Frequency representation

Part 2
Development of mechanisms involved in frequency representation
<table>
<thead>
<tr>
<th>Summary of development of frequency representation</th>
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<tbody>
<tr>
<td>• Frequency discrimination and frequency resolution are immature in humans at birth, at least at high frequencies</td>
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<td>• Frequency discrimination at high frequencies becomes mature at about the same time as frequency resolution, around 6 months</td>
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<td>• Frequency discrimination at low frequencies continues to mature to at least school age</td>
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<tr>
<td>• <strong>What are possible auditory physiological bases of immaturity? Are there other explanations?</strong></td>
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Overview of lecture

- Cochlear development (prenatal events for humans)
  - Anatomy
  - Physiology
- Neural contributions to the development of frequency representation
  - Frequency resolution
  - Temporal code for frequency
Development of the cochlea

Frequency resolution is established in the mature cochlea and the nervous system maintains that fine frequency resolution.
This drawing comes from chick embryos, but the basic sequence is similar across vertebrates. The cochlea starts as a disc of cells called the otic placode on the side of the fetal head. The little picture at the left shows about where that would be. It grows in to form a sphere that is called the otic vesicle and detaches from the surface of the head. All the cells in the cochlea, including the spiral ganglion neurons originate in the cells that line the otic vesicle. But early in development, the spiral ganglion cells migrate out of the vesicle, taking up residence just outside it. The vesicle elongates to form a duct, and while this is going on, the cells that line the duct begin to differentiate. The processes of the spiral ganglion cells grow back into the cochlear duct and contact the base of some cells. Those cells detach from the “basement membrane” the outside layer of the duct and migrate toward the inner surface. As they reach the surface, they begin to look more like hair cells, stereocilia form and the hair cell takes on its characteristic shape. The first hair cells to differentiate are formed near the base of the duct, but as additional hair cells differentiate, they push the older cells up toward the apex. The duct continues to elongate, and in mammals form the turns that characterize the adult cochlea. Notice that the tectorial membrane is a pretty late arrival, as is this structure labeled “tegmentum vasculosum”-- which in mammal we call the stria vascularis. “Basilar papilla” in birds is the basilar membrane in mammals.
Timing of cochlear development events in humans

<table>
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<tr>
<th>Table 2.1: Entepology Summary of the Ear</th>
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<tr>
<td>37th</td>
<td>Apical plate; auditory pit</td>
</tr>
<tr>
<td>46th</td>
<td>Auditory vesicle (represented)</td>
</tr>
<tr>
<td>9th</td>
<td>Auditory vesicle (intermediate)</td>
</tr>
<tr>
<td>11th</td>
<td>Utricle and saccule present; saccule begins to bulge</td>
</tr>
<tr>
<td>15th</td>
<td>Organic tissue present; sensory cells in utricle and saccule</td>
</tr>
<tr>
<td>18th</td>
<td>Ductus reuniens present; ductus utriculi in semicircular canals</td>
</tr>
<tr>
<td>21st</td>
<td>Nasal process of mesenchyme begins</td>
</tr>
<tr>
<td>23rd</td>
<td>Two and one-half cochlear coils begin to form and spiral bulla attach to cochlea</td>
</tr>
<tr>
<td>12th</td>
<td>Sensory epithelium of cochlea membrana limitans complete; organ of Corti begins to develop</td>
</tr>
<tr>
<td>33rd</td>
<td>17th</td>
</tr>
<tr>
<td>28th</td>
<td>Maturation of inner ear; inner ear adult size</td>
</tr>
<tr>
<td>21st</td>
<td>20th</td>
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Although it says “cochlea mature” at 20 weeks, that really only means that it is adult size. The cochlea begins to respond around 22 weeks, but it isn’t mature for some time after that.
Retzius published a series of drawings of the cat cochlea at different ages in 1884. We know a lot more about cochlear development since then, but Retzius’ drawing hit the major points over 100 years ago. This drawing is from a 30 day old cat - at this point the cat cochlea is mature for all intents and purposes.

Prenatal and postnatal development of the cochlea

This slide shows the sequence of gross morphological development in the cat from before birth to about 30 days after birth. To put this in perspective, the gestational period for a cat is around 63 days long. If you blast a newborn kitten with 90-100 dB, you can get a response out of its cochlea, but not much of one. By 10-14 days, you start to get responses at moderate levels.

The events shown in this slide all happen prenatally in humans.

In the 8 cm cat fetus, the cochlear duct is very small. You can see hair cells in there, but no tectorial membrane and no tunnel of Corti. By 12 cm the duct has increase in size, we see the tectorial membrane sitting on top of the Organ of Corti, but there is no fluid space between to tectorial membrane and the rest of the organ. At birth, the cochlea is still sort of glomped together, although now the tectorial membrane is separated from the rest of the organ of corti. By 7 days after birth, things look a lot more mature. You can see the tunnel of corti. The hair cells look to be where they belong and the tectorial membrane is positioned above them. 11 days-- things look pretty good. There are still some differences between the 11-day old ear and the mature ear, but the structures are much more differentiated and the arrangements of the structures is much closer to what we see in adults.

Notice that as the cochlea develops the basilar membrane goes form being a thick mass of cells to being the thin layer that we see in the adult ear.
Developmental events happen first at the base, and progress apically

Retzius was the first to report that this sequence of development did not occur at the same time in all sections of the cochlea. In these drawings we see the basal turn of the cochlea, the middle turn and the apical turn in an 8 cm cat fetus. The appearance of the base is more mature than that of the middle turn and much more mature than that of the apical turn.
Hair cell innervation

You might remember from hearing science that the innervation of inner hair cells is different from that of outer hair cells. Inner hair cells are contacted by an exclusive set of afferent nerve fibers (i.e., each hair cell has its own set of nerve fibers). There is efferent input to the inner hair cell system, but the afferents are small and actually contact the afferent fibers contacting the inner hair cells. Outer hair cells, in contrast, receive only a small afferent input, and one afferent neuron may contact several outer hair cells. The outer hair cells, however, receive a massive efferent projection, directly to their cell body.
Early in development inner and outer hair cells look more alike than they will when they are mature. Both of the hair cell types are contacted by many of what appear to be afferent fibers. While the inner hair cells maintain that innervation, the afferents retract from the outer hair cell and efferents subsequently reach it. You can see that the form of the efferent contact changes with age.

The other thing that may strike you here, is that at any age, inner hair cells look more mature that outer hair cells. That is another general phenomenon. Inner hair cells differentiate, grow stereocilia--do everything before the outer hair cells at the same place in the cochlea.


Some potentially important developments

- Changes in basilar membrane stiffness and other mechanical properties of the organ of Corti.
- Differentiation and innervation of hair cells
- Development of the stria vascularis and endocochlear potential.

So if we are interested in the development of frequency resolution, there are several things developing here that would be important for frequency representation-- the mechanical properties of the basilar membrane, hair cell development, particularly outer hair cells, and the endocochlear potential (because without a battery, the outer hair cells won’t work.)
At the onset of cochlear function, sensitivity and frequency resolution are poor.

These are tuning curves of auditory nerve fibers from adult cats and from kittens at 3 ages--3, 4 and 7 postnatal days. As expected, the adult tuning curves are sharply tuned to particular frequencies, and the nerve fibers response at pretty low levels at their best frequency. The tuning curves from young animals are very different. Immature auditory nerve fibers only respond to very high levels of sound and they exhibit almost no tuning at all.

Researchers generally argue that frequency tuning at the level of the eighth nerve matures earlier at high frequencies. You can see in these tuning curves measured in animals of different ages, that even at 8 days you can see a little tip on the high-frequency tuning curve, so if you just measure the width of that tip the high-frequency fibers are going to look pretty mature. But because the tip and tail of the tuning curve are so close together, a tuning curve like this won’t give you very good information about the frequency of a sound. A major trend in tuning curve development is the increase in this tip-to-tail distance. So other people argue that there is no real gradient in the development of cochlear frequency resolution.
You can study frequency resolution in the cochlea using otoacoustic emissions. For example, if you play two tones with frequencies $f_1$ and $f_2$ into the ear (assuming that you've picked the frequencies and levels of the two tones correctly), the sound you measure in the ear canal will include $f_1$ and $f_2$, but also the distortion product $2f_1-f_2$. Now you can introduce another tone with a frequency around $f_2$ (because that is where the distortion product is generated). That tone will suppress the distortion product emission, or make it smaller. As I change the frequency of the suppressor around $f_2$, it will reduce the level of the distortion product more or less, depending on how close it is to $f_2$. So I can find the level of the suppressor at each suppressor frequency that will reduce the distortion product emission by some criterion amount. And if I do that I get…. 
Measuring cochlear frequency resolution using otoacoustic emissions

A suppression tuning curve, which gives me an idea of how finely tuned the basilar membrane is around the f2 frequency.
Caroline Abdala obtained dpoae suppression tuning curves from premature and term infants. Her results are sown here. Three f2 frequencies were studied, 1500, 3000 and 6000 Hz. In each graph you’ll see lots of tuning curves overlying each other at each f2 frequency-- those are the tuning curves for all the individual subjects she tested. Essentially her results showed that even the youngest infants had mature cochlear tuning. She did find that in young infants, tuning was a little better at 6000 Hz than it was in adults, but subsequent work has shown that that is a middle ear effect (lower intensity tones give sharper tuning curves, and the immature middle ear doesn’t pass sound at some frequencies as well in infants as it does in adults).

So if cochlear tuning is mature, why do 3-month-old infants have immature frequency resolution?

So what’s going on here? We know that psychophysically, 3-month-olds have immature frequency resolution, at least at high frequencies. How could that happen with a mature cochlea?
Sanes and Rubel pointed out long ago that if you measured the tuning of neurons in the brain, you could find 2 neurons tuned to the same best frequency, but one of them would have mature tuning—mirroring that in the cochlea and auditory nerve, and another with poor tuning. This leads us to the idea that it’s not enough that the cochlea be finely tuned. The auditory nervous system has to be able to maintain the same level of selectivity as the cochlea.

Development of the end bulb of Held

For example, you might remember that the auditory nerve makes a distinctive contact with bushy cells in the anterior ventral cochlear nucleus, that’s called the end bulb of Held. Just one nerve fiber contacts each AVCN neuron, with a massive, “secure” synaptic specialization. That’s what is shown on the right of this picture, which shows the end bulb of Held in a 4-day post hatch chick. On the left, is what the AVCN looks like at embryonic day 14 (some 10-11 days before). You see that the auditory nerve fiber is contacting multiple bushy cells. So think about it, if each there are 10 auditory nerve fibers coming in to the nucleus here, and each one has a somewhat different best frequency, and each one is contacting multiple bushy cells, then each of the bushy cells will respond not to one narrow band of frequencies, but to the frequencies of all of the nerve fibers that contact it. It’s tuning will be poor. So what has to happen during development is that some of these nerve branches retract, so that finally each neuron will respond very selectively.

Development of neural tuning in human infants

These are ABR Wave V tuning curves that Folsom and Wynne obtained from adults and 3-month-old infants in the 80s sometime. These were recorded a lot like the OAE suppression tuning curves. You measure the ABR to a tone pip, at say 1000, 4000, or 8000 Hz. Then you present another continuous tone along with the tone pip. When the continuous, masker< tone is close to the tone pip in frequency, it will reduce the response to the tone pip. So then you figure out what masker level you need to have to reduce Wave V amplitude by a certain amount, for masker frequencies all around the tone pip frequency. And voilà-- a tuning curve. And what Folsom and Wynne found is that a 3-month-old and adult had very similar ABR tuning curves at 1000 Hz, but that the infants had broader tuning curves than the adults at 4000 and 8000 Hz. (At what frequency were 3-month-olds immature in frequency discrimination and resolution?)

Gilbert Gottlieb first summarized observations of the frequencies to which young animals first respond, compared to the range of frequencies to which adults of their species respond. Gottlieb noticed that every species he looked at tended to respond first to low to middle frequencies.

A paradox in development

How can it be that the base of the cochlea--which responds to high frequencies--matures first, but baby animals first respond to low-to-middle frequencies? Ed Rubel and Brenda Ryals had the idea that during the development the place code might change, that the base of the cochlea might actually be responding to low frequencies early in life. So what they did was to expose chicks of different ages to a high-intensity pure tone, and then look at where the hair cells died along the cochlea. So if the animal is adultlike, we expect high frequency tones to cause damage near the base, and low frequency tones to cause damage near the apex.
This graph shows the position of hair cell counts at different positions in the chicken cochlea. The shaded curve shows the hair cell counts in a chick that has not been exposed to intense sound. The other curves show the hair cell counts for chicks exposed to an intense 1500 Hz tone. E20 means embryonic day 20— that is right around the time that the chick would hatch. P10 is post hatch day 10 and P30 is post hatch day 30. For each age you can see that the hair cell count looks normal except for in one region. More importantly the position of the region of maximal hair cell loss shifts away from the base as the animal gets older. So that would mean that a 1500 Hz tone is coded near the base of the cochlea at the youngest age, but that as the animal gets older that same frequency is coded at more apically. So you might call this a “place code shift”. The place code seems to be shifting during development.

This slide shows the position of maximal hair cell loss as a function of the chick’s age for 3 frequencies. You can see that for all three frequencies (and chickens don’t hear very high frequencies) the position of maximal hair cell loss shifts toward the apex. At the youngest age, intense exposure to the high intensity sound doesn’t cause any hair cell loss-- apparently the cochlea in the youngest animal doesn’t even respond to that frequency.
Other observations about the place code shift

- Responses in the nervous system shift with the responses in the cochlea.
- The shift occurs in mammals.
- If an animal is trained to respond to a certain frequency early in life, they will act like they learned to respond to a lower frequency later in life.

Subsequent studies of the place code shift documented that as the shift in the cochlea occurs, the same shift happens in the nervous system. The tonotopic organization of the nervous system just reflects the tonotopic organization in the cochlea. In addition. The shift was found to occur in mammals as well as bird, and in one interesting study of rat pups, Hyson and Rudy found that if they trained an animal to respond to say, an 8000 Hz tone shortly after its cochlea began to function, they would get a bigger response to a 5000 Hz tone when the animal was older than they did to an 800 Hz tone, suggesting that what we remember is which neurons were responding.

So there were several explanations offered for the place code shift. Some people argued that it wasn’t about the cochlea at all but that the frequency response of the middle ear was changing with development, so that when you put in an intense tone, there was likely to be distortion and the immature middle ear might boost higher frequencies more-- so you were really putting in a higher frequency sound than you thought. On closer examination, though, that explanation did not work. The next idea was kind of attractive. Remember that when the outer hair cells are active, the peak of excitation on the basilar membrane shifts toward the apex. (You can see that in your notes from last week.) So if the outer hair cells weren’t working, the peak would shift toward the base. However, later studies showed that outer hair cell response was actually pretty good and that the shift caused by outer hair cells wasn’t big enough to create the observed effect. Finally, Mills and Rubel found that the answer was the stiffness of the basilar membrane. Over development, the basilar membrane becomes less massive and stiffer. Floppy things have lower resonant frequencies, so when the base of the basilar membrane is less stuff, it responds best to a lower frequency. And it might not even be stiff enough to give a decent response to a high frequency-- as we saw in the chick.

Timing in humans?

If the place code is shifting, then you would expect to see that the time it takes for a sound to travel to its best place in the cochlea will get longer as the “map” shifts toward the apex.

These data are from Eggermont et al. (1996) in which they measured the latency of distortion product otoacoustic emissions in preterm infants and adults. (All of the latencies are expressed relative to the latency for 10-11 kHz.) You can see that the adult latencies for frequencies above 2500 Hz or so are longer than seen in the infants, particularly the 30-33 week conceptional age group. So the place code shift may still be going on at this point in humans, but we believe that the map is adultlike by term birth.

Development of the temporal code for frequency

- Phase locking takes longer to develop than frequency tuning.
- Phase locking develops in the central nervous system later than at the periphery.

So we’re done with the place code, how about the temporal code for frequency? Could it be that it takes a long time for low-frequency discrimination to mature because the temporal code-- based on phase-locking--takes a long time to mature.

These graphs illustrate the development of phase-locking in the auditory nerve and AVCN of cats. Remember that the quality of phase locking is measured by a synchronization index that ranges from 0 to 1, where one means that action potentials only ever occur exactly at the same phase on every cycle of a tone, and 0 means the action potential could occur anywhere in the cycle with equal probability. The open symbols are for AVCN and the filled symbols are for auditory nerve.

In the youngest cats, phase locking is only good in either structure at the very lowest frequencies. As the animal gets older, phase locking improves at progressively higher frequencies, and in general, the auditory nerve gets good at a frequency before the AVCN neurons. In the oldest cats, both auditory nerve and AVCN show mature phase locking, although there remains a group of AVCN neurons that are not well phase-locked. It may be that those particular neurons will never be well phase locked-- in the central nervous system, some are and some aren’t.

These data then illustrate two general trends. First is that it takes longer for phase locking to develop than for frequency resolution to develop. Cochlear tuning looks pretty mature at 21 days in the cat, but phase locking takes 8 weeks. Second, the central nervous system tends to lag behind the periphery in the development of phase locking. So it may be that it takes even longer for phase locking to develop at more central parts of the brain.
Development of phase locking in humans

We don't actually have a lot of information about the development of phase locking in human infants. Some indirect evidence that phase locking continues to develop after birth, is that evoked potentials change as infants get older. Evoked potentials can only be measured if a lot of neurons are responding at the same time-- because they are all phase locked to the sound. Even in the ABR, for example, we see that the amplitude increase, the latency decrease and the morphology becomes more adultlike in the first year or so of life.

We also have a little bit of data about the development of phase locking to amplitude modulation. These are data from Levi et al. They recorded the amplitude modulation following response in 1 month old infants and in adults at different modulation frequencies for a 500 Hz carrier and for a 2000 Hz carrier. The AMFR is thought to arise from the midbrain and cortex. The measure on the y-axis, mean coherence, can be thought of like the synchronization index. 1 means that the evoked response is following the amplitude modulation very closely, 0 means that the evoked potential isn’t related to the modulation at all. You will notice that for adults, synchronization increases as the modulation rate increases, and that while the same is true for the infants, they do not achieve equivalent coherence-- their phase locking may not be as good as the adults’.

Now these are really low frequencies we’re talking about and we’re at a pretty high up part of the brain. It may be that infants don’t have any trouble following the fine structure of the sound at say the level of the auditory nerve, but the data on this are not complete.
Development of frequency representation: Conclusions

- Frequency resolution at the level of the cochlea is mature prior to term birth, but at the level of the brainstem is still immature until about 6 months.
- The cochlear map of frequency shifts during development; in humans this occurs prenatally.
- The development of the temporal code for frequency has not been studied extensively, but there is some evidence that it may take longer to develop than the place code.