical hair cells are larger than their DC counterparts, 8th nerve fibers are capable of phase locking their AP to the depolarizing phase of AC receptor potentials.
- AC potentials measured in basal IHCs are their CF and are extremely attenuated owing to the cell's membrane time constant. DC receptor potential of these cells is responsible for neural excitation.

Inner Ear Development (Joe Adams)

Torres (1998)

- Major Stages of Inner Ear Development (Fig 2):
 - Otic vesicle derives from an ectodermal placode.
 - Otic placode → earliest morphological evidence for the primordium of the ear

- Otic placode invaginates to form otic cup and the otic vesicle.
- Otic vesicle undergoes intense proliferative growth prior to differentiation.
- Acquisition of placodal competence → 1st step in ear development (starts at early gastrula stage)
- Next, specification of the otic field → determined by activity of early-expressed genes. Cell-to-cell communication and cell exchange is restricted so that otic ectoderm is functionally isolated from surrounding ectoderm.
- Otic field → acquires identity, becoming committed to otic fate and eventually reaching an irreversible state of determination [i.e., otic field will develop into an ear independently of the embryonic environment].
- First symptoms of regional and cell-fate specification of otic vesicle → earliest stage of regional gene expression and first events of cell differentiation, respectively.

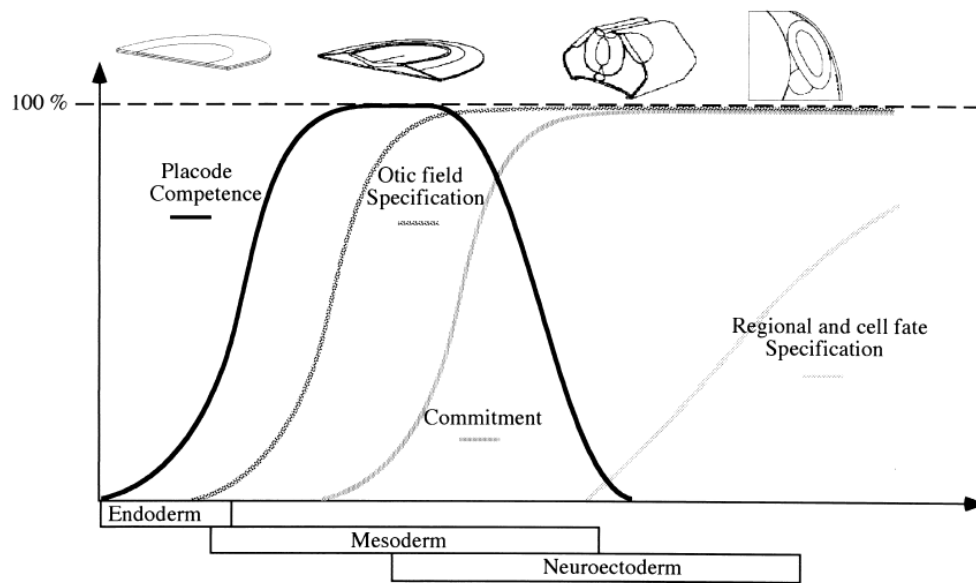


Fig. 4. Critical processes in ear development. The different curves represent the degree to which each of the processes is achieved. Icons above mark the approximate relationship between the processes represented by the curves and developmental stages (as defined in Fig. 2). The definition of the processes represented is detailed in the text. Bar diagram below the curves represents the time period during which each germ layer is believed to exert its main influence on otic development (Jacobson, 1963).

Groves *et al* (2000), "Competence, specification and commitment in otic placode induction, Development, **127** 3489-3499. [Sherry's presentation]

- Questions concerning this paper:
 - How long and in which region(s) is embryonic ectoderm competent to form an otic placode?
 - When does the presumptive otic ectoderm specified, committed to form otic placode?
 - Where are the regions of embryo that have otic placode-inducing activity, and how long does inducing persist?
- Many regions of the early embryo ectoderm are competent to form an otic placode.
- Cell / tissue is **competent** wrt a particular developmental fate if it can adopt that fate in an appropriate environment, regardless of whether it normally adopts such a fate *in vivo*.

(**specified** – if it adopts that fate in a neutral environment, free of any further signals;
committed – if it recapitulates that fate regardless of its environment.)

- Experiment used quail tissue because they have antibodies that are distinct from chick hosts → can separate the tissue regions from donor and host.
- Placode competence declines with age and is completely lost by the 10-12 ss (Somite Stages).
- Significant specification of the otic placode with respect to *Pax-2* and *BMP-7* occurs at 5ss and 7ss, respectively.
- The otic placode is committed to express *Pax-2* and to form an epithelial vesicle by 9-10ss.
- The region extending from the first 2 pairs of somites forward to rhombomeres 2 and 3 has the ability to induce *Pax-2* protein.
- The effects of the signals inducing or maintaining expression of these 4 markers are exerted over different time courses and may be redundant.
- NB: Formation of an invaginating otic placode or vesicle in embryos ≠ successful formation of inner ear.

Eddison *et al* (2000), Notch signaling in the development of the inner ear: Lessons from *Drosophila*, PNAS **97**, no. 22, 11692-11699. [Brad's presentation]

- Author argues that simple lateral-inhibition model of Notch signaling pathway in sensory and supporting cell is inadequate → more complex model including lateral-induction regulation.
- Sensory epithelium of inner ear differentiates into supporting and hair cells (but with the "King of the Hill" theory) → Primary fate is to become a hair cell → lateral inhibition model where developing hair cell delivers an inhibitory signal to surrounding cells.
- In sensory patch, every cell touching a hair cell is a supporting cell, every cell not touching a hair cell is a supporting cell.
- *Drosophila* (fly) model: Notch is expressed in all cells → nascent hair cells express greater amount of Delta than adjacent neighbors → increase Delta activates Notch in adjacent cells → Notch activation prevents cell to adopt primary fate, also downregulates Delta.
 - Mutations in loss of Delta-Notch function → lack of lateral inhibition → excessive proportion of population adopting primary fate.
- Problems of *Drosophila* model:
 - Delta is not the only Notch ligand at work → Serrate (another Notch ligand) is also present and must be mutated along with Delta for loss-of-function phenotype.
 - Delta expression remains high in the winning and losing cells
 - Intracellular protein Numb is distributed asymmetrically → makes cells that contain it deaf to lateral inhibition.
- Revised model of lateral inhibition:
 - Nascent hair cells have high levels of Delta 1 and Serrate 2 expression (both activate Notch receptors in supporting cells). Supporting cells express Ser1.
 - Notch upregulates Serrate 1, inhibits Delta 1 and Serrate 2, and inhibits hair cell differentiation.
 - HCs contain Numb and down-regulate their Notch 1 expression → despite exposure to Ser1 from all sides, their level of Notch ↓, Ser1 ↓, DI1 ↑ and Ser2 ↑

- Serrate 1 activates Notch receptors in adjacent supporting cells, causing an increased inhibition, also activates Notch in hair cell (Experiment: blocking Notch signaling resulted Ser1 expression lost; blocking Notch targets → Ser was lost)
- Eventually, hair cell becomes “Deaf” to the Notch signaling pathway by deactivating some component – perhaps through Numb. (Experiment: Use immunohistochemistry to look for Num homolog → once HCs have differentiated, Numb is seen at high concentration in HCs → Numb protein acts in nascent HCs to make them immune to Notch signaling.)
- Why 3 Delta Ligands? Ligand in supporting cell must be different from that in hair cell since Notch activation downregulates the other 2; Redundancy?; Serrate 1 might be important to prevent premature hair cell differentiation. (Experiment: Notch blockage should prevent lateral inhibition → expected excessive density of hair cells, but NO)
- Cells → HCs because of Notch ↓, supporting cells because of ↑ Notch activation.
- Model does not account for diversity of cell types in mammalian cochlea (2 types of hair cells, 6 types of supporting cells).
- Model does not account for signals coming from mesoderm
- Model does not explain how precise number of hair cells and supporting cells are regulated.

Inner Ear Damage (Joe Adams, refer to Apoptosis figure from notes)

- Apoptosis (controlled cell death) – sign as include cell shrinkage, nuclear fragmentation, plasma membrane blebbing.
- Necrosis (uncontrolled cell death) – occurs in cochlea when noise intensity causes physical disruption of the epithelium.
- Receptor mediated apoptosis: 7 of the 2 dozens tumor necrosis factor (TNF) receptors have a “death domain” (DD) intracellular component → DD bind their ligands → activate “initiator” procaspases, e.g., Procaspase-1, -8, -9 → in turn activate “executioner” caspases (caspase-3, -6, -7) → cleaving essential proteins and nucleic acids → apoptosis.
- Stress Pathways → Initiate by controlling efflux of cytochrome c and other enzymes from mitochondria → triggers Apaf-1 (apoptotic protease activating factor) → apoptosomes which attracts procaspase-9 → activation by dimerization and allosteric alterations → activated caspase-9 activating caspase 3 → apoptosis.
- Anti-apoptosis achieved by:
 - Decoy receptors binding TNF family receptors but lack a death domain
 - Compounds binding elements of apoptotic pathway → inhibit activity, eg viruses
 - IAP (inhibitor of apoptosis) proteins that inhibit caspases.
 - Up-regulation of anti-apoptotic members of Bcl-2 family → inhibit caspase activity and/or control stress-induced release of mitochondrial enzymes.
- Multiple redundant anti-apoptotic mechanisms must be active within cochlea since it is post mitotic and hearing usually endures for a lifetime.
- Large genetic component in the outcome for vulnerability, but specific genes not yet identified.
- Little is known about specifics of molecular pathways underlying protected states (heat / restraint / surgical) of cochlea (but can drawn from ischemia protection – lack of O₂ in blood)

- Drugs shown to protect cochlea from noise and ototoxic drugs: anti-oxidants; those that block reactive oxygen species, eg allopurinol, d-methionine; anti-inflammatory drugs; caspase inhibitors; growth factors, eg FGF, GDNF, and neurotrophins.
- Some drugs are effective in reducing permanent cochlear damage given AFTER noise or ototoxic challenge.
- Subtle Temporary Threshold Shifts (TTS) anatomical traits: stria edema, nerve fiber swelling, HC swelling, HC organelle disruption; high K^+ in perilymph; toxic levels of amino acid-like agonists present in perilymph (or glutamate agonists, eg kainic acid mimic effects of acoustic trauma)
- Anatomical correlates of PTS: stereocilia disrupted, ciliary rootlets broken, organelles within HCs are disrupted, HC loss (OHC and IHC), organ of Corti destruction, Schwann cell disruption, limbal cell loss, ligament cell loss, nerve fiber loss accompanies IHC loss, CNS degeneration. In extreme, de-differentiation of surviving cells to form a squamous epithelium.
- Impulse or low pass noise → predilection for 4kHz loss in human → acoustic basis in middle ear.
- Wideband stimuli → narrowband lesion → lesion spreads in time.
- Tinnitus usually accompanies TTS and PTS.
- Common ototoxic drugs are aminoglycosides (eg neomycin, gentamicin) → treating gram negative bacterial infections and platinum compounds, (eg cisplatin) → anti-cancer drugs.
- May reduce effect by same manipulations effective for reducing noise-induced trauma → anti-oxidants, growth factor → suggests stress and apoptotic pathways are shared by acoustic and ototoxic stresses → may lead to local treatments to spare side effects.
- Ototoxic antibiotics do not produce nerve degeneration → good implant candidates!
- Middle ear administration of gentamicin → currently used to treat symptoms of Meniere's syndrome.
- Non mammals → regeneration of hair cells by division of remaining supporting cells.
- Mammalian cochlear → signals maintain post mitotic state. (Goal: make tissue return to a mitotic state without making it more vulnerable)
- Math1 can induce interdental cells and other non-sensory epithelial cells to convert to hair cells, BUT problems: 1) devise means of inducing HCs to form in the right places (acoustically), 2) getting newly formed HC innervated, 3) getting cells to survive. (Research now into embryonic stem cells)
- HC needs proper functioning of supporting cells to survive → necessary to regenerate the entire organ. Difficult problem: how to induce formation of cochlea with the starting point as a de-differentiated remnant that previously was organ of Corti.

Yoshida (2000), Sound Conditioning reduces noise-induced permanent threshold shift in mice, Hearing Research 148: 213-219

- Background: 15 min of elevated body temperature induced a protection from subsequent acoustic overstimulation → protective effect correlated with rise and fall of heat-shock protein expression in cochlea.
- Hypothesis: any manipulation increasing whole-animal stress level for 15min will result in reduced acoustic injury during a subsequent time window extending 48 hours beyond manipulation.
- NB CAP as a measure of cochlear function has much finer resolution in the frequency and intensity domain than ABR.

- Both 15 min heat shock and 15 min exposure of almost-traumatic noise band result in a significant protective effect – however time course of protective effect was different (heat shock max protection ~ 6 hrs, sound conditioning, protective effect occurred from 6 hrs, grew until 24 hrs and disappeared around 48hrs)

Wang (2002), *Dynamics of Noise-induced Cellular injury and repair in the mouse cochlea*, JARO 03: 248-268

- Quantify hearing loss and cellular damage in noise-exposed CBA/CaJ mice ('normal hearing' in oppose to 129/SvEv, which is exceptionally resistant to acoustic trauma)
- Results: HC loss and stereocilia damage after narrowband exposure occurred in 2 foci: tonotopically appropriate (with half-octave shift basally) and the hook (extreme basal end)
- Cochlear mechanics provides no explanation for hook damage focus (no evidence is vibrating more, but there might be an inherent base-to-apex gradient in susceptibility of hair cells trauma; also there might be vulnerability due to proximity to round window.
- See Yoko's summary for detail list of effects for stereocilia damage; acute changes in organ of Corti, acute pathology in neural structures (eg swelling of spiral ganglion etc), Spiral ligament and excitotoxic effects, the limbus and stria vascularis abnormalities after acoustic trauma.

Definitions:

Cochlear Microphonics – AC responses to the acoustic stimulation and are generated at the cilia-bearing end of the hair cells. AC current measured anywhere in ear.

Endocochlear resting potential – Positive standing potential (70-100mV) that can be measured from the scala media (endolymph) referenced to the ground (perilymph). It is generated by the combined action of multiple cells within the stria vascularis.

Intracellular Potential – Resting potential exist without acoustic stimulation (Recording site: receptor potential, hair cell; ipsp – inhibitory post-synaptic potentials, hair cell; epsps – excitatory post-synaptic potentials, afferent fiber; Compound action potential: auditory nerve (VIII nerve))

Ectoderm – Embryonic tissue that is the precursor of the epidermis and nervous system.

Endoderm – Embryonic tissue that is the precursor of the gut and associated organs.

Endothelial cell – Flattened cell type that forms a sheet lining all blood vessels

Epithelium – Coherent cell sheet formed from one or more layers of cells covering an external surface or lining a cavity

Mesoderm – Embryonic tissue that is the precursor to muscle, connective tissue, skeleton and many of the internal organs

Hypotonic solution – solution having a low solute concentration (→ high water concentration) → expands (converse, *hypertonic*)

Inner Ear Development Glossary (Sherry's presentation)

Agenesis: A condition in which a part of the body (such as an organ or a tissue) does not completely develop or fails to develop at all.

Autocrine: Secretion of a substance, such as a growth factor, that stimulates the secretory cell itself. One route to independence of growth control is by autocrine growth factor production.

Delaminate: To split into thin layers.

Epiblast: The outer layer of the blastoderm; the ectoderm.

Homeobox: The homeobox consists of about 180 nucleotides coding for a sequence of 60 amino acids in a protein, sometimes termed the homeodomain, of which about 80-90% are identical in the various homeodomains identified from *Drosophila*. The homeobox codes for a protein domain that is involved in binding to DNA. Linear order within genome maps to order of expression in embryo.

In Situ Hybridization: Technique in which a single-stranded RNA or DNA probe is used to locate a gene or a mRNA molecule in a cell or tissue by hybridization.

Induction: The act or process of inducing or causing to occur, especially the production of a specific morphogenetic effect in the developing embryo through the influence of evocators or organizers or the production of anesthesia or unconsciousness by use of appropriate agents.

Mesenchyme: embryonic tissue of mesodermal origin.

Morphogenesis: The process of shape formation: the processes that are responsible for producing the complex shapes of adults from the simple ball of cells that derives from division of the fertilized egg.

Neurogenesis: Differentiation of the nervous system from the ectoderm of the early embryo.

Neurotrophins: Molecules with closely related structures that are known to support the survival of different classes of embryonic neurons.

Nodose Ganglion: the inferior (caudal) ganglion of the Vagus (10th cranial) nerve. The unipolar nodose ganglion cells are sensory cells with central projections to the medulla and peripheral processes traveling in various branches of the vagus nerve.

Otocyst: The embryonic vesicle from which the parts of the internal ear of vertebrates are developed.

Placode: Area of thickened ectoderm in the embryo from which a nerve ganglion or a sense organ will develop.

Plexus: A network or tangle, a general term for a network of lymphatic vessels, nerves or veins.

Primordium: An aggregation of cells in the embryo indicating the first trace of an organ or structure.

Rhombomere: Neuromeres or segments in the hindbrain region that are of developmental significance.

Somite: Segmentally arranged blocks of mesoderm lying on either side of the notochord and neural tube during development of the vertebrate embryo.

Transcription Factors: Endogenous substances, usually proteins, which are effective in the initiation, stimulation, or termination of the genetic transcription process.