Role of DNA Repair in the Cochlea, presented in partnership with the American Auditory Society Recorded December 16, 2019

Presenter: O'neil W. Guthrie, MS, PhD, CCC-A

AudiologyOnline.com Course 34189

- [Christy] At this time it is my pleasure to introduce Dr. O'neil W. Guthrie, who received his BS and MS degrees from the State University of New York campuses in Geneseo and Ordonia. He completed his research training in molecular biology and audiology at the University of Pittsburgh, where he earned his PhD. He then completed a postdoctoral research training in molecular genetics at Duke, where he was awarded the Hargansal Biology Fellowship. In addition to his research credentials, Dr. Gruthrie is a licensed clinical audiologist with over 17 years of experience. His research has been supported by the NAH, DOD, and also the VA. Welcome, Dr. Guthrie, and at this time I hand the mic over to you.

- [Dr. Guthrie] Good morning, everyone. My name is O'neil Winston Guthrie, and I would like to just thank the American Auditory Society and Audiology Online for providing this opportunity. It's always a pleasure for me to share my research with folks, both clinicians and scientists. I also wanna thank the participants today, because I understand that you could be spending your time anywhere, yet, you've chosen to spend your time with me this morning. So, I really appreciate that. During the question and answer period, at the end, I'm simply going to use that as an opportunity for me to learn from you, through your questions and your comments. And hopefully, in that same vein, I can provide you with some new information. So, to learn outcomes here are to, after this presentation hopefully you'll be able to define ways in which cochlear DNA becomes damaged. And also, describe the consequences of such damage. And state ways in which cochlear DNA can be protected. Now, throughout the years there've been several individuals that have come in and out of my lab, and each of these individuals have contributed their own unique expertise to the entire research effort. And I do wanna recognize those individuals here. The folks who are circled red. I'm going to display their research throughout this presentation, so that's Mohamed and Xiao-Ping. I would also like to just recognize the different institutions that have generously funded the research that I've done, ultimately, we could not do the research without the funding effort, so we do wanna recognize those folks as well. Okay, so,

contents, what exactly will be covered today? So, throughout this presentation there is only one message that I'd like you to walk away with. And that message is that DNA repair is an endogenous cochlear defense. And all that means is that this process, called DNA repair, that naturally exists in the cochlea, is a defense mechanism. And the reason why we care about this is because a DNA repair could be something that, if we understood it, if we are able to tweak it, if we are able to improve it, we could then deploy it as a therapy. In other words, we could, ultimately, effect clinical outcomes by using this defense mechanism to prevent hearing loss. One of the things that enticed us earlier on about DNA repair is the observation that a given cell can, during the process of cell death, so a cell that's already committed to cell death, you can reverse that cell death by augmenting DNA repair within the cell. And we found that to be an awesome power. And we thought that if we can control that power, if we can wield it when we want, from the therapeutic perspective, then it might be possible to limit the progression of certain diseases. And in this case, the particular disease that we're interested in is hearing loss.

So, that's why we're interested in DNA repair, and the single message that we want to convey today is that this DNA repair mechanism does exist in the cochlea. And, it might be a worthwhile research endeavor. Now, in order to tackle this message, there are four lines of research, four emerging lines of research that we will cover. The first line of research comes from human observations, where patients who have mutations in DNA repair genes tend to exhibit hearing loss. And when you examine the cochlea from these patients, we get a census to why there is hearing loss. Because the cochlear from these patients exhibit dead cells throughout the cochlea. And this provided some of the earliest indication that, under normal conditions, the cochlea relies on DNA repair. In other words, in normal conditions there must be DNA damage occurring in the cochlea and the presence of these DNA repair genes maintains the integrity of the cochlea. So, this is why human beings present with mutations to DNA repair genes they end up with hearing loss. So, we'll take a look at that particular line of research. The second line of research that we will evaluate is actually research that

comes from my lab, and this is where we find that when we stress the cochlea, the tissues in the cochlea, the cells within the tissues in the cochlea tend to respond by upregulating, increasing the expression of DNA repair proteins. So it's as if the cells are trying to protect themselves whenever they encounter a stress. And the third line of research also comes from research in my lab, and this is where we're focused now on spiral ganglion neurons, the neurons that connect the cochlea to the brainstem. And what we observe in these spiral ganglion neurons is when we stress the neurons, and it doesn't matter what stress we use, whether it's chemical stress, such as a ototoxic drug, or a physical stress, such as noise exposure, whenever we stress these spiral ganglion neurons they seemed to respond by regulating DNA repair proteins. And specifically, what they are doing is regulating the location of the DNA repair proteins. So, for instance in one type of stress proteins move from the cytoplasm of the cell into the nucleus, so as to protect the nucleus from a given stress. In another type of stress the proteins may move from the nucleus out into the cytoplasm to protect the cytoplasm from damage. And, DNA does exist in the cytoplasm, not just the nucleus, so that somewhat makes sense to us.

So we'll take a look at that particular line of research. And the last, or fourth line of research that we'll look at actually has two points. One point is that if we are able to limit the magnitude of DNA damage in the cochlea, then we can prevent, or limit, the magnitude of hearing loss. And the reason why that is important is because DNA damage occurs from ototoxic drug exposure, from noise exposure, and from the aging process, okay? So that's somewhat exciting to us that this possibility exists. The other point that this fourth line of research will make is that you can limit hearing loss by increasing the expression of DNA repair genes. Okay? And, in this line of research what we've done, for instance, is taken a given subject and exposed that subject to a damaging level of noise so that both ears are exposed to the damage. And then after the damage, we take one ear from the same subject and increase the expression of DNA repair enzymes in just the one ear. And then what we do is track both ears over time, and what we find is that the ear where we increased the expression of DNA repair

genes result in less hearing loss than the opposite ear, 'kay? So all of those are exciting and it suggests that we're in the right direction, and we'll take a look at each of these lines of research. Ultimately, none of these individual lines of research, by themself, is all that convincing. In other words, even though they're all in the right direction, by themselves, they don't convince us of much, but when you combine all four together they begin to tell us a message. And that message is that DNA repair is an endogenous defense in the cochlea. And from our perspective, if we can harness this defense, if we can control it, then we may be able to deploy it as a therapeutic strategy. But before we jump into it, let's figure out what is DNA repair? What exactly are we talking about? So, if this is a segment of DNA, here you have the five prime strand of DNA. And here you have the reversed three prime strand of DNA. If one were to study the morphology of the DNA molecule, if one were to study the physiochemistry of the DNA molecule, one thing that you will learn is that the molecule itself is poorly designed. And when I say poorly designed, I mean that it is vulnerable, it is highly vulnerable to damage.

And throughout this presentation we will represent damage to DNA as these dark circles. So, each of these circles represents damage to DNA. Now, there are two categories of damage that can occur to DNA. One category comes from endogenous stressors. Endogenous means things that occur within a cell. So, for instance, the ions that exist within a cell; calcium, sodium, potassium, chloride, the concentration of these can damage DNA. Also, simple things like water can hydrolyze bases within DNA and cause damage. 'Kay? There are many enzymes that exist naturally within a given cell that can damage, that can chew up DNA. So, there are several things that exist naturally, normally within a cell that causes damage to DNA. In fact, in several of your cells, right now, as you're sitting here, there is spontaneous damage occurring to your DNA. Right? So, endogenous stressors the normal physiology, the normal function of the cell, actually causes damage to DNA. A second category of damage comes from exogenous stressors. And these are things that exist outside the body. So, for instance, the food you eat, the quality of the air that you breath, some of the

medications that you take, physical exposures as well, the most prominent being when you walk outside in the sun, so exposure to the sun. All these things, both endogenous and exogenous constantly threaten your DNA and that leads to damage. Now, if that damage, and remember these black circles here represent damage. If the damage is allowed to accumulate and it's not removed, it's not repaired, then several bad things occur. For instance, you can have a gene function can be disrupted. DNA damage is a very efficient way of developing cancers. Also, DNA damage can lead to cell death. In fact, a precursor to cell death is damaged DNA. And ultimately, all these bad things can result in the development of various types of diseases. So from a health perspective, DNA damage, or the accumulation of DNA damage, is a bad phenomena. Now, it turns out that most cells have mechanisms for dealing with DNA damage and this, or these mechanisms are associated with DNA repair proteins.

So these are the proteins, or enzymes, that seek out damaged DNA and then facilitate the process of removing that damage from the genome. And when that occurs you could have preservation of gene function, you can avoid cancer development and you can limit the progression of cell death, and ultimately, limit the progression of certain types of diseases. So from that perspective, we are inspired by this process and we think that if we can deploy it in the cochlea, we could potentially limit the progression of cell death in the cochlea and also prevent or limit hearing loss. Okay. So, the first evidence to support our message comes from observational studies where you have patients who have mutations in DNA repair genes and that seems to be associated with hearing loss. So let's take a look at that. So, in these observational studies what's happening is a patient presents with a given mutation in a DNA repair gene. And then, what happens is the patient also presents with hearing loss. And here you can see two audiograms showing you left and right ears of a patient that has a mutation in a given DNA repair gene. In this case, the DNA repair gene is called XPA. And this patient has a significant hearing loss in both ears. Here is another example of a different patient who has a mutation in a different DNA repair gene, and you can see that the patient does have hearing loss. Now, if you try to understand the cellular basis of this, what we

see is under normal conditions if you take a patient, a normal patient, and you look at, examine your cochlea, if you take a specific segment from that cochlea here and you blow it up, you can see that there are several areas where there's appears to be healthy tissue where the cells are present and they look pretty good. All right? But if you take a cochlea from a patient who has a mutation in a DNA repair gene, there are several interesting things that you can find. Now, one interesting thing is that the spiral ganglion neurons have died away, so there's some atrophy of the spiral ganglion neurons. And if you take the organ of Corti and you simply try to blow that up, you can see that the hair cells have also atrophied. All right?

There's also dead cells, or atrophy of the cells of the lateral wall. So, several areas within the cochlea tend to die away if there's a mutation in a given DNA repair gene. And this really provided some of the first evidence that DNA repair is a defense against endogenous stress. In other words, under normal conditions the genome of the cochlea is under stress, and therefore, the cochlea needs DNA repair proteins, under normal conditions, to maintain its own integrity. Okay? So, under normal conditions DNA repair proteins are necessary for maintaining the survival of cochlear cells. Okay, so then the next, or the second line of research that supports our message that DNA repair is an endogenous cochlear defense comes from observations where we observed that when you stress the cochlea, the cochlea seems to be responding to a given stress by increasing the presence of DNA repair proteins. And we suspect that that then facilitates some level of protection of these cells. So let's take a look at that data. So here is a section through the cochlea, and, we're focusing on the tissues of the lateral wall. So, here you have the stria vascularis here, which is composed of basal marginal intermediate cells. And then next to it you have the spiral ligament, all right, which is composed of several different types of fibrocytes. And then, over here you just have the optic capsule, so this is just bone right here. Now, DNA repair proteins, and all proteins, are invisible to us. So, in order to see the proteins what we have to do is tag them with something so that we can see them. And in this example what we've done is we've tagged them with a chromogen so that we can see them. So where you

see black, or these dark reaction products, that represents the DNA repair proteins. Okay? So, this panel here, if you were to take this structure that's outlined here and you blow it up, this is what you see. So, what this data tells us is that under normal conditions those proteins exist and they're expressed in the cochlea. Now, when you introduce a stress, and in this case we're using cisplatin, which is an ototoxic drug, notice that there's a massive increase in the expression of these DNA repair proteins. All right, so wherever you see these black or dark reaction products, that's where we've tagged proteins so that we can see them. Now, it's easy enough to quantify this. And in this panel, what we've done here is this acts as, this is the level of expression of a given DNA repair protein. Down here what we have is we've treated with two cycles of cisplatin. So, one cycle just consists of four days of cisplatin therapy. That's similar to what you would do in humans who have cancer, for instance, you treat them with different cycles of cisplatin.

So we've done the same thing here. And what we notice is that for each treatment cycle there is a significant increase in the expression of DNA repair proteins in the cochlea. Okay? So, whether we treat with cycle one or we do a second treatment cycle, there is an increase in expression of DNA repair proteins. Now, saline is just a placebo. It's basically what we dissolve cisplatin in. Now, if we allow the cochlea to rest, so, after these treatment cycles, if we allow the cochlea to rest we still see an increase but it's not as dramatic as when cisplatin is present in the cochlea. And we've repeated these experiments several times, so here's a different DNA repair protein, but the pattern of results is the same. And essentially what this tells us is that each time we stress the cochlea it responds by mobilizing these DNA repair proteins. Now, we can look at a different tissue type in the cochlea. So, here is the spiral limbus. Spiral limbus is composed of stellate fibrocytes and medial, central, and lateral interdental cells. And we see a similar situation, where, when we stress the cochlea with cisplatin, these cells respond by mobilizing, or increasing, the expression of DNA repair proteins. And indeed, we can quantify this as well. And the pattern is the same. In other words, the story is that each time we stress the cochlea with an ototoxic drug, in this case

cisplatin, the cells tend to want to protect themselves by increasing the expression of DNA repair proteins. So that suggests to us that DNA repair could be an endogenous stress response or defense mechanism. Okay, so this is initial evidence that DNA repair is a defense against exogenous stress. All right, so then the third line of research comes from work that we've also done, and this is where we focus now on spiral ganglion neurons. Spiral ganglion neurons are the neurons that connect cochlea to the brainstem. And here, what we find is that the spiral ganglion neurons, in response to stress, they tend to relocate DNA repair proteins, presumably, to the area within the cell where there is stress. So let's take a look at that data. So, just to give you some background or some context here. So, here we have an outline or a cartoon of a cell and here would be the cell wall, or the cell membrane. So, inside would be the contents of the cell. Within the cell there is a nucleus, right? And the nucleus has its own membrane, called a nuclear envelope. And then within the nucleus you have chromosomes, and embedded within chromosomes are DNA, or genes, and each chromosome here is just represented by these black vines, 'kay? Now, most people are familiar with the notion that DNA is within the nucleus of a cell.

But there's also DNA outside the nucleus of cells as well. So, for instance, these green structures here are mitochondria. And mitochondria are organelles within cells that provide energy, or the power necessary for a cell to conduct its daily activities. Now, within each of these mitochondria there is DNA. In fact, mitochondria has its own genome. 'Kay? Now, there's another pool of DNA that exists outside the nucleus of cells, and some people would argue that this pool of DNA is probably more important than the mitochondrial DNA, and probably more important than the nuclear DNA. Now, I don't entirely agree with that, but I would say that this pool of DNA that exists outside the nucleus is vital, is definitely important because it is the precursor to DNA that exists in the mitochondria and DNA that exists in the nucleus. In fact, if you damage this DNA in the cytoplasm then it will result in damage to DNA in the mitochondria and also DNA in the nucleus. Because DNA here migrates to these other areas. Sometimes we call this pool of DNA naked DNA. So, because DNA exists in the nucleus and in the

cytoplasm, DNA repair proteins, which are represented by these circles, need to be in both the nucleus and the cytoplasm. Right? Because they need to maintain or sanitize the DNA that exists throughout the cell. Now, there's an interesting thing that most cells do and that is when they encounter stress most cells within a population of cell will respond in one way. So, for instance, one typical way is that most cells will send their DNA repair proteins into the nucleus to protect genomic DNA. And we observed the same thing when we look at spiral ganglion neurons. So, here is a field of spiral ganglion neurons, and when we stress these neurons with cisplatin, what we see is that the DNA repair proteins accumulate in the nucleus of the cells. All right, so wherever you see this black reaction product, that represents the DNA repair neurons, the DNA repair proteins, sorry. Now, it's easy enough to quantify this and in these panels what you're seeing here would be the proportion of neurons, spiral ganglion neurons, that are expressing DNA repair proteins in their nucleus. And we can look at neurons at the base of the cochlea, the middle of the cochlea, or the apex of the cochlea.

Now, under normal conditions there are neurons, and normal conditions would be the white bars, under normal conditions there are neurons that express DNA repair proteins in their nucleus, but when we treat with cisplatin, there's a significant increase in neurons that now express the proteins in their nucleus. And, the opposite of that would be to look at the cytoplasm. Under normal conditions we find that there's a lot of neurons that express the proteins in their cytoplasm. But, with cisplatin exposure, there is less cells that are expressed in the proteins in their cytoplasm. Essentially, what this tells us is that under normal conditions the proteins are expressed in the cytoplasm, but after noise exposure there is an increase in the nucleus. 'Kay? So the neurons are trying to protect their nucleus. That's how we interpret that. It turns out that this phenomena, where the majority of cells within the population of cells, respond in a single way and by a single way, in this case we mean they respond by relocating the proteins into the nucleus is not unique to auditory neurons, because when we investigate non-auditory neurons, such as trigeminal ganglion neurons we find the

same result. Also, when we investigate non-auditory cells, such as kidney cells, we also find the same outcome. So, we call this behavior herding. And it's like a herd of animals, where all of them follow a single pattern. It turns out that there are other examples of herding and we can, we've used noise exposure to demonstrate this. And, in this case, unlike cisplatin, where all the DNA repair proteins migrate from the cytoplasm to the nucleus, with noise exposure the DNA repair proteins migrate out of the nucleus into the cytoplasm. So, here's some data showing that. So, this photomicrograph shows a field of spiral ganglion neurons. And notice you can see that the proteins are now accumulated, they have accumulated in the cytoplasm, and the nucleus is now void of proteins. And this is typically what happens with noise exposure. So, it's as if the neurons are protecting their cytoplasmic DNA and neglecting their nuclear DNA. And it's easy enough to quantify that and that's what we've done here. This figure just shows that quantification. So, one of the things that we were particularly not impressed with this herding response and we questioned whether or not this is the best population response. Could there be a better way for cells to respond to stress?

One of the things that, there are two things, really, that forces us to question this response. So, remember that with cisplatin, what's happening is all the DNA repair proteins are migrating to the nucleus. So if all your forces have migrated to the nucleus then who is defending the DNA that exists in the cytoplasm? Because remember, the DNA is always under stress. Right? Even under normal conditions. So, it just doesn't seem like an efficient strategy to mobilize all your forces in one area. All right. The other thing that makes us think that that's not a good strategy is because we have developed assays where we can trace the cisplatin molecule, no matter where it is in a cell. So, whether it's bound to the cell membrane, or it's bound in the cytoplasm of a cell, or it's bound to DNA. No matter where that molecule is, we can trace it within a cell. And, here, we've shown that. So, this is a field of spiral ganglion neurons, and where you see these dense, black, metallic reaction products, that represents a platinum molecule. And what we learn from that is that cisplatin is found in the nucleus

and it also accumulates in the cytoplasm. So, the strategy of sending all your forces to the nucleus, to protect the nucleus, doesn't seem to make sense to us because you're gonna have damage to DNA in the cytoplasm and you're not providing protection in that area. The other thing that makes us believe that this herding response for the majority of cells responding one way is not an effective strategy also comes from noise exposure. So remember, noise exposure what's happening is the repair proteins are abandoning the nucleus to protect the DNA in the cytoplasm. Well, the problem we have with that is then the nucleus then is left undefended because there is endogenous stress, normal cellular stress that's naturally occurring in the nucleus. So, if everybody's outside the nucleus, who's providing protection? Not only that, but our experiments have demonstrated that noise exposure results in DNA damage. So, here is the outline of a spiral ganglion neuron. And where you see these punctate brown deposits, that represents broken DNA, or damaged DNA, within the nucleus of a spiral ganglion cell. 'Kay? So, if this damage is in the nucleus, it doesn't make sense for the cells to respond by relocating their proteins in the cytoplasm. 'Kay?

And there's another reason why we think this herding response is not an efficient response. So, we can go back to microbiology and look at bacterial cells and get reeducated about this. If you take a colony of bacterial cells and you expose them to a stress that's unfamiliar to the cells then if the cells respond in this herding way, in other words, the way that spiral ganglion neurons respond where the majority of the cells respond in a single way, either send in all your proteins to the nucleus or send in all your proteins to the cytoplasm. Then, what happens with bacterial colony, if they do that, is that they entire colony dies. All right. So, but if you take a given colony of bacteria and you expose them to an unfamiliar stress and that colony exhibits a heterogeneous response, where some cells responding one way, other cells respond another way, and other cells yet respond in a different way, then what you do is you increase the probability that some member of that colony will survive. 'Kay? So we think that this is a more effective response. A heterogeneous response might be a more effective response than this herding homogeneous response. 'Kay? The problem

is, would it be possible for us to entice spiral ganglion neurons to exhibit a heterogeneous response instead of their natural herding response? How would one go about doing that? And if we did that, would that lead to preservation of spiral ganglion neurons and their function? 'Kay? So, that's a particular line of question that we are interested in. So, we've tried many ways to do this and we've basically failed most of the time. Here is one way that seems to be effective. Here what I'm showing you is a cartoon of a cell. So, here's the cell wall. And within the cell you have the nucleus of the cell. It turns out that some cells have what's called EGFR. EGFR is a receptor that lives on the membrane of some cells. What's important about this EGFR molecule is that it can regulate various DNA repair proteins. And the DNA repair proteins are in brown, or light brown in this cartoon. And it can regulate the distribution of DNA repair proteins and the expression of DNA repair proteins.

So one of the first things we wanted to do was look to see if spiral ganglion neurons could possibly express EGFR and DNA repair proteins. And indeed, in our experiments what we found is that spiral ganglion neurons could express both, and they could express both at the same time. So here is a field of spiral ganglion neurons. And in green is a DNA repair protein called XPA. Here is the same spiral ganglion neurons, and in red is the EGFR protein. And when you merge these two together, wherever there is red, wherever red and green interact, or come together, you get yellow. So, the yellow tells us that, indeed, spiral ganglion neurons could express both EGFR and DNA repair proteins. EGFR and DNA repair proteins. The picture is actually more complicated than that because there are cells that, or spiral ganglion neurons that only express EGFR, and there appears to be spiral ganglion neurons that only express DNA repair proteins. And there are also spiral ganglion neurons that tend to not express either of those proteins. So it seems to be a complicated picture. But nonetheless, we got an answer to our question, that, indeed, spiral ganglion neurons could express EGFR and XPA. So, if spiral ganglion neurons could express both, could we then manipulate EGFR so that we could control the location of DNA repair proteins? And one strategy that seems to be effective is we used this small molecular molecule,

called CAE to stimulate EGFR so that we could control these DNA repair proteins. And, when we do that, what we find are some interesting results. So first of all, here is a field of spiral ganglion neurons. So, this is a single neuron here. This is another neuron there. And when we use CAEs, we see that we can control, we see that we can have a situation where the neurons express the DNA repair proteins throughout the entire cell. Right, so wherever you see brown, these brown reaction products, that is DNA repair proteins. And it's easy enough to quantify that so we can do a line scan across the cell and when we do that we can measure the level of these DNA repair proteins. 'Kay? Another outcome that we see when we treat spiral ganglion neurons with CAE is that we do see that they can also exhibit a cytoplasmic response. 'Kay?

And there's another response where the neurons respond with a nuclear reaction. And then there's a fourth response, that we call perinuclear, where the neurons respond by localizing their proteins throughout the cell membrane, or the nuclear envelope, excuse me. So, we think that this is a more heterogeneous response, as opposed to only responding in one way, you have some cells within the ganglion that respond in this diffused way. Some cells that respond in a cytoplasmic way, a nuclear way, and a perinuclear way. We think that this heterogeneous response then increases the probability that some cells, some neurons, will survive a given stress. It's easy enough to quantify this. So we've done that, so in noise exposure, as usual, we find the prominent cytoplasmic response. And when we treat spiral ganglion neurons with CAEs, no particular response is more dominant. All these responses tend to be prolific, or present. So, typically when we do that, most clinicians ask us, "So what, is that physiologically relevant?" And indeed, what we find is that there is some evidence that this might be physiologically relevant because when we measure compound action potentials from the cells, after noise exposure, what we find is that when we induce this heterogeneous response among spiral ganglion neurons we get a significant protection from hearing loss. Okay? Now, one of the things that bothers me with this line of research is I don't know if the protection is due to the fact that CAEs might be improving hair cells, or they are improving the spiral ganglion neurons. In other words,

what proportion of this protection, this reduction in hearing loss, is due to the fact that the hair cells might be involved. So, one of the experiments that we tried to do was to damage the hair cells first, then treat with, and damage them so that we can get them out of the way 'cause we were focusing on spiral ganglion neurons. So, damage the hair cells first and then look to see if the spiral ganglion neurons were protected. And we've done that, and I'm just gonna kinda hurry up 'cause we're already running out of time. We've done that and what we find is that when we remove the normal function of hair cells, we can still get a significant protection by inducing this heterogeneous response among spiral ganglion neurons. 'Kay? So this result isn't, this research, or this line of research isn't complete, we still need a lot more work to do, but it is definitely encouraging. So, the fourth line of research, and I'm speeding a little bit here because we could potentially run out of time. The fourth line of research has to do with data that suggests that if we are able to reduce the level of DNA damage, we can prevent hearing loss, or limit the progression of hearing loss. And on the flip side of that, if we are able to increase the expression of DNA repair genes, we could also limit the progression of hearing loss.

So let's take a look at some of that data. So, this is a photomicrograph, or radial section of the organ of Corti, showing you inner hair cells and outer hair cells. And, where you see these black reaction products, that is the expression of DNA repair proteins. Now, this data suggests to us that under normal conditions hair cells are expressing high levels of DNA repair proteins. And what's interesting about that also is that when we look at the level of DNA damage within hair cells, we see that under normal conditions there seems to be heavy amounts of DNA damage. So these brown reaction products, they label or identify damage within the outer hair cells. So, under normal conditions there is DNA being damaged, and as a result there is a heavy load of DNA repair enzymes within hair cells. So therefore, there's a balance. There's a balance between damage, the level of damage, and the level of repair that's happening within a given hair cell. Now, we know from the DNA repair field, that anything that biases the load of DNA damage, in other words, anything that increases DNA damage

above baseline will lead to cell death. Right? So, any type of chemical stress, or physical stress that leads to more DNA damage can lead to cell death. Now, we think that with noise exposure what's happening is that noise causes an increase in DNA damage and that can lead to the death of hair cells. Therefore, if we were to somehow reduce the level of DNA damage then we would find less cell death and that's actually exactly what we've done. So, in this panel here, here on this Y axis you have the level of DNA damage. And, what we can see here is that noise does cause an increase in DNA damage. And when we look at the functional significance of this DNA damage we see that DNA damage is associated with hearing loss, as revealed by otoacoustic emissions. And, when we look at the morphology we see that DNA damage is also associated with a loss of hair cells. So these are a set of cochlear grams showing you dead cells, the amount of dead cells within the cochlea. Now, when we treat with CAEs, what we find is that CAE results in less DNA damage. There is still an increase in DNA damage, but it's less, relative to noise by itself. And when we look at the functional significance of reducing the level of DNA damage, we see that we can actually achieve almost complete recovery of DPOES.

All right, so function tends to recover when we reduce the level of DNA damage. And when we look at the population of dead cells, we do see less dead cells when we reduce the level of DNA damage. 'Kay? So we find all of that to be extremely encouraging. Now, instead of trying to reduce the level of DNA damage, what if we tried to increase the expression of DNA repair genes? And we've actually tried dozens of ways to do this, and I'll share one, we've failed in almost all of them, but I'll share one way that seems to be encouraging. And this is where we've designed a synthetic molecule, called t-oligo, and this t-oligo molecule, we introduced it directly into the cochlea through transtympanic injections. And, we can verify that it's taken up into the tissues of the cochlea. So, we simply harvest the tissues and look inside the tissues to see that they have taken up the construct. What we also do is ensure that as you increase the concentration of the construct, so from one millimole to 10.8 millimole, there's a corresponding increase in the expression of DNA repair genes. So that's very

convincing to us. And we've done this for dozens of DNA repair genes now and the outcome is similar in the sense that as we increase the expression of DNA repair genes, as we increase the concentration of this construct, t-oligo, there is a further increase in the expression of DNA repair genes, 'kay? To further convince ourselves we show that even after exposure, after we've exposed the ear to a traumatic level of noise, we can still achieve a significant increase in the expression of DNA repair genes. Now, usually when we present this to clinicians they'll say, "But, so what, is this relevant?" So, to get a sense of relevance, we've conducted experiments where we take groups of animals and we expose them to loud traumatic noise. Then we take one cochlea from these animals and we treat that cochlea with t-oligo and increase the expression of DNA repair genes, and then measure, in this case we're using ABR, measure auditory function to see if that increase in DNA repair genes has any protection.

And what we see here, so in this panel, this axis is ABR threshold, and at baseline, both the right and left ears of these animals have similar auditory function. But at one week after exposure, the ear that receives t-oligo has a modest shift in hearing. But the opposite ear of the same animal that did not receive the t-oligo construct, has a significant loss of hearing. We've also verified this with otoacoustic emissions, and even with cortical evoked potentials and other measures. So, this is highly encouraging to us and suggests that our initial sense that DNA repair could be a powerful defense in the cochlea, might be a realistic fantasy. So, I see that I'm running out of time, so I would like to just summarize here by saying, so what we've done is we've reviewed four emerging lines of research and each of these research lines by themselves is not that convincing. It's only when you combine them together that they begin to reveal a message, and that message is that DNA repair is an endogenous cochlear defense. And the reason why anyone should care about that is because if we can harness this power, if we can tweak this power, if we can manipulate this power, then we might be able to deploy it in such a way that we can effect clinical outcomes. And I have three

more minutes and I'm gonna end right there by thanking you and take any questions you may have.

- [Christy] Thank you, Dr. Guthrie. That was a wonderful presentation. At this time we're gonna go ahead and open up the floor for any comments or any questions for Dr. Guthrie. We do have a question here from Anne. Anne asks, was it mentioned where, in the cochlea, the enzymes are located?

- [Dr. Guthrie] Yeah, so we find that the enzymes are located in the various tissues of the cochlea, so both the sensory epithelium and the non-sensory epithelium. So, in essence, all the cells seem to have DNA repair proteins.

- [Christy] Thank you, Dr. Guthrie. And thank you, Anne, for your question. We have an additional question here. Which tissue or cells in the cochlea have DNA repair enzymes?

- [Dr. Guthrie] So, it seems like all the tissues and all the cells have DNA repair enzymes. Each tissue and each cell utilize those DNA repair enzymes in different ways. I can see that there's another question. This question says which animal models are you using typically? So, in our experiments we use rats, different strains of rats and we also use mice.

- [Christy] Hi, Dr. Guthrie. It does look like that Patrice has another question. She says cortilymph, endolymph, perilymph, or stria vascularis. And then she said, or all four? Which tissues, or cells in the cochlea?

- [Dr. Guthrie] Oh, so all tissues, all the cells express DNA repair proteins. Now, cortilymph, endolymph, and perilymph aren't tissues, they're fluids in the cochlea. Yeah, so three of those options are not tissues or cells, they are fluids in the cochlea. And then one option is actual tissue.

- [Christy] Excellent. Well, thank you so much. Dr. Guthrie, we really appreciate your time and expertise with that. I think we're gonna go ahead and wrap up the course for today. So, thank you everyone for attending today. Thank you Dr. Guthrie for your time and we hope everyone has a great day.

- [Dr. Guthrie] Thank you so much.