Beyond Audibility – Diverse Cochlear Pathophysiology Profiles in SNHL, presented in partnership with the University of Pittsburgh

Presenter: Hari Bharadwaj, PhD; Samantha Hauser, AuD

- [Christy] It is my great pleasure to welcome to you two new presenters to AudiologyOnline. This course is presented in partnership with the University of Pittsburgh. Annually we hold two courses with the University of Pittsburgh where they highlight their research and their researchers. And today we welcome Dr. Hari Bharadwaj and his mentee Dr. Samantha Hauser. And Dr. Bharadwaj is a assistant professor in the Department of Communication Sciences Disorders at the University of Pittsburgh. And his lab generally investigates how sound information is encoded and analyzed by our ears and brains in complex and noisy environments. Very interesting. And if you'd like to learn more about Dr. Samantha Hauser or Dr. Hari Bharadwaj, you can read their bios on the course registration page. And at this time, it's my pleasure to welcome Dr. Hari Bharadwaj. Over to you.

- Thank you, Christy. It's a pleasure to be here and with my colleague Dr. Samantha Hauser. And today we want to talk to you about two recently recognized phenomena, kinds of damage that can occur in the cochlea that would fall under the umbrella of sensorineural hearing loss. But the effects of those kinds of pathophysiological changes can go beyond audibility and affect how we listen in more complex environments. And these are our disclosures. And the learning outcomes for this course. After this course, participants will be able to identify key factors besides audibility that are important for successful listening in noisy environments. Describe the suprathreshold functional consequences of noise and age-related cochlear synaptopathy and distorted tonotopy from noise-induced outer hair cell damage.

And also describe the need for precision diagnostic tools based on different cochlear components of sensorineural hearing loss. Let's jump in. So I want to start by taking a moment to reflect on an amazing ability that healthy auditory systems can bestow us with. So if we are in a complex environment such as a busy street, crowded restaurant, bar, sports game, we are bombarded with sounds from many different sources. And the amazing ability that we have that AI can't do yet is be able to ignore many different

sources of sound and focus on one source of sound and then process that and understand, say, your friend and ignore all the background noise. So this is an amazing ability, but it's also fragile.

It's a common problem for lots and lots of people, billions of people around the world that listening in such complex environments is challenging. And of course, many of us in the field are interested in understanding how we listen in such complex environments and treating problems for people who find these situations challenging. And it's useful to keep in mind that it's not just hearing loss that can challenge your ability to understand speech and communicate in these complex environments, but there can also be many other factors that play into making this situation very challenging for you. So, for example, many neurodivergent groups, children with autism, for example, find communicating in environments of multiple sources very challenging. Elderly people, even if they don't have much hearing loss, can find the situation challenging.

If you have concussions, you can find the situation challenging. So if we try to unpack why this is such an amazing ability for a healthy auditory system and what are the different processes that need to all happen and all go well for us to be able to successfully listen in these environments, there's actually a lot that needs to go on under the hood. So, for instance, the first thing that needs to happen is our cochlea, the inner ear should be able to amplify soft sounds and provide stimulation to our auditory nerve in a frequency-specific manner, in a tonotopically specific manner. And then once that happens successfully, there are regions of our ascending auditory pathway that extract different features from the sound mixture that we hear.

And it's important to keep in mind that what we hear in these complex environment is not its source separately, but it's a mixture. So, for example, we have neurons that try to extract spatial information, neurons that try to extract modulation information and so forth, right? And that needs to happen successfully. And then that brings us to the next

big step, which is actually quite challenging. So if you think about a scenario where I'm speaking, and let's say someone else is also speaking on a few feet away, my voice would be broken down by the auditory system into thousands of different pieces and many, many neurons responding to different bits of my voice. But then the same thing is also happening for the other person's voice who's also speaking.

And their voice is also broken down into thousands of pieces and being represented by thousands of different neurons in the ascending auditory pathway. So now you have many thousands of pieces of two sources, but then our brain needs to figure out which pieces go together to make up my voice and put them together, group them together, or bind them together, and then which other pieces go together and form the other person's voice. So this is a fairly challenging phenomenon that our brain accomplishes readily if you have a healthy auditory system. And this is called the object formation problem. And this can be challenging on its own, and that's also a part of the process that could fail on its own.

And once the brain has grouped the information from my voice and represented it as one object, and then the information from the other person's voice represented it as another object, then cognitive processes like attention need to come in. So, for instance, a third person was in the room with me and the other speaker, for the same information that their ears are getting, they could choose to listen to me and ignore the other person, or do the exact opposite, like ignore me and choose to listen to the other person. So that cognitive process of attention is very important as well. And if attention is successful, then, of course, linguistic processes have to take over integrated with our experience and memory and then make sense of what's being said by me or the other person.

So this is a complex process that goes well beyond the cochlea or the inner ear. Many aspects of the brain processing are important and as a result, can also fail in many

different ways. So what we will focus on today is changes that can happen in sensorineural hearing loss at the level of the cochlea, and that'll be the focus of our presentation and discussion today. But it's useful to keep in mind that there are other brain processes like object formation, selective attention that can fail as well. So, for instance, we have done some work looking at children with autism spectrum disorders, and even when they don't have hearing loss, they can find complex situations like this with multiple sounds.

So that's very challenging. Through our collaborators they also had done some work in mild traumatic brain injury, concussions, and that can also be challenging. So although we'll focus on the cochlea today, it's important to keep in mind that listening in a complex environment goes well beyond the cochlea. So when we think about hearing challenges and sensorineural hearing loss, audibility of course is a big factor. So we do audiometry to figure out that someone needs increased levels of sound to be able to detect them in the first place. And that's of course a huge factor. Audiometry is the cornerstone of audiology. And treatments for hearing loss, of course, focus on restoring audibility because that's absolutely a very important thing to do.

But even if we restore audibility by amplifying sounds, or sometimes even when someone doesn't have loss of hearing sensitivity, so if their audiograms are normal, it's still possible to have situations like this challenging due to physiological changes that happen in sensorineural hearing loss that affect suprathreshold hearing and not just audibility. And we can see that in many different ways. So one of the ways in which we know that the effects of sensorineural hearing loss go beyond audibility is that even when we provide amplification, listening in noise in people with sensorineural hearing loss, it lags a little bit behind the level of performance that someone with normal hearing can achieve. So in this study by Woods et al., you have three groups of subjects that they studied.

So older individuals with normal hearing, that's the blue line. Older individuals with hearing impairment, that's the red dotted line, with aided listening. So this is when they heard speech and noise with their hearing aids. And then the same individuals, unaided. So older hearing-impaired listeners unaided in green. So as you can see in these results, as the signal-to-noise ratio increases, all groups get better, and amplification does help. So the red curve is above the green curve, and amplification does help quite a bit, but it's still lagging behind the performance that you see in similarly aged individuals but without hearing loss. That's despite amplification. And this also shows up in other ways.

So if you ask hearing aid users as was done in the MarkeTrak surveys, for example, only about a third of hearing aid users are satisfied with what their hearing aids can do for them in these challenging cocktail-party type environments. And this drops even further if you specifically ask them about large group settings. And another way in which we can see this is if you're doing a QuickSIN, which is a sentence-based speech-in-noise task. The SNR that the average individual with hearing loss that they need, the average SNR that they need to get about 50% of the words correct is four and a half dB or so. And only about 10% of people who use hearing aids seem to be able to break down below the three dB SNR loss number.

But then normal hearing individuals on average are at zero dB and most of them are able to recognize half the words with less than three dB of SNR. So in many different ways, we see that when you're listening in noise, despite amplification, performance or our ability to understand speech is a little bit behind those with normal hearing. And it's not just in background noise. Even listening in quiet data, you can see these effects. So, for example, this is data from about 94,000 patients from the Massachusetts Eye and Ear Infirmary clinic from the Grant et al. paper. The pink line is the word direct scores. So this is the maximum performance that they get at a suprathreshold level for people with various degrees of pure-tone average thresholds.

So the pink line is for patients with conductive hearing loss, and the black line is for patients with sensorineural hearing loss. And as you can see from these results, even for sounds presented at a high level, presumably audible, clearly audible level in sensorineural hearing loss, the performance even in quiet can lag a little bit behind those with conductive hearing loss. So from this, we can guess that sensorineural hearing loss has changes in the cochlea that affect your hearing beyond just reducing audibility. And I'm going to pass it over to my colleague Samantha to talk about that.

- All right, so I think it's very obvious from everything that Dr. Bharadwaj said about how complicated sensorineural hearing loss really is, not just in terms of the audibility and how much audibility we can restore as audiologists in getting the amplification there, but all of the components that really can be disrupted that affect how somebody is going to hear in their everyday life at normal everyday levels. And so that's what I want to get into next is what's really going on in the actual cochlea. So, obviously, sensorineural hearing loss is going to involve both the sensory and the neural components. And so here would be a normal hearing organ of Corti. And you can see in the red, the outer hair cells, which are the biological amplifiers.

They're going to be making those soft sounds louder so that you're able to detect those sounds. In the blue you have the inner hair cells, which is going to transmit that basilar membrane motion into the electrical impulses that get sent to the brain. And in green, you have the auditory nerve fibers that are going to collect that information from the inner hair cells and send that up. Now the other piece I want to draw your attention to that we're going to talk a little bit more about today are those little kind of bubbles on the end of the auditory nerve fiber and that connection there between the inner hair cell on the auditory nerve fiber. And so it's really all of these pieces working together to be able to create the hearing that we have and be able to do all of the amazing things that we can and be able to understand in these really noisy environments.

And so the conventional view of sensorineural hearing loss has always been that the most susceptible part of this system is the outer hair cells. And so in this cartoon here, you can see things like noise or ototoxic drugs are going to primarily affect these outer hair cells and you'll actually lose the whole cell body there. Or even if they remain, sometimes that stereocilia is non-functional. And then you don't really get that amplification that you need to be able to hear those soft sounds normally. And obviously, if those outer hair cells are affected, that add to the amplification that's going to lead to changes in threshold. And so that's something that we do notice. And as audiologists doing the hearing test, you can see these effects.

And that's because they're not able to detect those soft sounds as well as they used to. And so we get effects on the audiogram, and that's what we consider to be sensorineural hearing loss. And this has sort of been the pervasive theory of what really is underlying sensorineural hearing loss for a really long time. But I think we often forget that there are all of these components to the system that really need to be working together and correctly at all times in order to do all of the amazing things that we can do with our hearing. And so over the last decade or so, a little bit longer, there's really been a new form that's been talked about quite a bit on the research side of things that will hopefully sort of start to explain some of these complexities that we see in the audiology clinic.

And so rather than the outer hair cells being the most susceptible to noise exposure or to ototoxic drugs, it actually looks like those connections between the inner hair cell and the auditory nerve fiber might be even more susceptible. And so what this particular group saw back in 2009 was that something that caused a temporary threshold shift. So something like going to a loud concert and your hearing's not quite as good as it was before, but then it recovers back to normal that that type of noise exposure didn't necessarily change the outer hair cells, all the outer hair cells were

intact, but that connection between the inner hair cell and the auditory nerve fiber was damaged. And that connection there is absolutely critical for getting all of that sound information up to the brain.

And so it really kind of turned things around where we were really thinking that the first sign of any sort of measurable hearing damage was outer hair cell loss. It turns out that it's possibly this connection that's actually the much more susceptible to this problem. But what makes it difficult to find this is that it really doesn't result in any threshold shift. So even though the hearing thresholds recover back to normal after the noise exposure, you might think that there's not necessarily any major lasting damage to the person's hearing. But the actual anatomy, when they studied this in animals did show that there were these major changes to the auditory nerve fibers and that connection to the inner hair cell.

So I didn't know, as an audiologist, I saw patients all the time that would come in and complain that they were having all of these difficulties hearing in background noise, having trouble understanding speech, but then doing the audiogram and getting normal hearing thresholds, it's hard to reconcile those two facts. And this was a potential explanation for what's kind of going on underneath in that there's just enough auditory nerve fibers and enough connections left for something like detection of soft sounds and the outer hair cells are still working. So you still have the amplification. So detection might be fine, but if you're really trying to tax the system in the way that everyday life does with lots of background noise, this type of problem could really be more of an issue.

And so this is a really kind of newer area over the last decade to really force us to examine the more complex system that all needs to be working correctly and really looking at what might be the most susceptible to this type of damage. And so if you zoom in on this, this is sort of a side view of the organ of Corti there, you're looking at

the connection there between the green part, which is going to be your auditory nerve fiber, and then the blue part, which is your inner hair cell. And you can see that this connection has happened. And so I kind of think about this as like unplugging. If you unplug the vacuum from the wall, you might still have power in your house and the vacuum might still be perfectly functional, but if those two things aren't connected, then that's where we start running into the issues.

And that's exactly what's going on in this synaptic problem. And so sort of been termed synaptopathy because it's really the synapse and the connection that is the issue. And what happens at the beginning of this, so soon after your noise exposure, you might see that the initial sort of terminal, the unmyelinated part of the auditory nerve fiber that connects to the inner hair cell that sort of starts to pull apart, but the cell body itself actually remains for years or decades. And we take advantage of this fact for people who go on to receive cochlear implants that the auditory nerve cell body sticks around a lot longer. And so it's sort of that same idea with this type of synaptopathy is even though those connections might not be there, the actual components still should be working and are there.

And this has important implications for how we think about this particular problem might be treated. So being able to determine that it's this connection will help give us some ideas about what we might need to be able to do in order to fix this problem. So we don't necessarily need to regenerate new hair cells. We don't need to necessarily regenerate new neurons themselves. We just need to be able to restore this connection. So being able to sort of target this gives us some ideas about how we might look forward for new treatments. And another piece that we're going to talk about today as well is distorted tonotopy. And it's well known that part of the auditory system's beauty is that it is well organized across the frequency range.

You're having that high frequencies in the base going up all the way to the low frequencies in the apex of the cochlea, and that each part of the basilar membrane responds to its particular part of sound. And this is a very important feature of being able to separate out the different frequency components of sound. But what we've recently seen from some animal work and as well as some human work that we'll discuss too, is that this sort of nice tonotopy that's normally laid out in hearing loss after outer hair cell loss, the lack of outer hair cells doesn't just affect how audible something is, but it actually affects that tuning and how sounds are sort of distributed across the cochlea and where things in the cochlea respond to different frequencies.

And so we'll get into this a little bit more here too, but you can see that there's some nice tuning in the control example towards the top. But as we have more hearing loss, the response sort of broadens across the frequency range. In this particular feature, even though it's affiliated with the outer hair cells, it's not exactly the same as an audibility problem. And that's really what we want to talk about today is how there's so much more to this than just being able to detect a sound, but it's how things are processed at a more higher level. And so we'll get into this here in just a little bit, but there's some shifts that kind of happen and how this tonotopic organization works after hearing loss.

And so I've kind of been thinking about this as a reformulation or so, of how we think about sensorineural hearing loss more broadly. And because the traditional view of sensorineural hearing loss is very outer hair cell focused, I've sort of been thinking about sensorineural hearing loss more as complex sensorineural hearing loss, kind of in the way that we describe other sorts of genetic problems or other sort of health issues, that it's really this constellation of problems that's going on. And each individual is probably a different balance of those different problems. And I think as audiologists, it's really important to how we're looking forward to treating patients and how we diagnose

these different types of problems instead of it all kind of being lumped into one category of sensorineural hearing loss.

And I think this is going to have a big difference for things like amplification strategies. So, you know, right now we're definitely doing a lot of things to try to fine-tune hearing aids for an individual, but if we were able to have more diagnostic information to make better decisions about that from the get-go, if you're knowing what the underlying pathology is and being able to apply the right features and prescriptions beyond just what their threshold say, I think that's going to make a big difference for individuals and their individual satisfaction with things like hearing aids. This is also a huge emerging field with pharmacological treatments, and really being able to target the right problem and the right cochlear pathology is incredibly important for something like a drug therapy.

And there's been lots of new drugs kind of coming about with this synaptopathy problem. People have really felt that that was a good thing that they could kind of work on towards drugs. But what has been hard with that is making sure that you have the right candidates. If you don't have the right diagnostic tools, it can be difficult to know that you're giving that particular drug to the person who is going to be most affected by it. And so you might have a 50% success rate, but it might actually be a hundred percent successful for the right person. And so really being able to select the right candidates for treatment, knowing who is going to be the most likely to benefit from a particular drug, as well as just evaluating the efficacy while we're developing these drugs and having more diagnostic tests is going to be huge for how we're able to treat hearing loss in the future. So I'm going to pass it back here to Dr. Bharadwaj.

- Thank you, Dr. Hauser. So I want to jump in and talk to you about two studies that we did in our lab. The first one is about this phenomenon of cochlear synaptopathy where it seems to be the case that the most vulnerable structures in the inner ear might be the

synapses that connect up the inner hair cells to the auditory nerve. And I want to acknowledge the contributions of amazing people in the lab and our collaborators, Mike Heinz and the Purdue audiology clinic, and funding from NIH for this work. So we were interested in the question of, is cochlear synaptopathy a widespread phenomenon in humans? And if so, what measures might be able to sort of show changes in people with cochlear synaptopathy?

And to do that, we performed a cross-species study. So this is study on both human subjects and chinchillas. And that we can see one of our chinchilla subjects here with an OAE probe and a couple of ABR electrodes. And this is the human subject with a 32-channel cap for measuring ABRs and other evoke potentials. And this is an ongoing collaboration with our lab and the lab of some of my clients at Purdue University. So the first study is about cochlear synaptopathy. And what we did was we recruited three groups of human subjects. One group was just young individuals in the West Lafayette area in Indiana, about 55 subjects aged 18 to 35, general population of normal audiograms clinically.

And then we also recruited two test groups of people who are at greater risk for cochlear synaptopathy based on what we've learned from animal models. So animal models, the most work, for example, told us that noise exposure even at levels that can lead only to temporary threshold shifts can lead to cochlear synaptopathy. So one group that we recruited was young individuals, but with more regular and substantial exposure to more intense sounds. So these were individuals who were part of the large marching band at Purdue University and the various gun clubs in and around the area. But one key thing in the study was that all the subjects in the young noise-exposed group or sound exposed group were audiometrically matched to the control group.

So there were no differences in the audiogram all the way up to 16 kilohertz. And then the other group that's at higher risk for cochlear synaptopathy is just the virtue of their

age. So from the animal work, we also know that even in normal aging, you can lose these synaptic connections between the inner hair cells and the auditory nerve. And so we recruited 58 people. We call them the middle-aged groups, so 36 to 60 years of age, but crucially, their audiograms are also matched to the younger group up to eight kilohertz. So the idea is that if the groups are audiometrically matched but are at greater risk for synaptopathy owing to either more exposure to loud sounds or due to normal aging, what differences can we then pick up in their auditory systems and maybe that's attributable to synaptopathy.

And it's the chinchilla experiments that we're going to provide the basis for how we interpret the human data. So apart from the three human groups, we also studied one chinchilla group. And with the chinchillas, we can induce cochlear synaptopathy in the lab. So we had a group of chinchillas, we would expose them to noise, so 100 dB SPL, one octave band for two hours. And then we would perform measurements, the ABRs, DPOAEs, and reflexes, and we'll get into that a little bit more, before the sound exposure. And then we would expose them to noise and induce cochlear synaptopathy and measure the same metrics one day post-noise exposure, and then two weeks post-noise exposure. And we know from looking at the cochlear tissue of the chinchillas under a confocal microscope with immunostaining, that we are in fact able to induce this connection of the inner hair cell and the auditory nerve.

So the image that you see on the left is what normal pre-exposure tissue might look like from a chinchilla subject. So each red thing here is an inner hair cell. And then in the inner hair cells we have immunostained the synapses so that we can count them under the microscope. And so you can see each hair cell has about 15 to 20 synapses or so. And this is what tissue from a chinchilla subject looks like after noise exposure. So we can, again, stain the tissue and count the number of synapses. And visually you might get the sense that there are fewer synapses. And that's exactly what it is. So if

you actually count out the number of synapses, this two-hour 100 dB noise exposure in chinchillas is able to disconnect about 40 to 50% of the synapses.

So this is like having your full complement of hair cells and possibly your full complement of the cochlear neurons. But then this connection between them, as Dr. Hauser mentioned, is gone for about 40 to 50% of them. The synaptopathy has been induced. So what do we see in the different non-invasive measures that we do in the chinchilla that we can also do in humans? So one measure that we were interested in is the classic ABR. And as many of you know, the wave one of the ABR is sort of the population response that come from the auditory nerve. And the expectation is that if you lose 40 to 50% of those synapses and they're disconnected and not responding to the stimulation anymore, the size of the wave one would drop.

And so we looked at the ABR wave one, and we sort of normalized it by the ABR wave five and looked at the wave one and five ratio to sort of reduce some of the variability and things like head size and so forth. So that's one measure that we looked at. It's the ABR wave one. The other measure we looked at is the middle-ear muscle reflex. The one sort of technical addition that we did was we used a wideband approach to measure the reflexes. So in the clinic, we would measure immittance using a 226-hertz probe tone in adults and maybe like 1,000-hertz probe tone if you're measuring very young individuals. Here we are using clicks to measure the immittance changes that happen when you play a sound and elicit the reflex.

And what you see here is as you increase the stimulus level and play it to the ear, the immittance changes continuously and at some point about 65 to 70 DB for this individual, the reflex kicks in. So that's their reflex threshold. And then as you keep increasing the stimulus level, the reflex gets stronger and stronger. So it's the same acoustic reflex that is used in the clinic. It says that it's being measured with a wideband probe that we think gives us a little bit more sensitivity. So what did we find

in the chinchillas? So when we look at DPOAEs, and this is our way of checking that any threshold shifts or cochlear outer hair cell level changes are temporary.

It fits the histology. So the pre-exposure DPOAEs are the green curve. So you can see that there are nice robust DPs of about 30 dB SPL. So chinchillas tend to have larger DPOAEs than humans do. So the green is before we expose the chinchilla to noise. And then the violet curve, which is down here that you see it's a little bit reduced is the one-day post. So we expose the animals towards the noise, and then the next day we measure DPOAEs and the DPOAEs are reduced, but then that's temporary. Because when we measure two weeks post again, the DPOAEs are back, right? So this is consistent with something like a temporary threshold shift. So any audibility changes, threshold changes, outer hair cell level changes that we can measure with DPOAEs, those seem to be temporary.

And we also know that from looking at the tissue under the microscope, that the hair cells are intact and it's only the connections that are gone. And this is another way of confirming that OAE changes are temporary, come back in about two weeks. But when you look at the middle-ear muscle reflex, the wideband middle-ear muscle reflex that I talked about, the green is again the pre-exposure data. So the animals have a nice robust reflex starting at about 65 dB or so and grows as you increase the stimulus level. One-day post, there's a big drop in the strength of the reflex and an increase in the threshold of the reflex within two weeks after the noise exposure.

Unlike the OAEs, they don't come back, right? So the reflexes go down and stay down. So we thought that might be a very good measure for us to do in humans and see if the human groups with either more noise exposure or normal aging show these changes in the middle-ear reflex as well. So this is what the data in our human subjects look like. So this is the middle-ear muscle reflex measure just like the one I showed you in the chinchilla data. And we have our three groups. This is the young control group

and then the young noise-exposed group, which is the marching band group, and then the middle-aged. And then the middle-aged group is in the square sort of violet color here.

So when we use a broadband stimulus, broadband noise to elicit the reflex, we see big differences between the two younger groups and the middle-aged group. Despite the fact that the audiograms between these groups are well-matched, in the middle-aged group, you'll see a large reduction in the strength of the middle-ear muscle reflex. So this tells us that if we go by what we saw in the chinchillas, just from being a little bit older, you could have lost a good proportion of your synapses. So it does seem to be the case that cochlear synaptopathy is happening at least in middle-aged humans. This is when we used a broadband noise. So half to eight kilohertz to elicit the reflex.

And what we did next was to use a highpass noise. So this is three to eight kilohertz to elicit the reflex. And now we see that all three groups separate out. So even in the young noise-exposed group who have really good audiograms matched to the control group all the way to 16 kilohertz. You see a reduction in the strength of the reflex that happens. So what this tells us is that in the middle-aged people, you can see indications of something like synaptopathy even if you use lower or high-frequency stimuli to elicit the reflex. But in the young noise-exposed group, when we restrict our reflex eliciting stimulus to have just high frequencies, then we see some reductions.

So the way we interpret this is as a function of age, as we get a little bit older, we are losing synapses across the board. So at all frequencies across the low and high-frequency portions of the cochlea. But with noise exposure, it seems to be affecting the high frequencies synapses more. So what we learned from this is that even young individuals with good audiograms and middle-aged individuals with good audiograms show signs of synaptopathy. So it sort of confirms what we saw in animals in our human subjects that it's not the outer hair cells that are the most vulnerable. You

can lose the synapses before you lose the outer hair cells and have audiometric elevation and sensorineural hearing loss diagnosed in the clinic.

So this is useful even as a diagnostic measure. As Dr. Hauser talked about, the more we can disentangle the different components of hearing loss and say, okay, so this person has maybe this much outer hair cell loss and this much loss of synapses and much inner hair cell damage and so forth, the more precision we can achieve with that kind of diagnosis, the more targeted our treatments can be. And the middle-ear muscle reflex is one candidate that can help us think about cochlear synaptopathy. And we see sort of analogous changes with the ABR wave one. So it's not just the reflexes that seem to get weaker. The wave one which reflects the cochlear nerve response also seems to get a little bit weaker consistent with cochlear synaptopathy.

And it's not just with these wideband measures in the lab that you see these changes. We were able to look at a large database of about 4,000 subjects from the CDCs, the NHANES repository. And from the 4,000 data points that the CDC had, we sub-selected about 1,800 people's data based on the fact that these 1,800 people had really good audiometric thresholds all the way up to eight kilohertz. And even when we did that, as age went up, the strength of the reflex continuously went down. So it really does seem to be a widespread phenomenon that these cochlear synapses can be lost even when the audiograms look normal. And so you could be losing about 40% of the information being sent up to the brain.

And although it doesn't affect the audiograms, as Dr. Hauser mentioned, it can reduce the quality of information that your brain is getting, which is going to be make it more challenging in complex environments with background noise. So that's one phenomenon, cochlear synaptopathy, that's happening under the umbrella of sensorineural hearing loss. But the other phenomenon we want to talk about today is distorted tonotopy that Dr. Hauser mentioned. And this occurs with outer hair cell

damage. So with outer hair cell loss, because outer hair cells are the amplifiers, your audiograms do get elevated. You can't detect really soft beeps anymore, but that's not the only change that happens with outer hair cell loss. So what we have learned is that losing outer hair cells can mess with the tonotopic organization of the cochlea quite a bit.

So if you measure, say, the response of an auditory nerve from a chinchilla. So this is, again, ongoing cross-species studies. You can see that the tuning curve for an auditory nerve fiber in a control animal is nice and sharp, and you have these most sensitive frequencies and it's badly responsive to other frequencies. But then as more outer hair cell loss happens, the particular spot in the cochlea, this particular auditory nerve is starting to respond to lots of other frequencies. And it's not selectively responding to just the high frequencies that it should be responding to preserve this tonotopically resolve the information. And we can see this not only in tuning curves. So if we play broadband sounds like speech or speech-shaped noise and ask what information in the broadband sound is the particular auditory nerve responding to?

So in the control animals, the tuning curve that's supposed to respond to four kilohertz when you play a broadband sound is still only responding to four kilohertz. But then if you take this example of the bottom right plot, which is from an animal with moderate sensorineural hearing loss, moderate degree of outer hair cell loss, when we play a broadband sound, a neuron that's supposed to be responding to about three kilohertz is instead responding to about 680 hertz or so. So this is what we call distorted tonotopy. So the tail of the tuning curve, which is sort of the low-frequency portion of the tuning curve, has gotten sort of wider and sort of shallower that it's taking over the response in the sense that instead of the neuron responding to the high-frequency information like it's supposed to, it's instant responding to the tail information, to the low-frequency information.

So what this would mean is that if you had a broadband sound in situations of background noise, there is a lot of low-frequency noise neurons, and the high frequency portion of the cochlea which is supposed to help us hear and encode this detailed high-frequency content, are instead going to be responding a lot to low frequencies. And so we get this loss of information even though the sounds are audible. And this was confirmed in great detail in the chinchilla work. So what our collaborators did was they recorded from chinchillas and measured from thousands of different neurons and asked, when you play a speech, what is the content from the speech that each neuron is responding to?

And it turns out that the largest effect was that this distorted tonotopy where the neurons respond into low frequencies even when they are supposed to be responding to the high frequencies. So this is distorted tonotopy and this could have a very large effect on understanding speech-in-noise. The question is, does it happen in humans? And my colleague Dr. Hauser is going to take over.

- Yeah. So we're obviously not going to be measuring auditory nerve responses in the clinic. So we need a good behavioral or non-invasive measure to be able to figure out if this is going on in humans. And so one particular measure that works well for this is a psychophysical tuning curve. And so what we do for this test is to play a beep of a certain frequency, in this case, is four kilohertz, and we're playing that at a loud enough level to hear, so about 20 decibels above the person's threshold. And what we're measuring is the level of masking noise that is required to mask that tone. And so a four-kilohertz tone is going to be most easily masked for normal hearing by a four-kilohertz band of noise.

And if you change that frequency of the noise, it'll get easier or harder to hear the tone. And what this figure is plotting here is how much noise is required to basically cover up that tone that the participant is listening for. So what you'll notice in the shape of the

green line, which is people with normal hearing, is that you get this nice tip, this nice big peak in the response right around four kilohertz. And so that means that the masker could be the lowest level to mask that four-kilohertz tone. But as you move to lower frequency noises, it takes more and more masker to cover up that tone. And so you get what we consider the tail of the response, which would be the response at frequencies far away from our probe tone.

And so in this case, we're looking at around 2,000 hertz. You can see that you need a lot more masker to cover up that four-kilohertz tone. And so we're really considering these two points on the curve. The tip, which would be what the level of masking is at the dip at four kilohertz, and the tail, what the level of masking needed is at two kilohertz. And really comparing these two to come up with this tip-to-tail ratio. And you can do the same kind of thing in the auditory nerve fiber responses, but this is an easier more behavioral measure to do for our human participants. And so in the green curve for people with normal hearing, that tip-to-tail ratio is going to be about 40 dB.

So you have 70 dB-ish of masking at 2K and then about 30 dB of masking at 3K. 70 minus 30 is 40. And so that's how we're getting that tip-to-tail ratio. As you look at the different hearing loss groups, you can see changes just in the overall shape and in the amount of masking that's required to have the same effect though. So in our mild hearing loss group, people with thresholds between about 20 and 40 dB, you can see that that whole thing is kind of broadened out and flattened out a bit in the orange color there. And so that tip-to-tail ratio might be about 20 dB at the tip and about 50 dB at the tail, so about 30. So 40 to 30.

And then if you look in the purple here, if you have a more moderate hearing loss, it's really become completely distorted. And so that tip really isn't even at 4K anymore. It looks like it shifted to slightly lower frequencies and that tip-to-tail ratio looks more like just a couple of dB. So we've seen quite a bit of change there. And this is different

even across individuals with similar hearing losses. So our groups, especially, you can see in the moderate hearing loss category, there's quite a little cloud around there. So there's a lot of variability even with a given degree of hearing from the audiogram on how somebody might perform on this particular test. And so that's what we wanted to look at is what is this indicative of?

And does this help us to predict how somebody might do when it's a more noisy complex situation? But because we are working with people with hearing loss, we do want to account for that audibility problem. So obviously being able to hear the sound in the first place is going to be incredibly important. And so we're looking at how speech-in-noise is affected even when we're going to compensate things with the amplification. And so to do this, we worked with one of our colleagues, Dr. Joshua Alexander at Purdue and used the hearing aid simulator. And it's very similar to what you would do for a real ear verification of a hearing aid, but instead, it's for our psychophysical test.

And so you can still put a probe tone in the ear, but you have the headphone in as well and you can do some speech mapping to really make sure that you're providing what a real amplification would for our speech-in-noise tasks. And so what we did here was do speech mapping even with the normal carrot passage to really make sure that the right amount of amplification for a given hearing loss was being provided while the participants did a speech-in-noise task. And we fit these sort of simulated hearing aids at 65 dB and then we're able to get an SII of about .9 for about everybody here. So everybody has good audibility of the sound.

And then for the actual test, it was presented at 70 dB SPL. So, clearly, the signal should be audible and at least as audible as hearing aids would make it for them to do the test. And so what we see here when our subjects are listening for words in a low-frequency noise at different SNRs. And so we tested at five different SNRs plus 6

plus 12 minus 6 minus 12 and 0, and at plus 6 and plus 12, with that amplification, it's pretty easy. And just about everybody was at ceiling. They were all scoring about a hundred percent able to detect all of those words at that SNR. For the harder SNRs though, minus 12 and minus 6, we see a lot of variation in how people do.

So just across our range of all participants, some people were scoring at 90%, some people were scoring at 50%. And as you might think, that does have something to do with the hearing loss itself. So you can see from these different colored dots that they are kind of group, but they are spread out based on what the tip-to-tail ratio is. So if you take those numbers that we got from the tuning curve and that's plotted along the x-axis, the more sort of normal would be the bigger numbers. So further to the right here. And that's what we see is that the people that had a more normal and a bigger tip-to-tail ratio had much better hearing in noise scores versus people that had a much poorer tip-to-tail ratio.

So that much flatter tuning curve had much poorer speech-in-noise scores. And even for our people in the middle, this mild hearing loss category who all had similar hearing thresholds to fit in that mild hearing loss category, they had a range of tip-to-tail ratios from about 5 here to about 30. And that was strongly correlated here with how well they did. So the people in the mild hearing loss category with a small tip-to-tail ratio did much poorer than those who had a better one even when audiometrically we still would've considered them all to have mild hearing loss. And so this type of information was done looking at this statistically, the tip-to-tail ratio was a lot better at predicting the speech-in-noise outcomes than even the audiogram or the distortion product otoacoustic emissions.

And so really lending some credence here to the fact that these sort of other measures might give us more information about what's differentiating these people that have similar hearing losses. And so I think it's a really good takeaway of kind of what other

tests that we might want to be doing to get some more information about what's going on sort of underneath. But there's still a lot to do here to kind of get all of this sorted out and find something that's really clinically feasible too. And so just to kind of summarize everything today. Sensorineural hearing loss is obviously very complex and I think more complex than we often realize or appreciate. It goes far beyond outer hair cells alone and lots of different components.

And even when it is related to the outer hair cells, it might be more complex than just an audibility problem here too. And so there's many different possible underlying profiles that might separate out individuals that look somewhat the same in terms of the audiogram. And it's really this cochlear pathophysiology that may relate and explain some of these deficits that we see and some of the variability that we get in the clinic. These different profiles have sort of distinct suprathreshold effects too that all need to be considered and should hopefully help to shape how we're able to treat and better assist our patients. And lastly, that precision diagnostics ones that really to help disentangle these different types of profiles so we can figure out who has more or less distorted tonotopy or more or less synaptopathy are really going to help with how we do this treatment and new treatments that might come forward.

So helping us to individualize our amplification strategies beyond just what our normal prescriptive targets are, as well as improving our candidacy and outcome measures for some of the emerging pharmaceuticals that we hope to see come down the pike in the future. And I believe that is all that we have, but I know we are definitely both happy to answer any questions. So I think you can throw any questions in the Q&A. We do have one here. So, can you speak to the effect that frequency lowering and transposition will have with widening tuning curves and distorted tonotopy, especially for individuals with severe plus high-frequency sensorineural hearing loss? Do you want to go ahead, Hari?

- Yeah, that's a great question.

- Yeah.

- So the idea of frequency lowering, of course, is that the high-frequency portions of the cochlea have so much loss that the only way to provide high-frequency content is to transpose them to lower frequencies and then have the brain sort of recalibrate and adjust for that information. And that's definitely an alternative way of thinking about how to provide high-frequency content. With the distorted tonotopy, one thing that we should emphasize is that it's not so much that the high-frequency parts of the cochlea have so much loss that they can't respond to anything. It's that the tonotopy is affected so that even when there are hair cells, say, mild high-frequency loss or moderate high-frequency loss, that they are not responding specifically to high frequencies and instead, or in addition, responding to low frequencies too.

Yeah, it's interesting to think about how we might alter hearing aid prescriptions or strategies to address that, but as a phenomenon, it applies even to mild to moderate hearing loss. And the other sort of challenge with non-linear transposition techniques or even compression, is that they come with their own distortions of sound. And so at some point, there's a lot of figuring out for us to do as a field I guess is where I'm going here, is that the more we understand at an individual level how their cochlea is likely to respond to some complex mix of speech and noise, the more we can target those specific strategies to that individual. That's a great question.

- We have another one here that's I think is also a great question of might this research shed any additional light in tinnitus? I think the answer's definitely yes.

- Do you want to say more?

- You can go.

- Yeah, it's a great question. So with tinnitus, I mean, we know for instance that not everybody with tinnitus has a measurable hearing loss. So that's sort of one case where this could be directly applicable. So if you have an individual reporting tinnitus, maybe after a concert or something like that and their thresholds recovered back to normal, it could totally be the case that the hearing loss that's triggering their tinnitus is one of these cochlear synaptopathy-like phenomena. It's not apparent in the audiogram, but could nonetheless, by reducing the information the brain gets can trigger things like tinnitus and hyperacusis absolutely.

- Yep. So I think that was our last question, but we definitely appreciate your attention and I think are also happy to answer any questions at any additional times. We both really like this stuff, so, but we've had a great time and we appreciate everyone coming.

- Thank you all.