Summary of the lecture „Disease and Molecule“

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Kapitel 1

Development of a sensory organ / Development of the inner ear I

All sensory systems develop from a special part of the body, the rhombomeres, from day E58 on. Rhombomeres build our neural tube; a ectodermal region (the neural plate) invaginates and builds the neural tube. The formation of the neural tube is influenced by surrounding cells. Before the neural tube closes ectodermal thickenings, the placodes, grow – they are the precursors of our sensory organs.

Neural crest and placodes have a non-ectodermal origin. Neural crest cells have the potential to differentiate into wide spectra and move to other parts of the body. The neural crest and otic placodes are not only found in vertebrates, but also in other species, for example Amphioxus (German: Lanzettfischchen) or Ascidian (German: Seescheiden). They have the genes for sensory organs, so they have receptors, of course. Placodes forma ll kinds of sensory systems.

The ear is developmentally very old – the first rudiment of the inner ear appeared before the eye!

(whatever that should mean) Prior to auditory plate the very early genes that also stem cells have: SIX, OTX

Kapitel 2

Development Inner Ear II

The ear develops from non-ectodermal tissue. During development the placode elongates: the upper part forms the vestibular system, the lower part forms the cochlea (the elongation winds up). On day P0 in rodents and week E10 in humans the auditory cortex is already connected to the inner ear, although it's not yet mature: hearing occurs on day P12 (rat) / embryonal week E 20 - 24 (human). Even the current potential develops by time: measurement of potentials shows four waves, each wave belongs to a special part of the auditory system. After one or two years of life humans hear like an adult.

As soon as the cochlea has reached its full size thyroid gland function and so thyroid hormone secretion starts. Thyroid hormone (TH) is necessary for maturation and growth. Thyroid hormone receptors (TR) are distinguished in two groups, TRα and TRβ. TRα and TRβ are transcription factors that bind thyroid response elements (TREs) at the DNA. Without TH growth is retarded, but a TRα deletion rescues that phenotype $\rightarrow$ TRα is a suppressor.

TH controls FGFR1 and Runx2. FGFR1 and FGFR2 are found in cranial bones, Runx2 is a transcription factor for osteoblast differentiation from mesenchymal progenitors and is responsible for bone loss in estrogen deficiency.

Kapitel 3

Physiology of Hearing (Engel)

3.1 Hearing in Mammals

Animals analyse frequencies and amplitudes of sounds to hear. The evolution of hearing has its climax in mammals. Mammals have

- largest frequency range (16 Hz (elephant) - 150 kHz (whale), human: 20 Hz - 20 kHz)
- best frequency resolution
- highest sensitivity
- large dynamic range (10^6)
- best binaural temporal resolution

The sense of hearing is the most sensitive and most complex sensory organ. As our communication is based on speech hearing is the base of our everyday communication.

3.2 Physical basics of hearing

The source of a sound is a vibrating body, so the sound wave itself is a vibration of (air) molecules. Sound waves can be described as longitudinal waves, waves that have vibrations along or parallel to their direction of travel.

Sound pressure is the local pressure deviation from the ambient (average, or equilibrium) pressure caused by a sound wave. Sound pressure can be measured using a microphone in air and a hydrophone in water[1]. Sound pressure consists of oscillations and is measured in N/m^2 or Pa.

[1] from Wikipedia, the free encyclopedia (http://en.wikipedia.org)

As unit for sound pressure N/m^2 is not usable: this ranges from 1 to 10^6, but speech, what we have to hear most, ist in between 100 and 1000 Hz. The sound pressure level (SPL) describes long ratios using the unit Hertz (Hz):

$$L = 20 \lg \frac{p_x}{p_0} \quad [dB] \tag{3.1}$$

You get this definition by transforming the following equation (I_0 is the reference pressure, usually $p_0 = 2 * 10^{-5}$ Pa):

$$\begin{aligned} L &= \lg \frac{I_x}{I_0} \quad \text{[Bel]} \\ I \approx p^2 \rightarrow L &= \lg \frac{p_x^2}{p_0^2} \\ &= \lg (\frac{p_x}{p_0})^2 \\ &= 2 \lg \frac{p_x}{p_0} \quad \text{[Bel]} \end{aligned} \tag{3.2}$$

The numbers you get out of this equation are quite small, so you don't use Bel as unit but **deci**Bel:

$$L = 20 \lg \frac{p_x}{p_0} \quad \text{[dB (SPL)]} \tag{3.3}$$

Using dB SPL never forget it's a logarithm:

$$\begin{aligned} p_x = p_0 \quad &\Rightarrow \quad L = 0 \quad \text{dB SPL} \\ p_x < p_0 \quad &\Rightarrow \quad L = -x \quad \text{dB SPL; log < -1 is negativ} \\ p_x = 10 p_0 \quad &\Rightarrow \quad L = 20 \quad \text{dB SPL} \\ p_x = 100 p_0 \quad &\Rightarrow \quad L = 40 \quad \text{dB SPL} \\ p_x = 2 p_0 \quad &\Rightarrow \quad L = 6 \quad \text{dB SPL} \end{aligned}$$

That's why a 10 fold increase in loudness is shown by 20 dB SPL more.

Sound consists of soundwaves going through (in our case) air molecules - the nearer to the source we are the more concentrated the soundwaves are $\rightarrow$ the sound is louder. As the sound energy is in square to the sound distance sounds quite quickly fade out when the distance to their source increases.

The sound shadow is the equivalent to the shadow of the light - if there is something between the source of the sound and our ear we don't hear it as well as without an obstacle. This fact is more significant for high frequencies than for low ones.

3.3 Tones

- pure tone – a pure sinus wave
- harmonies – the sinus wave is a bit tremulous
- noise – the wave doesn't resemble a sinus wave at all and is very irregular

3.4 Basic Anatomy

Pinna	discrimination between sound from above or below and in front or backwards
outer ear	transmits sound to the tympanic membrane
middle ear	„translates" sound from air to water and scales the vibrating area down from the large tympanic membrane to the 17 fold smaller oval window
inner ear	contains the vestibular organ that enables us to balance and the cochlea which lets us hear

Kapitel 4

Development of the inner ear III

The period of final differentiation occurs in every organ before the onset of function. In rat on day E18, in human on embryonal week 20 the thyroid gland starts working and the level of thyroid hormone (TH), a kind of „master gene“ regulating many maturation processes, raises. In the organ of corti TH is needed for the maturation of the inner sulcus.

How long is TH required for the development of normal hearing? To answer that question thyrostatika were given to the mothers of the animals used for the experiment. These drugs need three days to reach the right level. If the mothers are fed with thyrostatika during the pregnancy the pups are born deaf - but if the pups get TH **the first six days of their lifes** they hear normally.

In embryos TH and its level act like a kind of clock: If TH level raises too late, embryos develop a hearing impairment. If the level raises too early, the onset of hearing is earlier.

The longer TH lacks the higher brain stem nuclei are defect: IHC → OHC → nerve → brainstem → colliculus.

The identification of TH-dependent processes will show new gen loci of terminal differentiation in the peripheral nervous system and the central nervous system as well as new targets of hearing loss.

The receptors of TH (thyroid hormone receptors, TR) consist of TRα and TRβ subtypes (splice variants? –> lookup):

- TRβ mutant mice are deaf
- TRα1 mutant mice hear normally
- TRα1 and TRβ mutant mice are deaf

TRβ mutations create a resistance to TH. If there are problems with the TH level hearing of all frequencies is impaired.

Kapitel 5

The inner ear

vestibular organ	senses linear acceleration in three directions
cochlea	senses sound; it consists of a lot of bone; why a coiled cochlea? → increasing curvature redistributes wave energy, the travelling sound wave has a very assymmetric shape
oval window	a cellular membrane that prevents the liquid from flowing out
round window	lets mechanical energy out of the cochlea
Reissner's membrane	one cell layer, between *Scala vestibuli* & *Scala media*, *Scala vestibuli* is filled with perilymphe, *Scala tympani* too
Scala media	is filled with endolymphe, unique in mammalian body, mimics intracellular lymphe (Ca^{2+} concentration 10x lower than normal inner, 100x stronger than normal outer cell lymphe)
basilar membrane	the name of the cochlear partition in English, the inner siede of Reissner's membrane
Stria vascularis	very special epithelium that needs much ATP, makes currents consist, secrets K^{+}, cells are unique in human body („a bit strange", Jutta said)

There is a connection between cochlea and vestibular system, fluid and currents are coupled.

The location of the peak of the travelling wave depends on the frequency → frequency selectivity.

High frequencies are heard in the basal cochlea (stiff basilar membran & active amplification by OHCs). Low frequencies are heard in the apex where the basilar membrane is less stiff. This effect is called tonotopy.

Humans have a hearing of 20/60 - 20.000 Hz, mice, rats, gerbils hear 2 - 70.000 Hz.

IHC: thick afferent myelinated nerves, OHC: thin efferent and afferent nerve fibres

(afferent function of OHC still unclear)

Stereocilia have side and tip links –> mechanoelectrical transduction channel (MET); K^+ and Ca^2+ influx on excitation, during inhibition –> no flux?

Current of -70 mV in IHC, 0 mV outside (normal extracellular current)

Current of +85 mV between *Scala tympani / vestibuli* ans endolymph; not consequence of high K^+ concentration.

–> 155 mV potantial difference between IHC and endolymphe –> huge electrical force

–> electric gradient drives K^+ into IHC (chemical gradient is quasi non existing)

Function of IHC: sound wave induces K^+ influx –> needed for transfmission to auditory nerve –> graded receptor potential (not all-or-none like activation potential)

K^+ recycling by supporting cells and fibrocytes of spiral ligament.

Kapitel 6

Hearing impairment

Most common sensory defect in humans (Germany: ca. 14 million people).

OAE = otoacoustic emissions = response to acoustic signals.

Salicylate causes reversible hearing loss (Aspririn!).

Dihydrostreptomycin causes hearing loss.

Risc factors:

- infections
- meningitis
- underweight
- premature birth

Syndromic = associated hearing loss + disorders in the body
Non-syndromic = no features besides hearing loss
Most syndromic and non-syndromic forms of hearing loss are genetically caused (ca. 60 % of affected newborns).

Today we know about 45 genes that are involved in hearing (loss).

Connexin 26 is the most important of the recent identified genes - in Italy 70 % of all deaf newborns have a mutation in this gene, in Germany about 15 %.

Usher syndrome

- type 1: born deaf, blind from the 10th year on (because of retinitis pigmentosa), no sense of balance
- type 2: milder than type 1
- type 3: like type 1, but sense of balance

The same gene can cause prae- or postlingual, stable or progressive hearing impairment.

Kapitel 7

Hearing impairment II

Most common genetic cause of deafness: homozygotous Connexin26 mutation 35delG (35delG/35delG).

Why do we do genetic research?

- early diagnosis
- early intervention
  - hearing aid
  - cochlea implant
- advice

Otoferlin: big gen, there are 50 examined families with different (!) mutations in this one gene.

Wolframin: wolfram syndrome. Homozygous mutations cause diabetes insipidus/-mellitus, optional atropy & hardness of hearing high frequencies. Heterozygous mutations cause automsomale dominant hardness of hearing low frequencies, non-syndromic.

A1555G mutation: mitochondral heredity, causes gentomycin (etc.) sensibility. Getting gentomycin impairs ATP production so hair cells die because of too less ATP.

7.1 Testing

There are objective (for babies & animals) and subjective (for adult humans) tests.

Distortion otoacoustic emissions (DOE), 0 - 40 kHz (?), are the „answer“ of the hair cells to a sound.

Acoustic evoked brainstem potentials are signals in the brain evoked by sounds of specific frequency & loudness.

7.2 Presbyacusis (German: Altersschwerhörigkeit)

Age related hearing impariment = ARHI

Tübingen is a member of the ARHI consortium.

Risc factors for ARHI:

- analgesics
- noise exposition duration
- exposition to solvents
- drinking of alcohol
- weight
- BMI (body mass index)
- size
- susceptibility for sunburn

Association technique: to compare well hearing with bad hearing people from the same group and scan over all chromosomes → model-free analysis of genes.

Kapitel 8

Pathology through channelpathy

Singularity is caused by genes, their subtypes and splice variants, not by the number of genes.

Corti organ: K^+ potential between endolymphe & IHC (?) is +155 mV.

PDS: anion & iodid transport; in ear: role in *stria vascularis*; KCNJ10 channel is missing in Pendret syndrome.

Jervell & Lange-Nielsen syndrome: LQT syndrome (LQ (QT?) distance too big, deionisation can't take place because of missing KCNQ1/KCNQE1 channels/channel, both cause deafness when lacking; they were found in heart, kidney and ear (*stria vascularis*)

Megalin knockout causes severe kidney defects. Megalin is colocalized with KCNQ1/KCNE1.

LIMP2 (lysosomal integrated protein 2) decides (?) if reuptake to membrane or garbage. Knockout mice suffer from progressive hearing loss.

Women have a better hearing than men – perhaps because of estrogen.

Turner syndrome causes (next to other things) progressive hearing loss, β-estrogen receptor in *stria vascularis* in marginal cells –> where megalin & KCNQ1/KCNE1 is! –> estrogen is the ligand (one ligand) of megalin.

Kapitel 9

Genetic prevention

„Genetic prevention“ is to prevent the development of a disease. This allows possibly early diagnosis and is done by genetic screening.

A special form of deafness is caused by mitochondrial mutations in combination with aminoglykosid antibiotics. If persons with those special mitochondrial mutations get aminoglykosid antibiotics they'll turn deaf. Genetic screening of persons with a affected relative can prevent the development of this desease.

Retinoblastom is caused by a RB mutation. Children with a mutation in this gene have to visit the ophtalmologist very often to check the retina – if an area in the retina is peculiar, it is burned out to prevent the development of a retinoblastom.

Genotype diagnostic is indirect genetic testing because you test for markers, not for mutations.

In silico analysis: mutated sequence is simulated in the computer and checked for changes 3D structure.

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Trisomie 8: enlargement of extremities (e. g. very long feet).

Benennung des X- und Y-Chromosoms: 1891 Henking / 1905 Stevens: in der Feuerwanze X-Körperchen gefunden, im Mehlkäfer irgendwas, das nicht paart (da X schon vergeben war, Y)

Kapitel 10

Prions and disease

Normally information is given from one generation to the next by the DNA that is in the nucleus. But there are also other possibilities, the so called „non-mendelian transmissions“:

- mtDNA coded (mtDNA = DNA in mitochondria)
- epigenetic effects
- non-DNA/RNA agents

The last possibility is the most surprising one. An example for a non-DNA/RNA agent is a **prion** (**pr**oteinaceous **i**nfectious particles, -on in analogy to virion). Prion diseases affect livestock, non-domesticated animals, and humans. Those diseases are termed *transmissible spongioform encephalopathy* (TSE). An example is scrapie, found in sheep during the 1700. 1946 scrapie was found to be transmissible. Its human equivalent is the Creutzfeldt-Jakob disease that ripps holes into brain, cortex, glia, causes a amyloid plaques and a loss of neural cells. Creutzfeld-Jakob is quite rare (about 50 new cases per year in the UK), many of its early symptoms resemble to Alzheimer.

In the 1960s a new version of TSE was found in tribes of the Kuru region in New Guinea. Those people ate deceased relatives. Since those traditions are abandoned because of the spreaded christian religion the TSE version termed „Kuru“ stopped existing and can now be looked at as extincted.

In 1985/1986 BSE came up. It is possibly transmitted to humans as Creutzfeld-Jakob disease (the cases rose up coupled to cases of BSE).

Human TSEs:

- Kuru
- CJD (Creutzfeld-Jakob disease)
- GSS (Gerstmann-Sträussler-Scheinker disease)
- FFI (fatal familiar insomnia)

  - rare, 1:1.000.000
  - occuring sporadically (only few examples for familiary CJD)
  - iatrogenic (ill health or adverse effect or complication caused by or resulting from medical treatment)
  - not caused by viruses, bacteria, RNA or DNA → protein-only hypothesis

1997 the nobel prize for medicine was given to S. Prusiner who named prions.

Prions:

- single protein (PrP, **Pr**ion **P**rotein), stable topology
- produces normal prion molecules (also PrP^c, c = cellular)
- PrP^c is a Cu^{2+} binding brain protein at neuron membranes
- sensitive proteolysis, forms β-sheets
- abnormal isoform (PrP^{sc}), sc = scrapies
- mechanism of conversion unknown
- possibly PrP^{sc} converts PrP^c in PrP^{sc}
- without PrP^c no neurodegeneration

Genetics:

- 10 - 15 % of TSE is CJD, onset at 60 - 65 years
- GSS, CJD, FFI are autosomal dominant diseases
- from PrP via DNA probe to gene (the gene is called PRNP like prion protein)
- linkage in CJD, GSS and FFI families: 20p
- 5 different mutations are known in GSS, >5 in CJD
- CJD also with 1 - 9 repeats of a 8-aa-motif
- FFI only when D178N occurs together with M129V
- no PRNP mutations in sporadic diseases!

Still open questions:

- what is the function of PrP^c?
- how is PrP^{sc} formed initially?
- which is the mechanism of conversion?
- is PrP^{sc} causing disease or is it depletion of PrP^c?
- how is PrP^{sc} connected with GSS (cerebellum), CJD (cortex) and FFI (thalamus)?
- effect by abnormal folding of PrP^{sc} or fragmentation?

Kapitel 11

Genetic prevention strategies

Fehlt mir irgendwie.

Kapitel 12

Coongenital and progressive hearing loss

K^+ transport in the organ of corti is crucial for hearing - the channel / protein that is involved has to be very important for hearing.

Stereocilia: shearing → K^+ influx. What is the mechanotransducer? This mechanotransducer is one of the oldes proteins that not have been cloned or identified yet. Three very high impact papers have been published about mechanotransducers that had to be withdrawn after short time because it was shown that the published protein was not the sought-after mechanotransducer.

TRP channel is a candidate for the mechanotransducer. TRPA1, the most promising candidate, failed in the second test: knockout mice hear normal. By the way: 20 years of taste research was damaged by finding out that TRP channels are important for tasting :-)

Dysfunction of multiply stereocilia proteins induces hearing loss → ciliopathy.

If Ca enters the inner hair cell (IHC) there's a glutamate output. The IHC is a secondary sensory cell (no direct connection (to the brain?)) and a synapse (it releases transmitter).

The glutamate receptors 2,3 and 4 are AMPA receptors at the base of the IHC.

Ca changes in the cell leads to changes in the mRNA (splicing etc.) → acoustic trauma etc. can induce RNA edition and so on.

$Ca_v1.3$ channel influences development of hair cells, knockout mice are deaf → Ca is required for maturation. If a knockout in humans causes deafness is unclear. The role of $Ca_v1.3$ in maturation or function is not known yet, but it's interesting that more than 90 % of Ca^{2+} currents in IHC flow through $Ca_v1.3$ channels.

IHC: Ca-activated K^+ channel → BK channel, important for phase locking. Phase locking is up to 2000 Hz, that's why IHC are supposed to have some of the fastest ion channels ever known.

The BK channel has 7 transmembrane domains, its β subunit has 2. The BK channel is at the top of the IHC, but the Ca channel is at the bottom - how can it activate the BK channel about such a distance? This answer is still not known. A BK knockout causes progressive hearing loss.

Also not known is what function spontaneous APs have. When the BK channel comes up hearing starts and spontaneous spiking stops.

Only prior to hearing acetylcholine from the brain sends signals. The acetylcholin receptor is not understood yet, but it modulates the frequency of spiking.

SK2 is a K^+ channel and a signal for immature IHCs. In $Ca_v1.3$ knockout mice SK2 is present.

$Ca_v1.3$ knockout: no BK channel, still acetylcholine, still SK2.

Otoferlin

- Ca binding protein
- responsible for deafness
- DFNB9 patients, congenital and/or progressive hearing loss
- vesicle protein for release of transmitter Ca (this is perhaps not true, the IHCs of animals with hypothyroidism have no otoferlin, but vesicles are released, too (known by capacitance measurement))

OHCs amplify the resonance behaviour of the whole basilar membrane structure. They moved too quickly for each protein known until then. 10 - 15 years ago people found out that the OHC membrane is so fully packed with proteins that it's nearly cristal - what was that protein? There were three candidates: 1. a anion exchanger, 2. a glykose transporter, 3. prestin.

Prestin = SLC26A5, multiple splice variants in humans. SLC26A1 - SLC26A11 cause diseases in humans when they mutate.

When Cl^- binds to Prestin it changes its conformation (thick stage, hyperpolarized). So there are two possiblities how it can make the cell motile: it could be anchored to the cytoskeleton or the membrane is so densly packed that conformational changes cause motility.

Also in OHCs K^+ has to go out - therefore KCNQ4 was found. Mutations in KCNQ4 cause autosomal dominant, progressive deafness. Because it is progressive there has to be another K^+ ion channel.

Kapitel 13

Complex Diseases II

Hier war ich leider krank - wenn jemand was mitgeschrieben hat, würde ich mich freuen, es haben zu können :-)