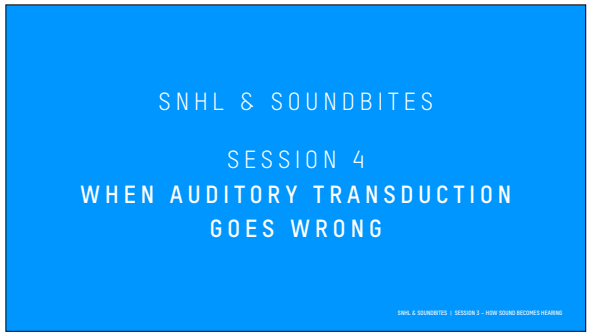
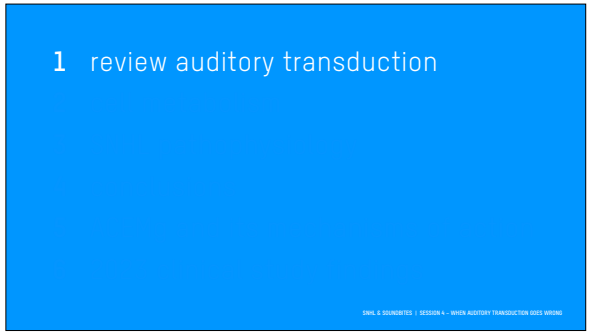


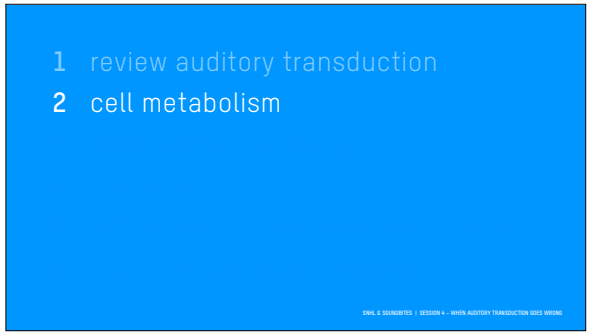
1

Hi, and welcome to Session 4, When Auditory Transduction Goes Wrong. In this session we'll cover the research on cochlear metabolism and metabolic dysfunction and the development of ACEMg.



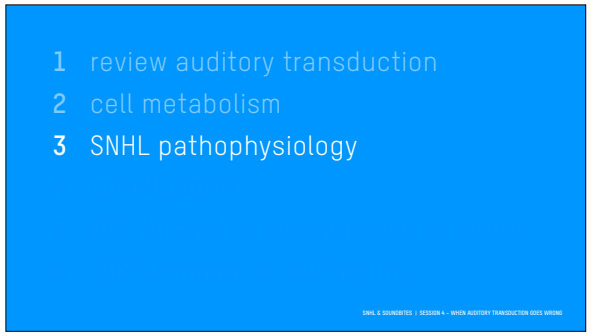
2

I'll start with a quick review of normal auditory transduction and the two take-home messages from Session 3.



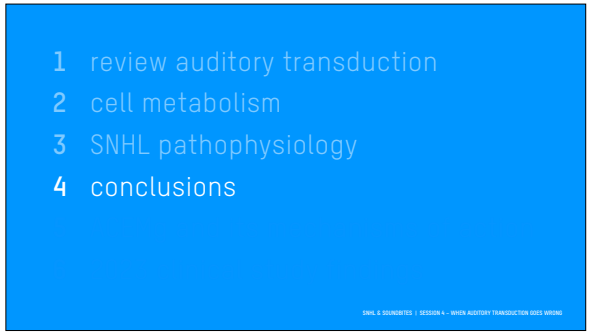
3

Then we'll cover cell metabolism.



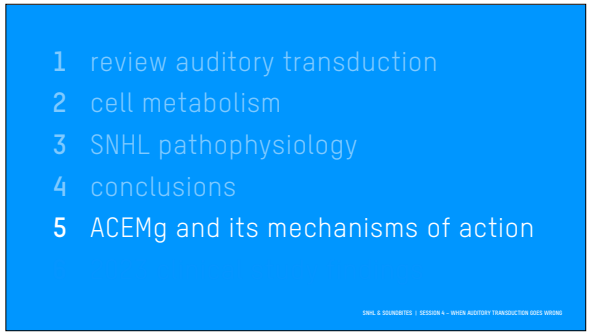
4

Then we'll learn about the root cause of SNHL pathophysiology by reviewing basic research findings from studies conducted in the Miller lab and others.



5

Then I'll summarize the conclusions that provided the roadmap for the research aimed at finding a solution.



6

Next, we'll learn about the ACEMg formula, its mechanisms of action to block the root cause of SNHL, and the findings from the ACEMg animal study published in 2007.

1 review auditory transduction
2 cell metabolism
3 SNHL pathophysiology
4 conclusions
5 ACEMg and its mechanisms of action
6 clinical data

SNHL & SOUNDBITES | SESSION 4 – WHEN AUDITORY TRANSDUCTION GOES WRONG

7

Finally, I'll fast forward sixteen years to summarize real-world clinical data demonstrating that ACEMg, Soundbites, preserves or improves hearing.

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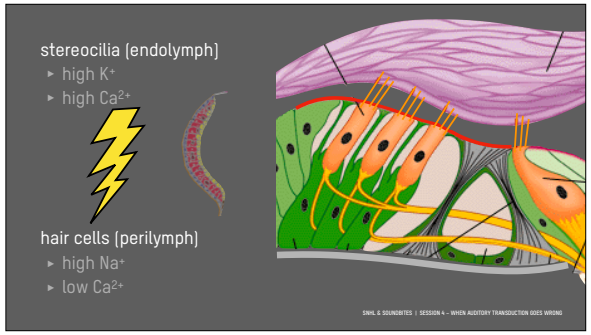
1 review auditory transduction

SNHL & SOUNDBITES | SESSION 4 – WHEN AUDITORY TRANSDUCTION GOES WRONG

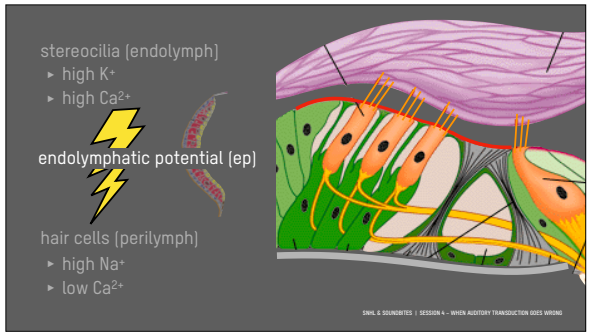
8

Let's quickly recap Session 3.



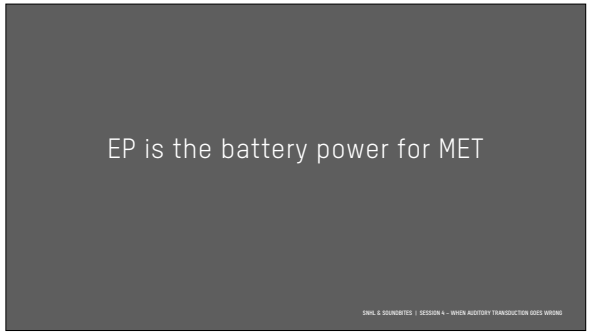
9

The difference in the ionic composition of the perilymph and endolymph creates a large difference in electrical voltage between cochlear compartments.



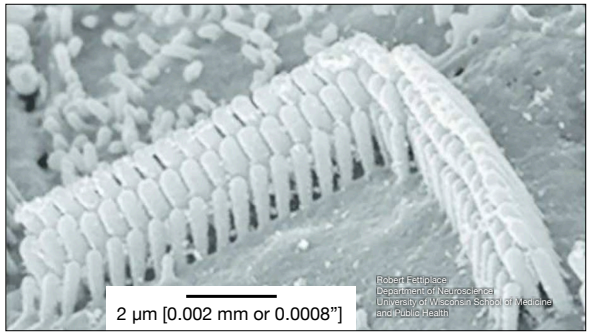
10

Electrical voltage of the endolymph in the organ of Corti is expressed as endolymphatic potential, or EP.



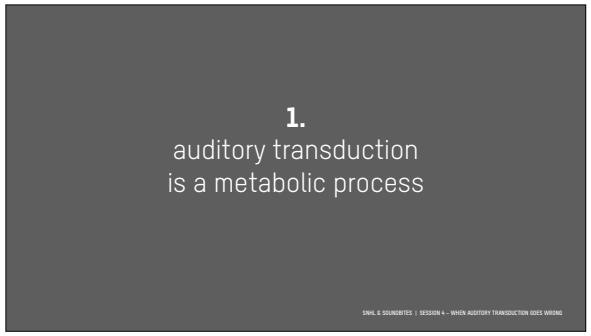
11

EP is the battery power for MET.



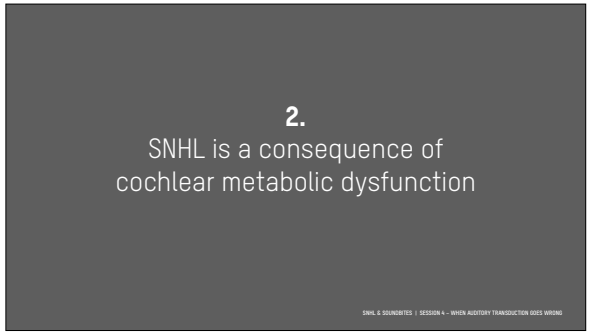
12

Outer hair cells convert sound waves into their equivalent electrical impulses and separate them into their component frequencies in the MET process.



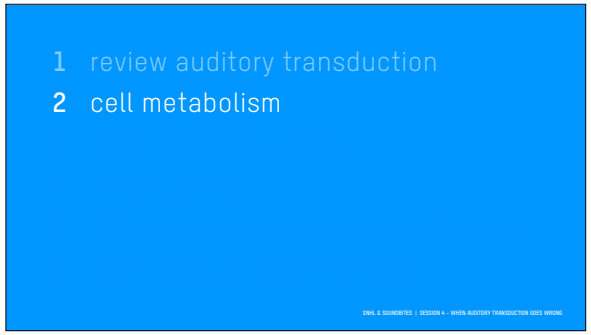
13

Session 3 ended with two take-home messages. First, auditory transduction is a metabolic process.



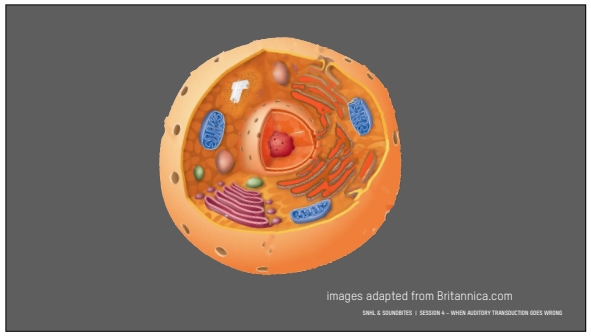
14

Second, the research that resulted in ACEMg focused on cochlear metabolism because SNHL is a consequence of cochlear metabolic dysfunction.



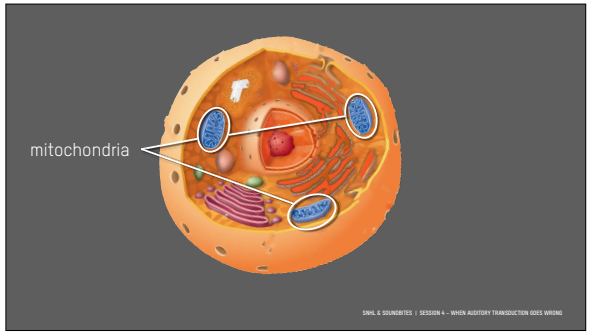
15

Now let’s take a few minutes to review normal cell metabolism.



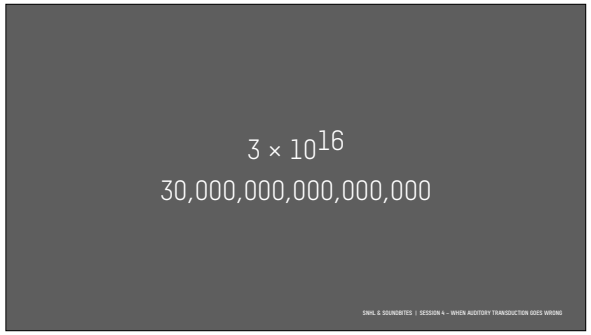
16

Adult humans have about 30 trillion cells of about 200 different types.



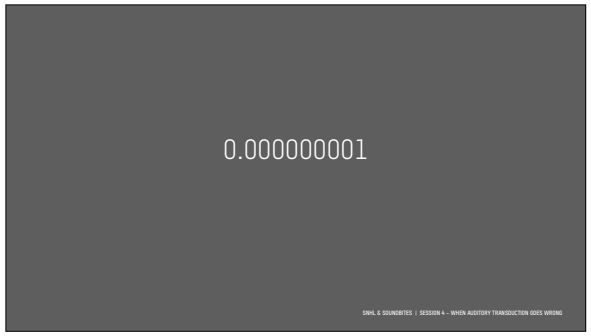
17

Energy generation in human cells, including cochlear cells, mostly happens in mitochondria, the so-called power plants of the cell.



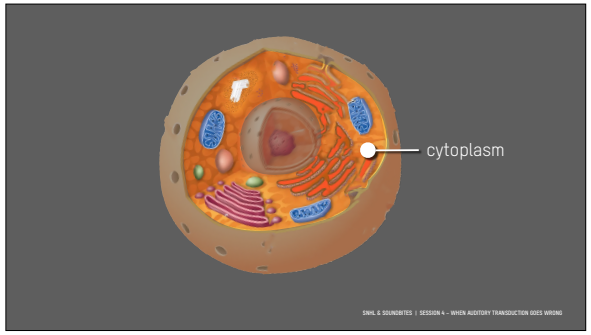
18

A human cell can contain more than 1,000 mitochondria, which means there are more than thirty quadrillion mitochondria in an adult human.



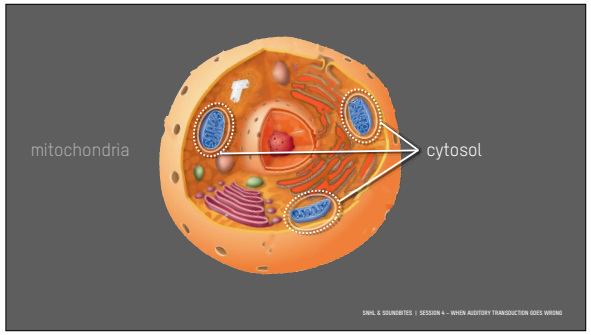
19

Cochlear cells account for about one trillionth of the total cells in a typical adult human. The number of mitochondria in inner ear hair cells is currently unknown.



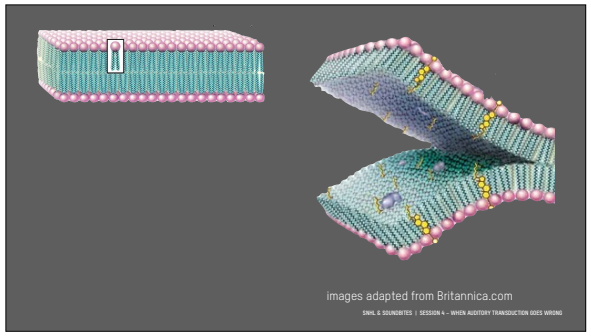
20

Mitochondria and other cell organelles are in the cytoplasm, a water-based fluid.



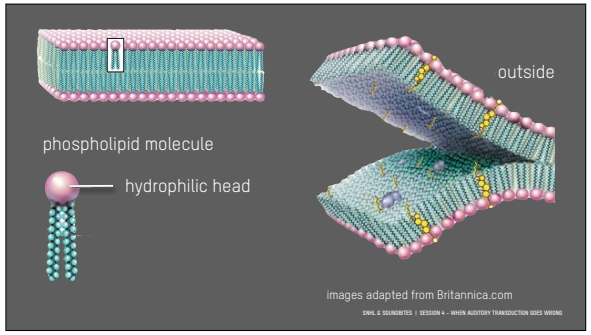
21

Mitochondria are surrounded by cytosol, which is a also a fluid sub-compartment of the cytoplasm.



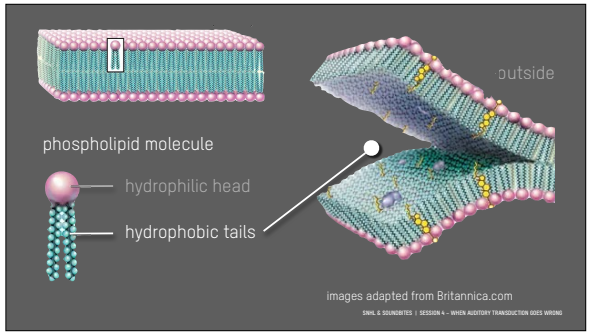
22

The mitochondria and other organelles are bounded by a phospholipid bilayer membrane, separating it from the cytosol and the cytoplasm.



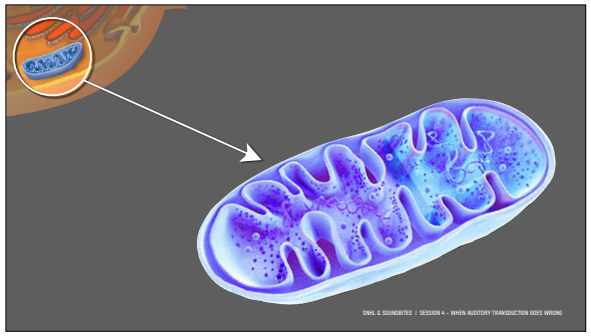
23

The membrane is comprised of lipids, which are fatty acid molecules that have a hydrophilic end, or head, that's soluble in the watery cytosol and cytoplasm outside the membrane.



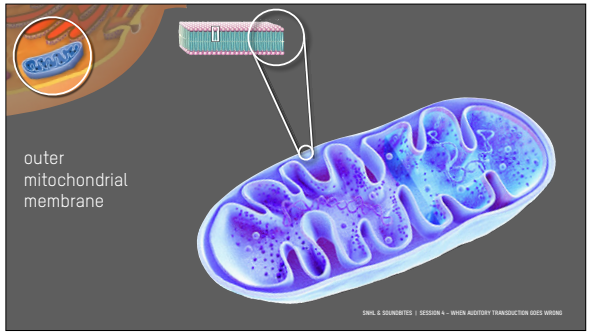
24

The hydrophobic ends, or tails, are insoluble in water, in the interior of the membrane.



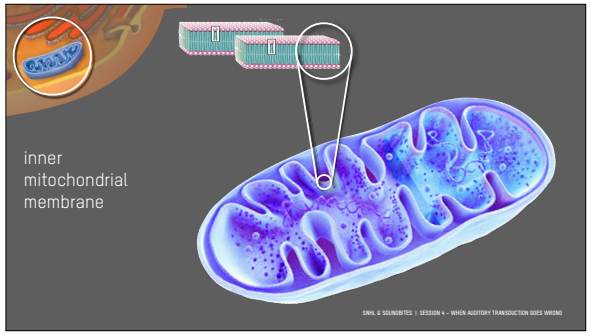
25

Let’s zoom in on a mitochondrion.



26

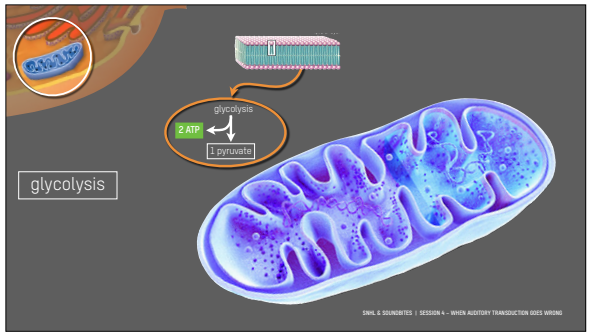
The outer mitochondrial membrane is permeable to a variety of large and small molecules.



27

The inner mitochondrial membrane is completely impermeable and separates the intermembrane space from the area within the mitochondrion which contains the mitochondrial DNA.

The inner membrane contains the transport proteins to enable energy production.



28

The three-stage metabolic process starts in the cytosol surrounding the mitochondrion in a ten-step series of chemical reactions called glycolysis.

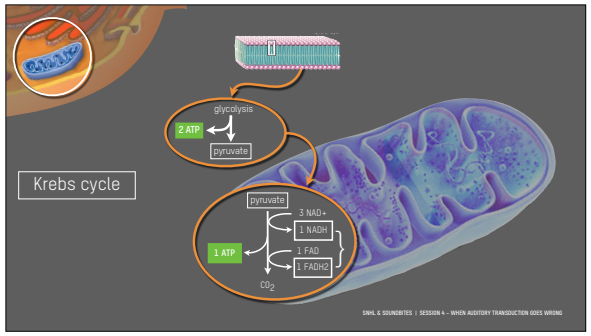
Glycolysis breaks down one molecule of glucose – a simple sugar – into one molecule of pyruvate, the salt form of pyruvic acid.

Adenosine diphosphate, ADP, is also present in the cytosol. ADP is the low-energy molecule from which adenosine triphosphate, or ATP is produced.

**Pyruvate diffuses across the outer mitochondrial membrane and the intermembrane space. Independently, ADP also diffuses across the outer membrane and the intermembrane space. **

Only a few seconds worth of ATP is stored. Mitochondria make ATP constantly.

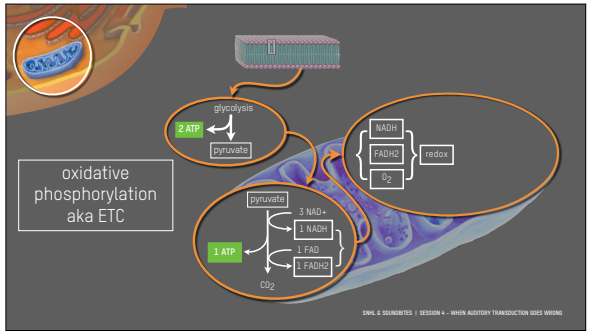
Pulitzer Prize science writer Jonathan Weiner describes ATP this way – Without ATP it would be useless for us to breathe air, to drink and to eat….Our bodies would slow down and stop.



29

The second step starts with pyruvate and ADP being actively transported across the inner mitochondrial membrane which starts the Krebs cycle.

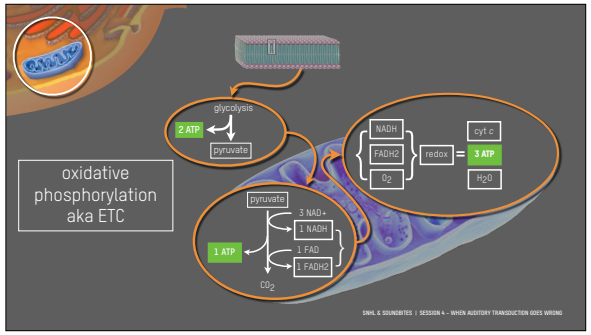
The Krebs cycle is named for the biologist who discovered it in 1937 and was awarded a Nobel prize for it in 1953. Currently it's called the citric acid cycle.



30

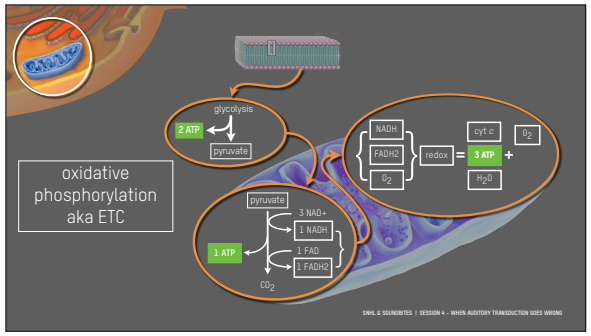
The third and final step is oxidative phosphorylation, sometimes called the electron transport chain, or ETC because of the way it works. The ETC was discovered by Peter Mitchell, who won a Nobel Prize for it in 1978.

Essentially, the ETC is the biochemical battery. It generates ATP and allows it to be actively transported through the inner mitochondrial membrane into the intermembrane space so it can diffuse out to power the rest of the cell.



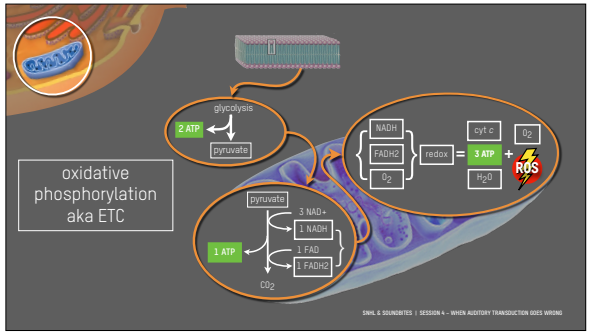
31

The ETC uses cytochromes and enzymes in reversible reduction oxidation reactions called redox to gradually reduce the large amount of available energy in redox cofactor molecules NADH and FADH2 and donate them to oxygen. If you know what those abbreviations stand for you know why I'm using them. And if you don't know, don't worry.



32

Redox reactions also produce molecular oxygen as a byproduct. Molecular oxygen is in the air we breathe, for example.

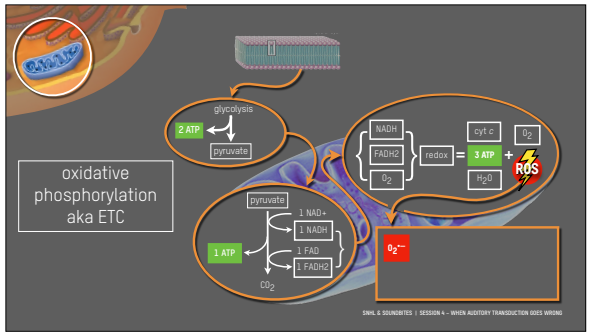


33

Redox reactions also produce atomic oxygen, unstable, highly reactive, partially reduced forms of oxygen called reactive oxygen species, or ROS.

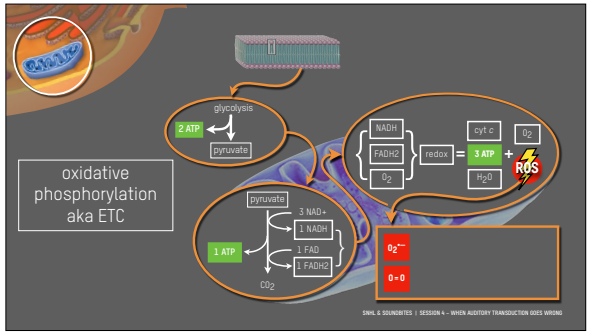
34

ROS is the root source of oxidative stress in cells. ROS is also called free radicals. ROS is our central topic.



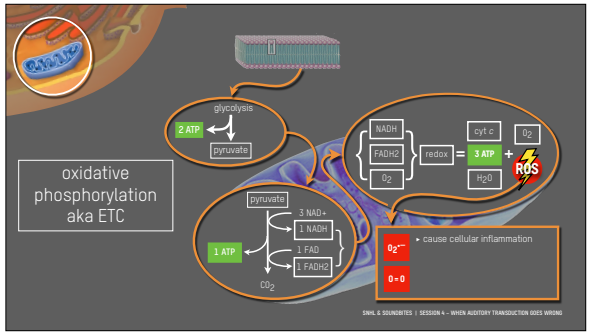
35

ROS is the pathway to free radicals including, for example, superoxide, a one-electron reduction of molecular oxygen…



36

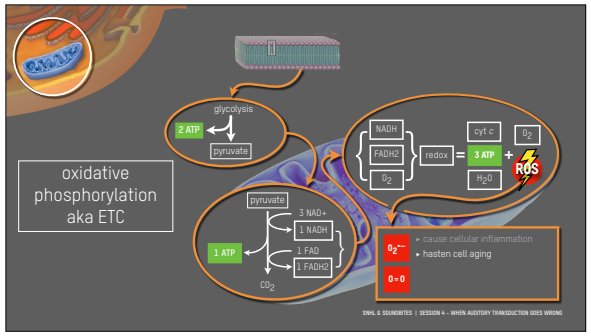
and singlet oxygen, among the most highly reactive forms of atomic oxygen. Among other things, singlet oxygen steals electrons from lipids, which are the fatty acids in cell membranes, leading them to rupture. That process is called lipid peroxidation.



37

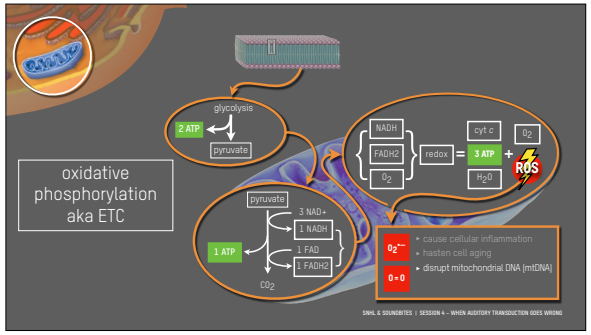
Free radicals switch cell functions on and off by altering proteins.

Excess free radicals cause cellular inflammation…



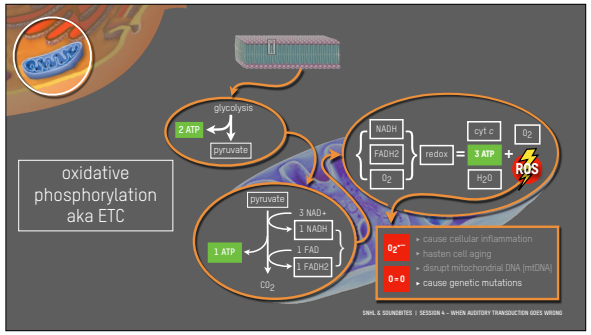
38

hasten cell aging…



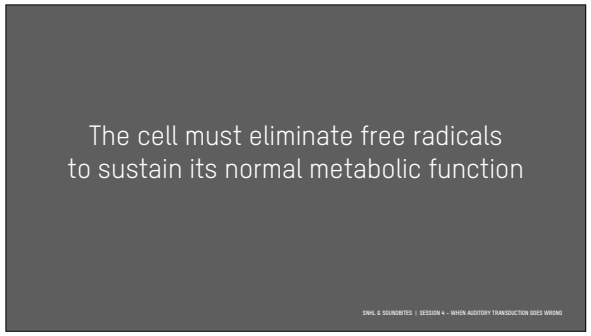
39

disrupt mitochondrial DNA…



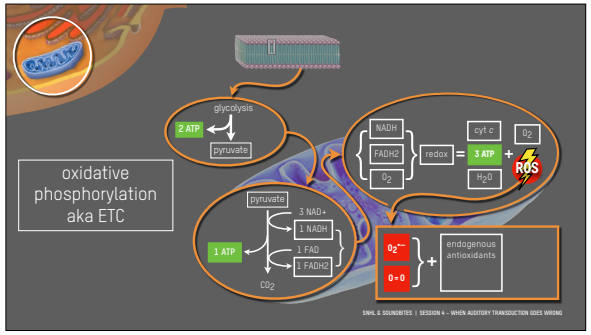
40

and cause genetic mutations.



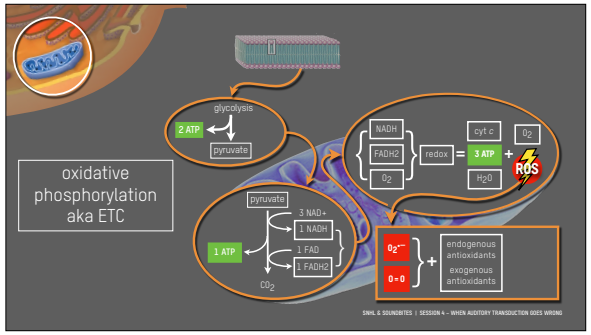
41

The cell must eliminate free radicals to sustain its normal metabolic function.



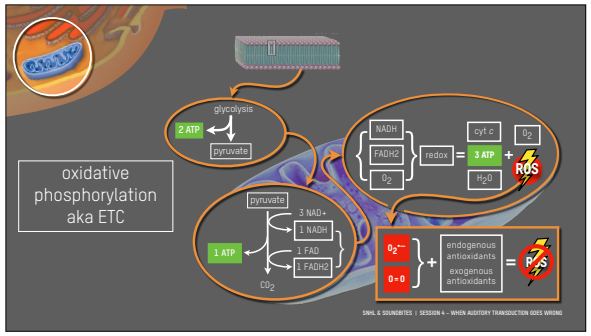
42

The job of eliminating free radicals is done by the cell's antioxidant system, comprised of a variety of endogenous antioxidants – antioxidants of internal origin, like glutathione…



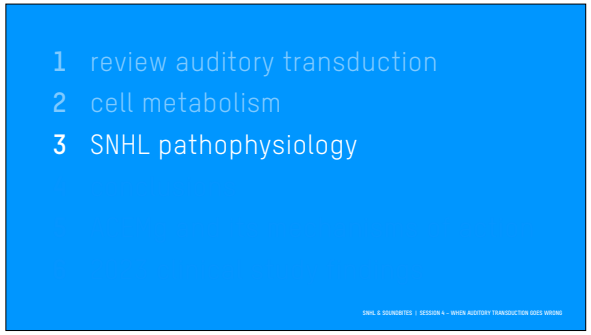
43

and exogenous antioxidants, which are antioxidants having an external origin, the vitamins and minerals we consume, collectively called micronutrients.



44

The antioxidant system scavenges and neutralizes free radicals, preserving normal cell metabolism by eliminating excess ROS as a potential source of oxidative stress.

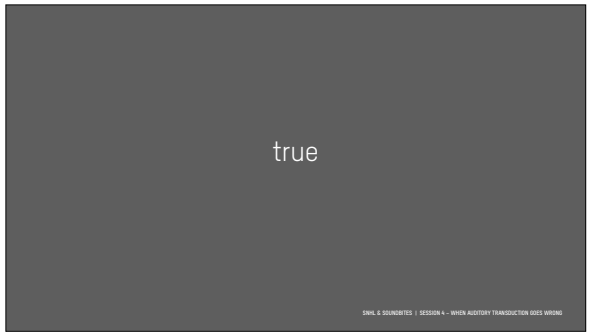


45

Now let's learn what happens when things go wrong in inner ear metabolism, when cells cannot neutralize excess ROS. This section summarizes about sixteen years of basic research in Dr. Miller's lab and others.

46

When Dr. Miller started lab research in 1987, medical schools were teaching that hearing loss was caused by damage to the physical structures of the ear.

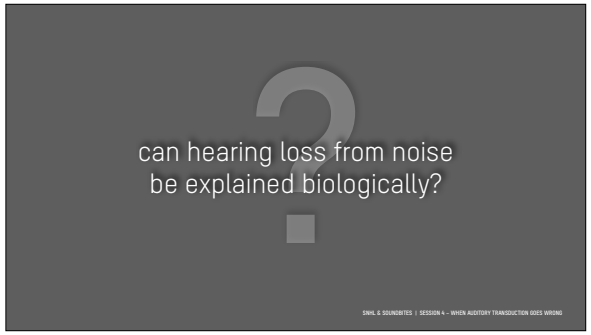


47

That’s true.

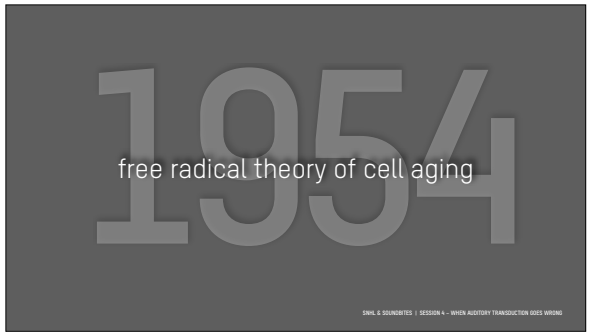
48

Explosion trauma can break the ear. But that explanation doesn’t account for the role of inner ear biology in hearing loss.



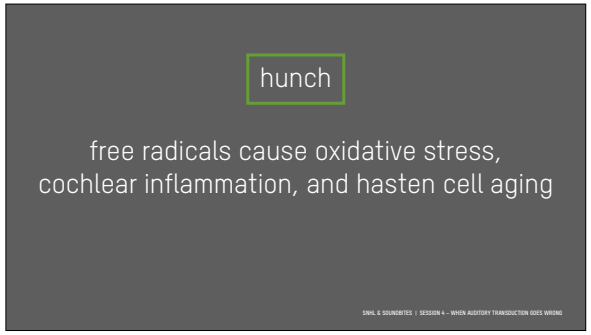
49

As a biochemist, Dr. Miller asked if hearing loss from noise could be explained biologically.



50

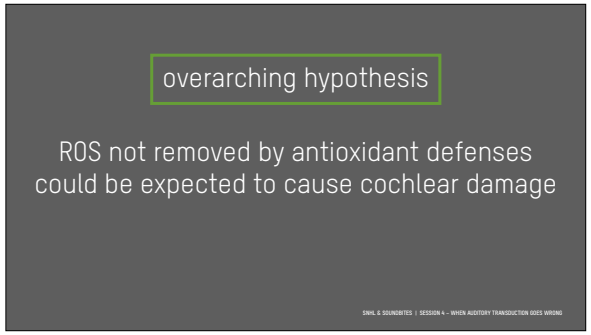
The aging process had been recognized as the major risk factor for disease and death since the free radical theory of cell aging was postulated as an overarching biological concept in 1954.



51

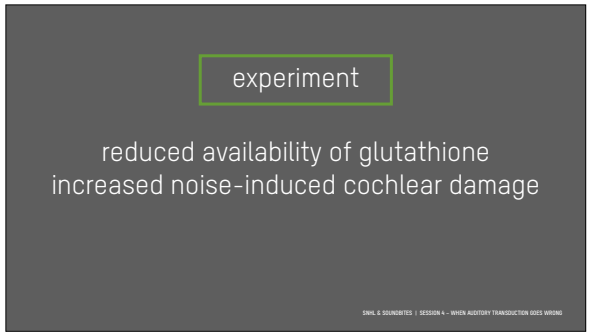
Dr. Miller wondered if the free radical theory of cell aging could be applied to the inner ear.

That hunch was ultimately confirmed by many labs worldwide. The scholarship in this arena is ongoing, and includes many hundreds of articles.



52

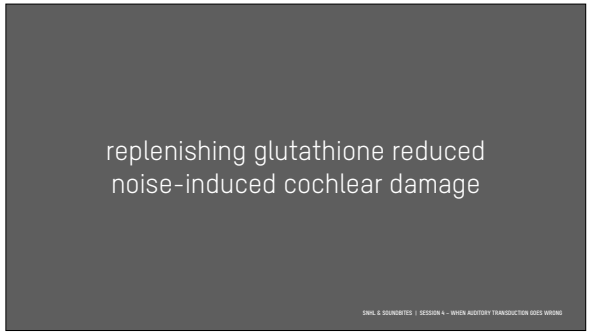
If the free radical theory was accurate, ROS not removed by antioxidant defenses could be expected to damage sensory cells of the cochlea, and that damage may be a potential mechanism of hearing loss from acoustic overstimulation – noise.



53

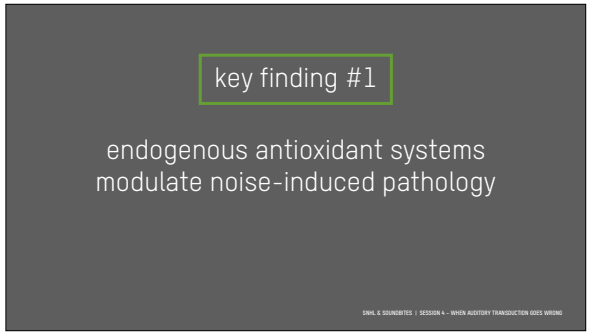
The endogenous antioxidant glutathione was studied to test the hypothesis.

The study found that reducing availability of the antioxidant glutathione did indeed increase noise-induced damage in the cochlea.



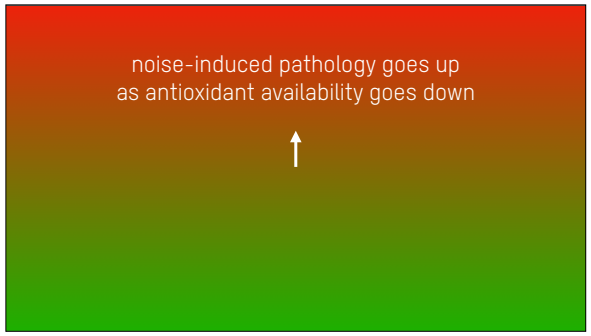
54

Conversely, replenishing glutathione reduced noise-induced cochlear damage.



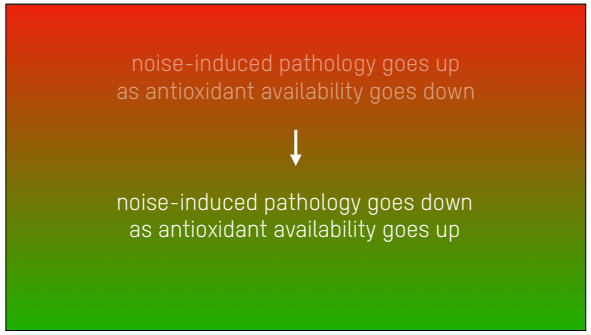
55

The study demonstrated a key finding that endogenous antioxidant systems in cochlear cells modulate noise-induced pathology.



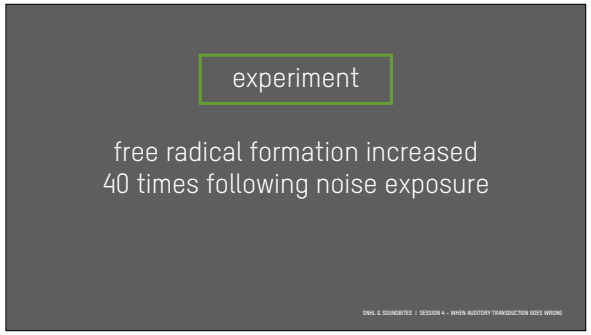
56

This means noise-induced pathology goes up as antioxidant availability in cochlear cells goes down.



57

And it works the other way. Noise-induced pathology goes down as antioxidant availability in cochlear cells goes up.

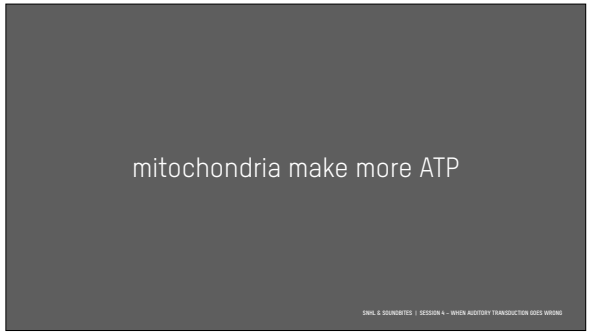


58

Another study quantified the surprising finding that free radical formation in the cochlea increased by 40 times following noise exposure.

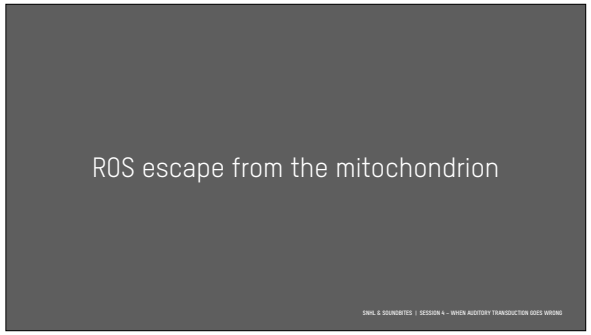
59

Noise increases cochlear metabolism.



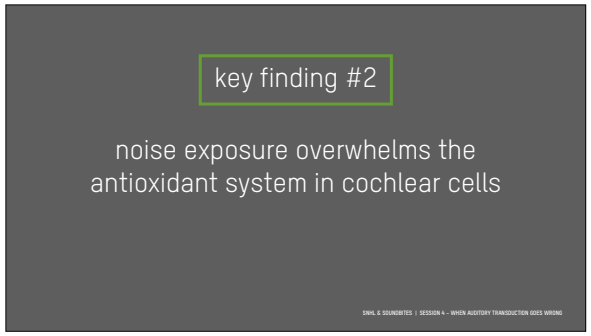
60

Mitochondria produce more ATP.



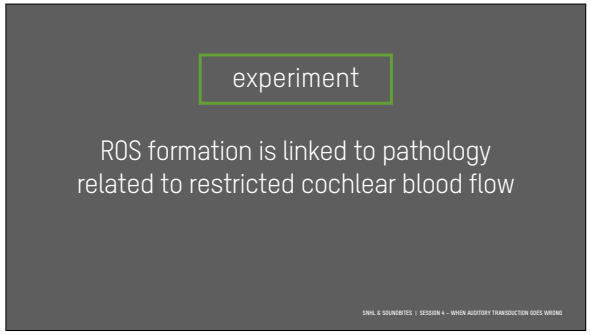
61

ROS leak out from the mitochondrion and initiate cell damage. Recall mitochondrial membranes are constructed to prevent that, so this is abnormal.



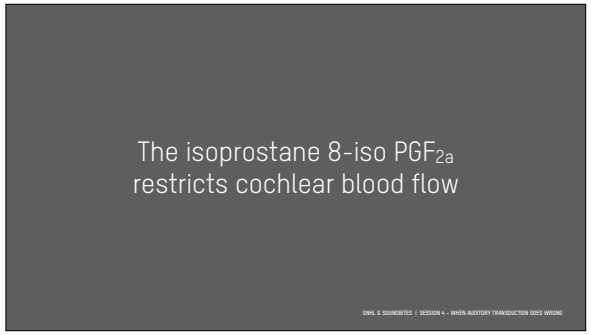
62

So the second key finding was that the vast amount of excess free radicals generated by noise exposure overwhelms the antioxidant system in cochlear cells.



63

A 2003 study demonstrated ROS formation is linked to pathology related to restricted cochlear blood flow.

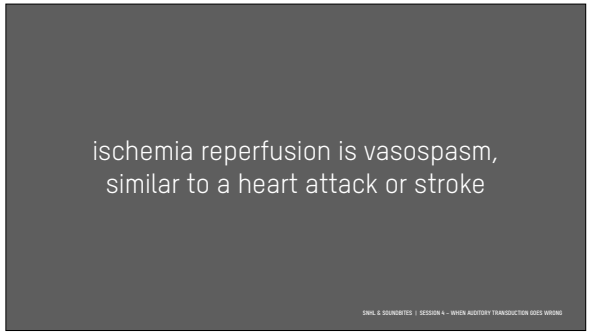


64

The study revealed that the isoprostane 8-iso prostaglandin F_{2a} restricts cochlear blood flow in response to noise exposure.

65

blood supply ‘overshoot’, called reperfusion, follows restricted blood flow, called ischemia.

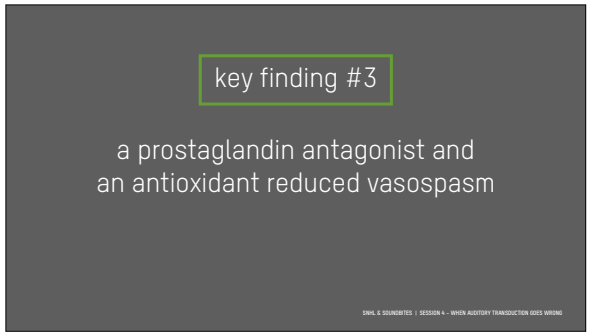


66

Ischemia reperfusion injury, or vasospasm, is similar to a heart attack or stroke in the inner ear.

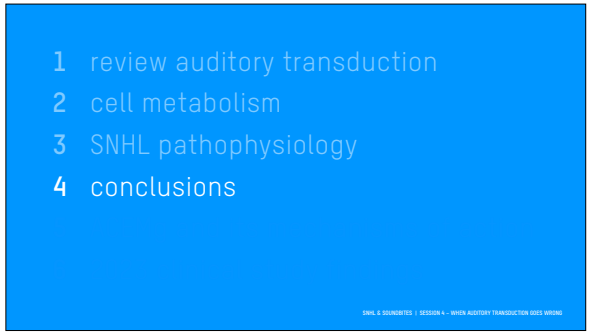
67

In experiments, administering an 8-iso PGF2a antagonist drug and the glutathione antioxidant prior to noise exposure prevented cochlear ischemia reperfusion.



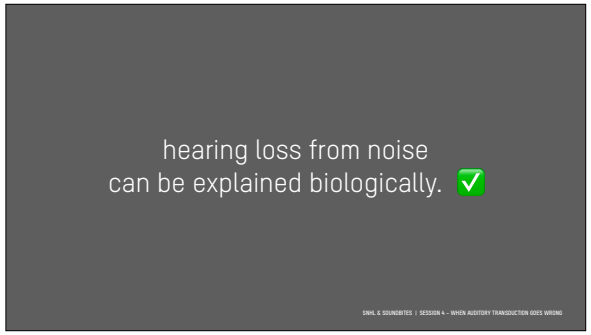
68

So a third key finding was that the combination of a prostaglandin antagonist and an antioxidant reduced vasospasm.



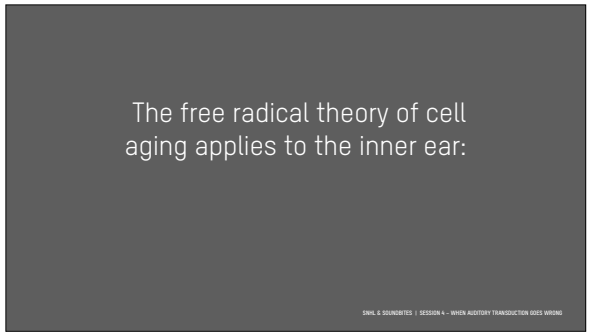
69

Let's summarize the conclusions that provided the scientific rationale and the roadmap for developing therapeutic interventions.



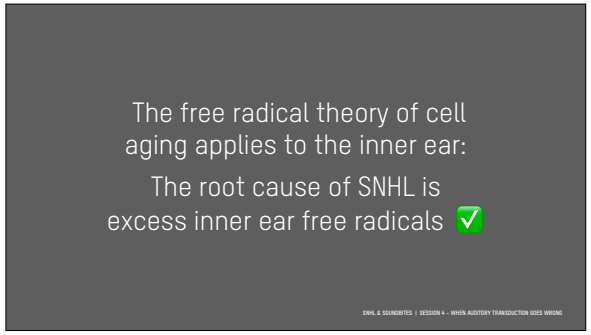
70

By 2003 these studies and many others, ultimately replicated by many labs worldwide, confirmed that hearing loss from noise can be explained biologically.



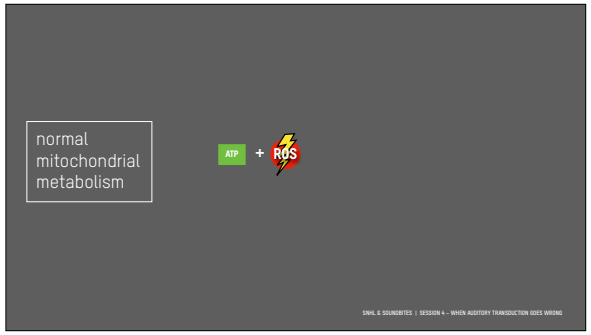
71

Lab findings emphatically validated the hunch that the free radical theory of cell aging applies to the inner ear.



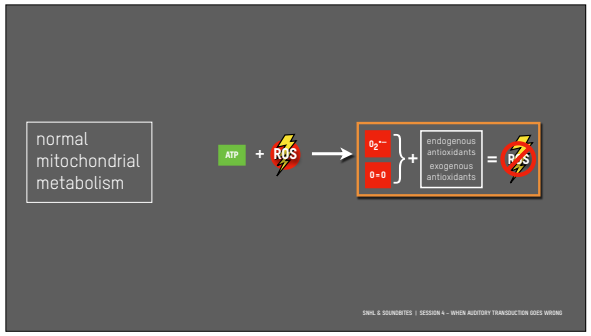
72

The root cause of SNHL is excess inner ear free radicals.



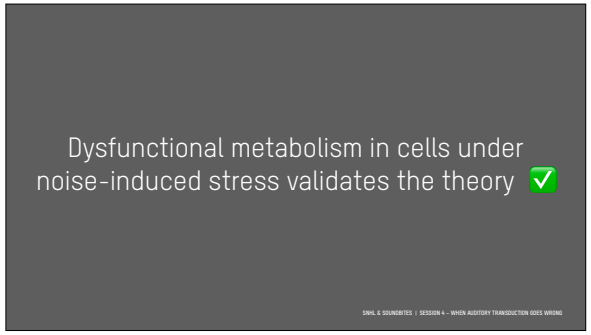
73

Recall normal mitochondrial metabolic function produces ATP, and free radicals form residual ROS.



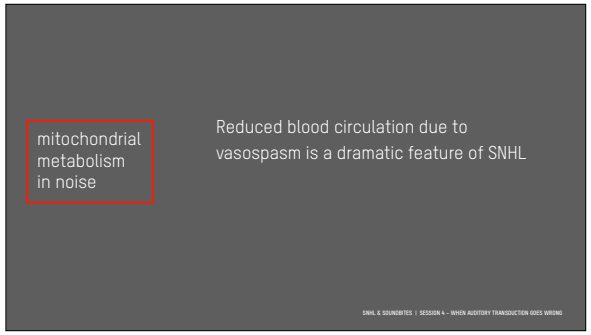
74

Normal metabolic function is preserved by endogenous and exogenous antioxidants in the cells's antioxidant system scavenging and neutralizing excess ROS, reducing a potent source of oxidative stress.



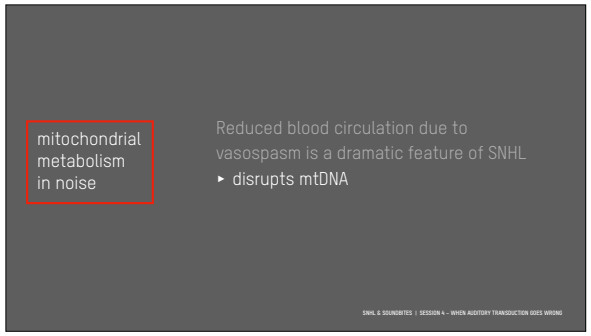
75

Dysfunctional metabolism in cells under noise-induced stress validates the free radical theory of cell aging.



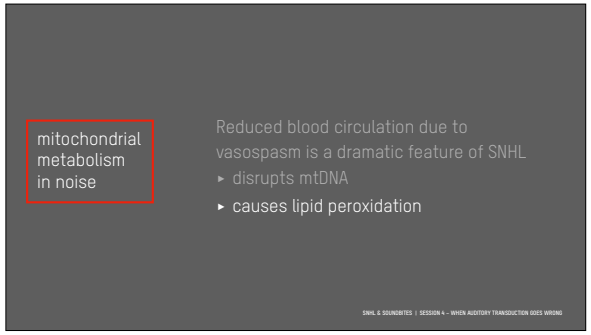
76

Reduced blood circulation due to vasospasm is a dramatic feature of SNHL. Software engineers would call it a bug.



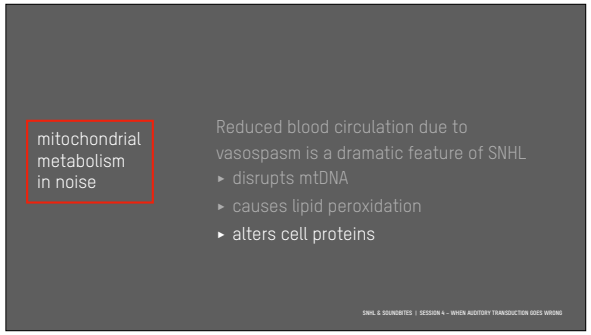
77

Noise creates oxidative stress that disrupts mitochondrial DNA…



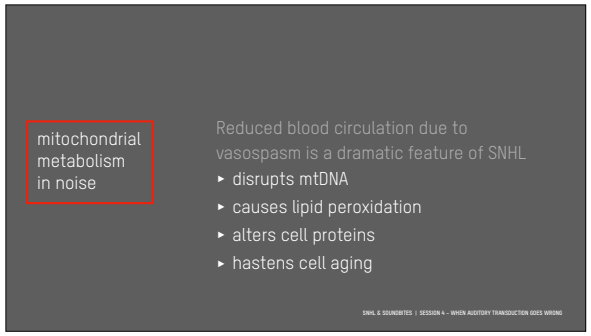
78

causes lipid peroxidation that weakens membranes…



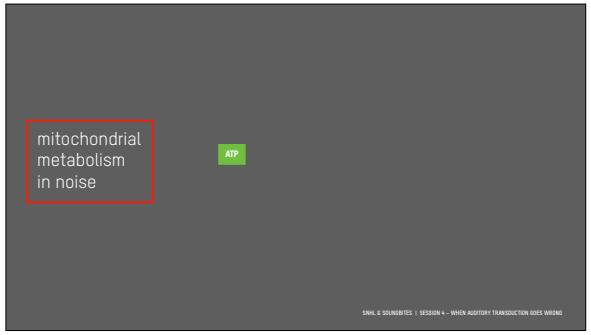
79

alters cell proteins…



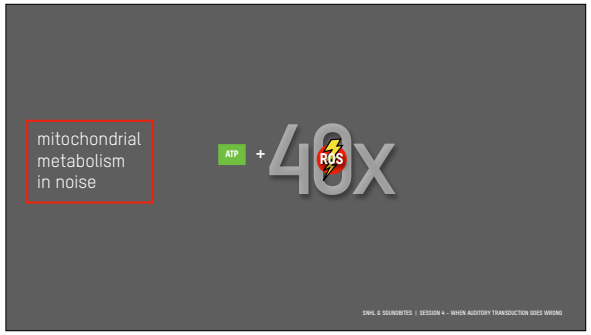
80

and hastens cell aging.



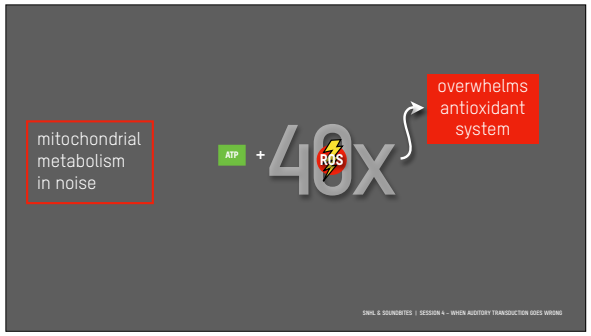
81

Cochlear cell mitochondria increase their metabolic activity to produce more ATP…



82

generating a stunning 40-fold increase in free radicals.



83

The vast influx of ROS overwhelms the capacity of the endogenous antioxidant system to neutralize excess inner ear free radicals including superoxides and singlet oxygen. Excess free radicals remain in the cochlea.

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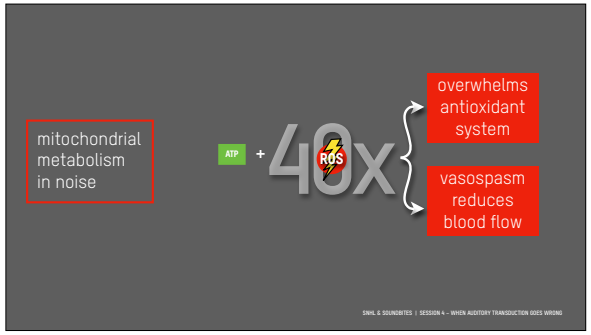

84

And just when the delivery of antioxidants and removal of waste products is most needed, vasospasm due to excess ROS also reduces cochlear blood flow.

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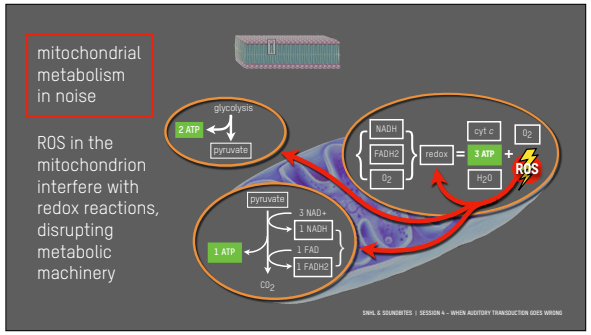
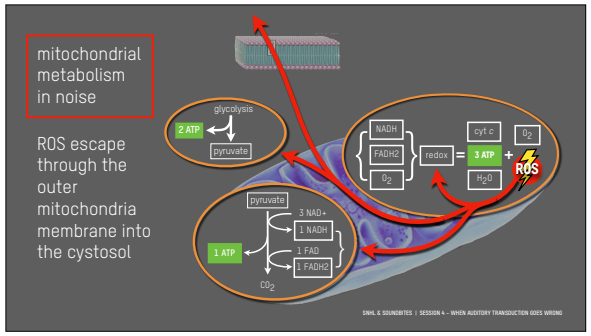


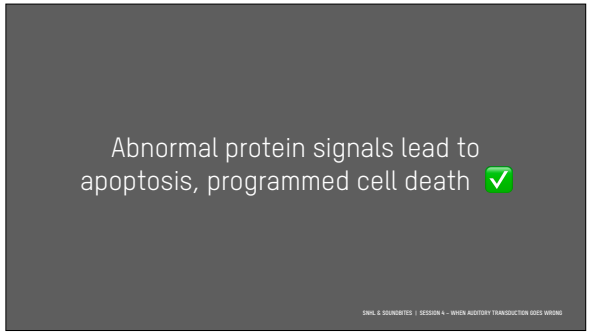
85

Excess free radicals in the cochlea interfere with redox reactions in the electron transport chain, disrupting cochlear metabolic machinery.



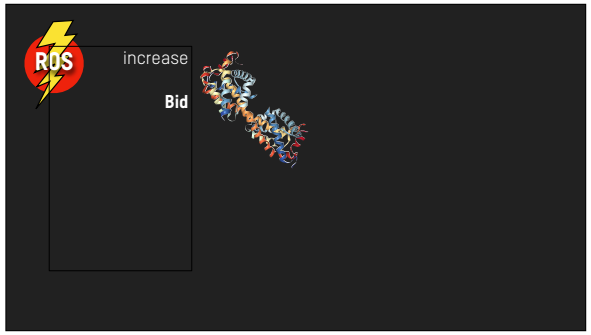
86

Excess free radicals escape through the outer mitochondrial membrane into the cytosol, where they interfere with the function of other organelles.



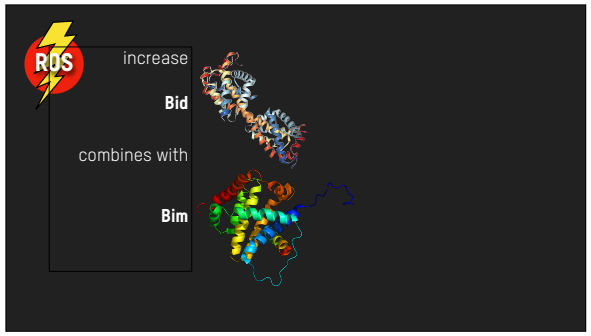
87

Abnormal protein signals initiate a cascade of cellular events leading to apoptosis, alternative programmed cell death pathways.



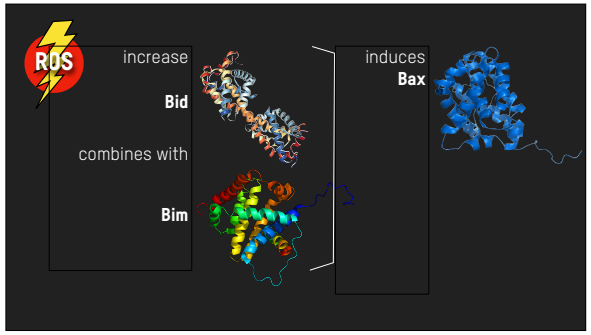
88

Excess free radicals increase formation of the protein BID…



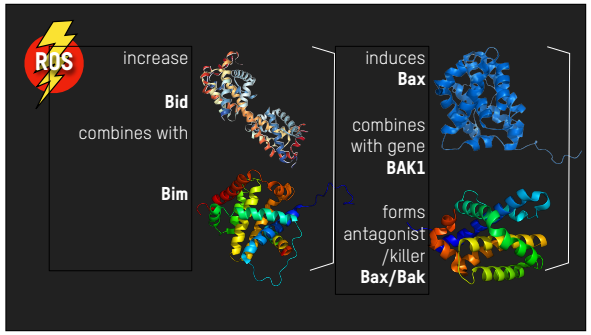
89

which combines with the protein BIM.



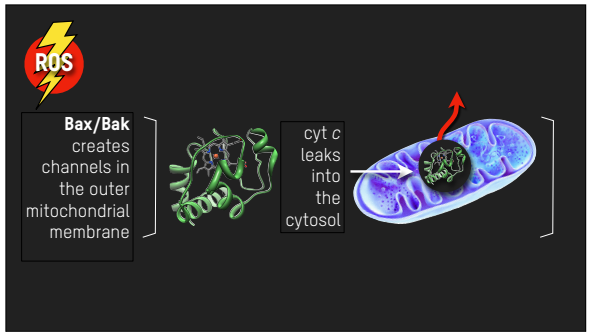
90

Their combination induces formation of the apoptosis regulator protein Bax. Bax is the basis for encoding several different proteins and apoptotic pathways.



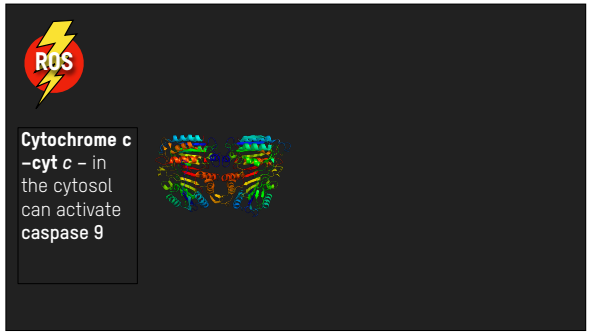
91

Bax can combine with the protein encoded by the BAK1 gene to form the protein BaxBak. BaxBak is called an antagonist/killer protein because it can either prevent or initiate apoptosis.



92

In apoptosis, Bax/Bak creates channels in the inner mitochondrial membrane that allow Cytochrome c, or cyt c, to leak into the cytosol. Recall cytochromes are redox agents that belong inside mitochondria, not in the surrounding cytosol or the cytoplasm, where ATP circulates.



93

Cyt c in the cytosol can activate caspase 9, which immediately starts the caspase cascade that commits the cell to apoptosis

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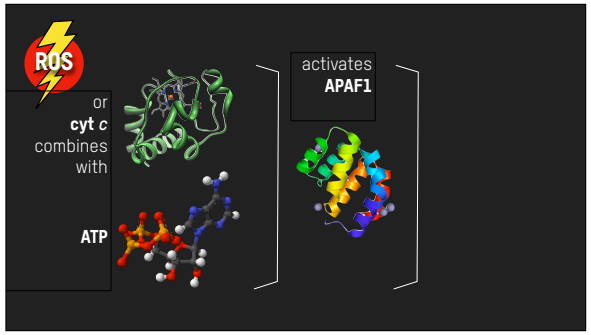
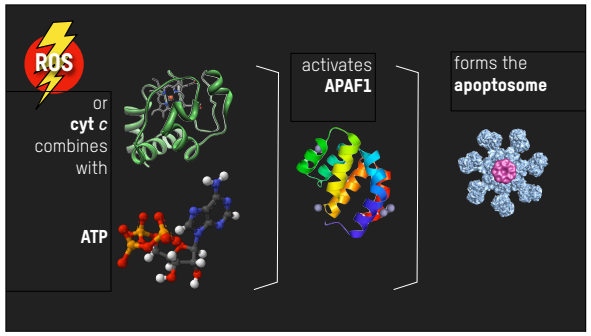


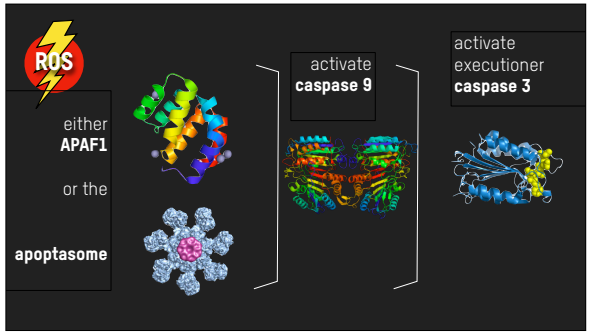
94

or cyt c can combine with ATP, which activates the apoptotic protease activating factor 1, called APAF1…



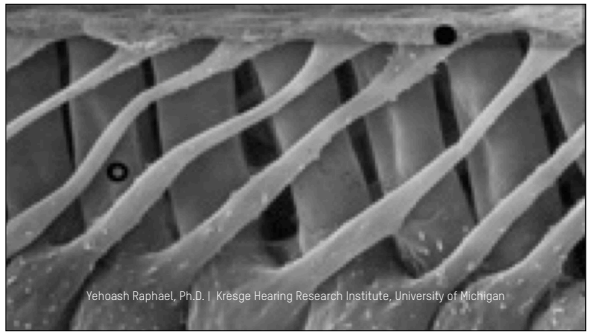
95

and forms the apoptosome.



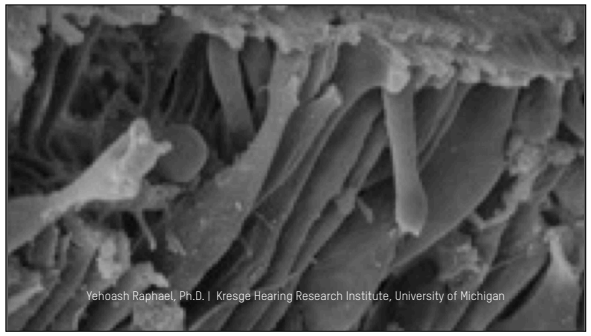
96

Either APAF1 or the apoptosome can activate caspase-9, committing the cell to apoptotic death by stimulating the caspase cascade that ends with the executioner protein caspase 3.



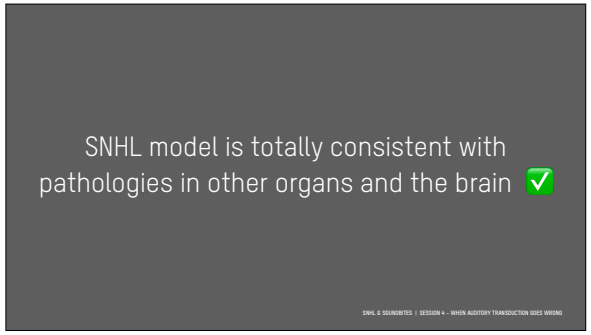
97

This is an electron micrograph of normal, healthy hair cell stereocilia extensions.



98

This electron micrograph demonstrates what can happen to stereocilia extensions after noise exposure when when the sequence of genetic events end in apoptotic cell death.

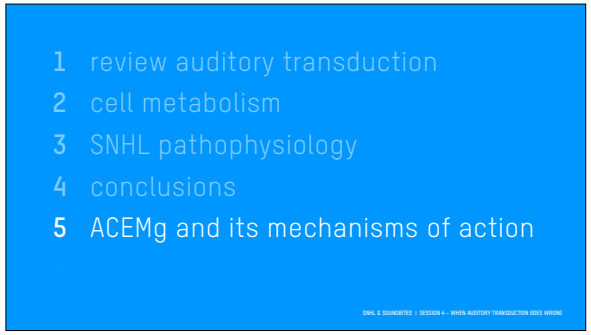


99

The SNHL pathophysiology model is totally consistent with pathologies in other organs and the brain, including peripheral and central nervous system disorders, disorders related to aging, and environmental stress like smoking of any kind.

100

Aside from limiting noise exposure, what can be done about SNHL?

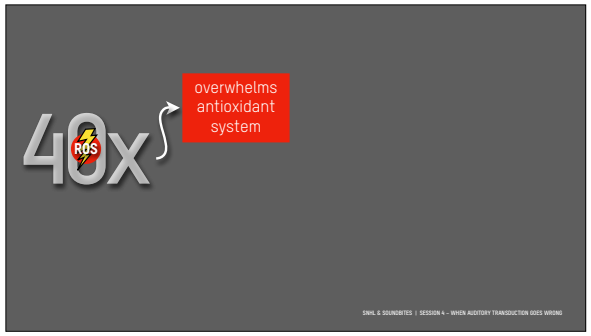


101

Next, we'll learn about the ACEMg formula, its mechanisms of action to block the root cause of SNHL, and the findings from ACEMg animal studies published in 2007.

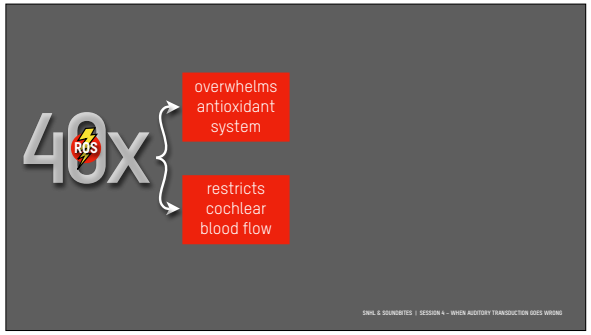
102

Research key finding provided a roadmap to solutions, starting with the finding that noise exposure causes a vast influx of excess inner ear free radicals.



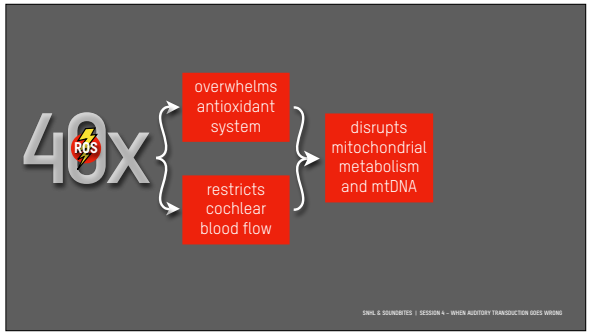
103

The influx overwhelms the antioxidant system, creating oxidative stress.



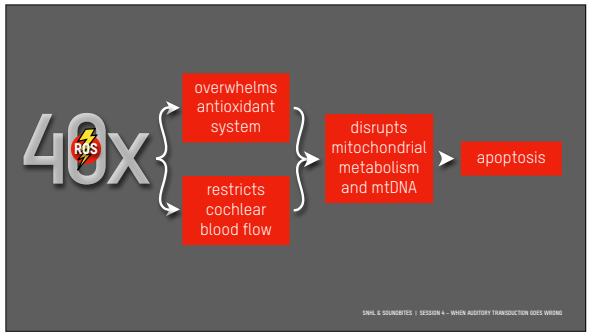
104

It restricts cochlear blood flow, causing ischemia reperfusion injury.



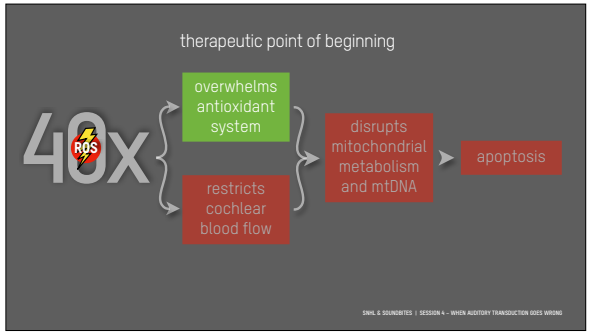
105

and it disrupts mitochondrial metabolism, causing mitochondrial DNA dysfunction.



106

which leads to apoptotic or programmed cell death, experienced as hearing loss.

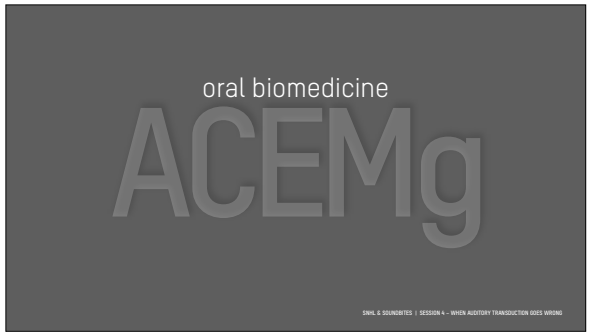


107

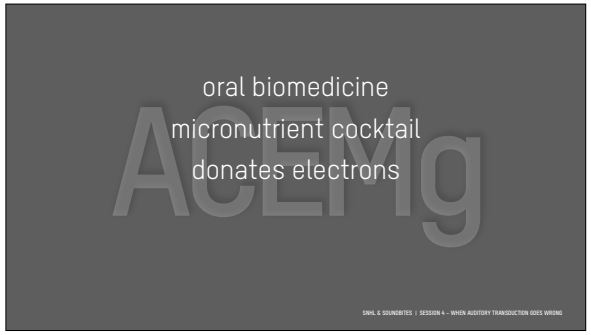
The finding that SNHL is triggered by excess reactive oxygen species (ROS) motivated interest to use antioxidants as a pharmacological intervention for hearing preservation.

108

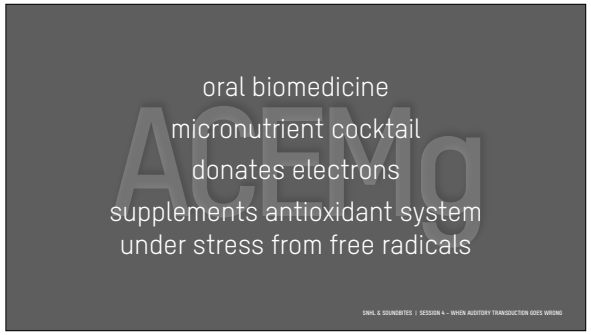
ACEMg was among the early candidates aimed at blocking SNHL. The ACEMg formula was developed from 2003 to 2005, disclosed in a 2007 peer reviewed paper.



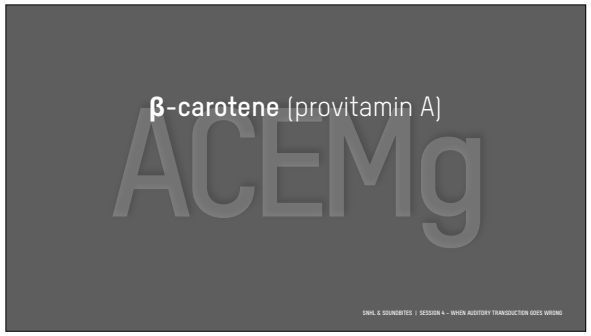
109 ACEMg was devised as an oral biomedicine,



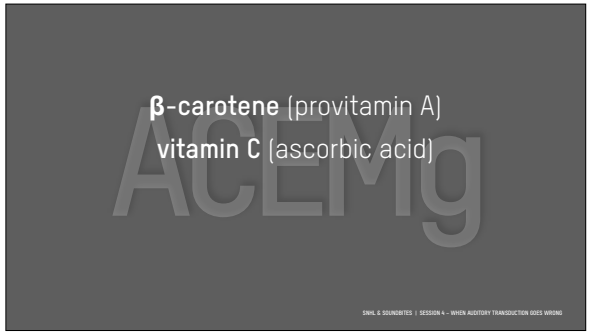
110 a micronutrient cocktail of antioxidants and a mineral that donates electrons



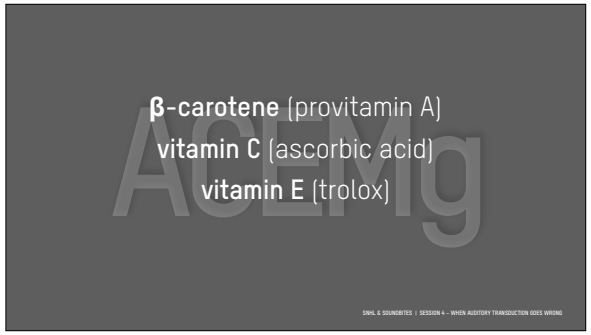
111 that supplements the endogenous cochlear antioxidant system under stress from excess free radicals.



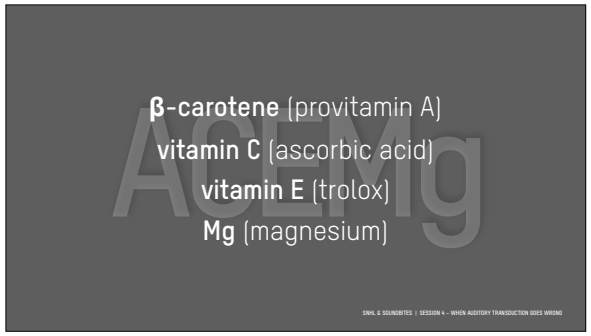
112 ACEMg is comprised of β-carotene (converted in the body to vitamin A).



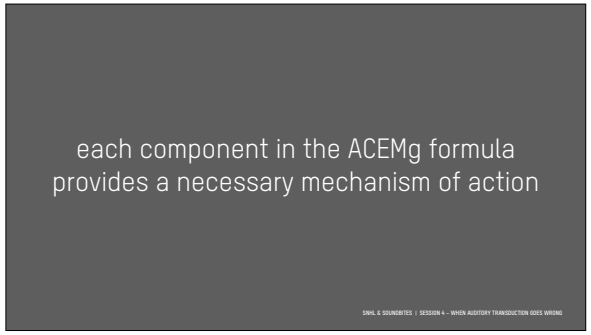
113 ascorbic acid (vitamin C),



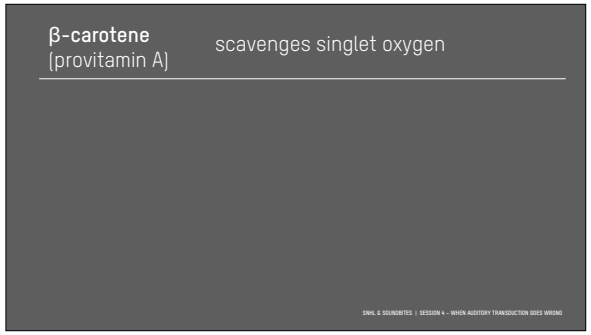
114 trolox (vitamin E),



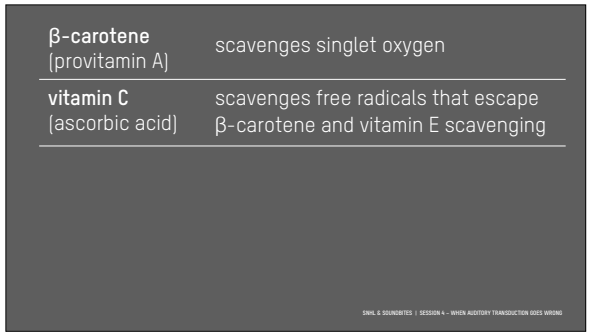
115 and magnesium, a vasodilator mineral.



116 Each component in the ACEMg formula provides a necessary mechanism of action.

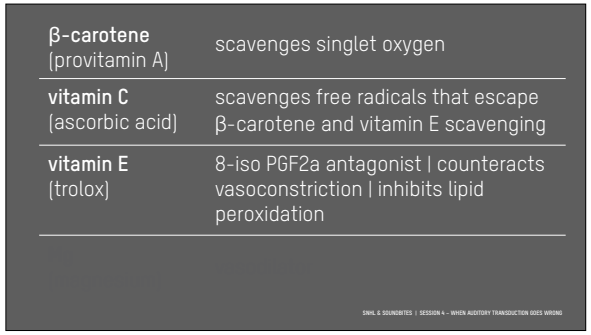


117 **Beta carotene scavenges singlet oxygen**



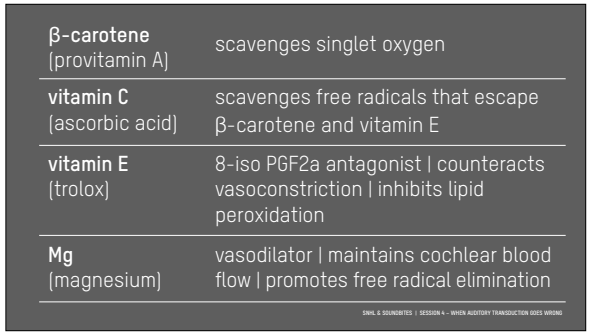
118

vitamin C scavenges free radicals that escape scavenging by beta carotene and vitamin E



119

Vitamin E is an 8-iso prostaglandin F2 alpha antagonist, which means it counteracts the action of that vasoconstrictor. Vitamin E also inhibits lipid peroxidation from redox reactions that damage fatty acids in cell membranes.

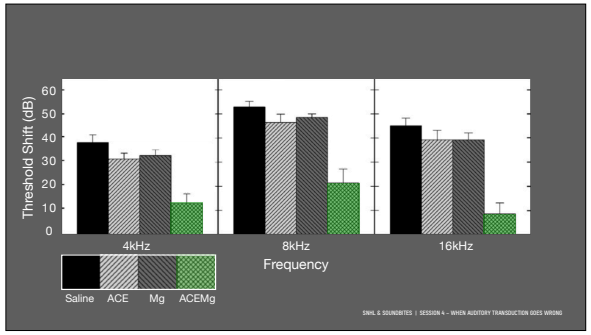


120

The mineral magnesium is a vasodilator, widening blood vessels to maintain normal cochlear blood flow, and promoting free radical elimination by donating electrons.

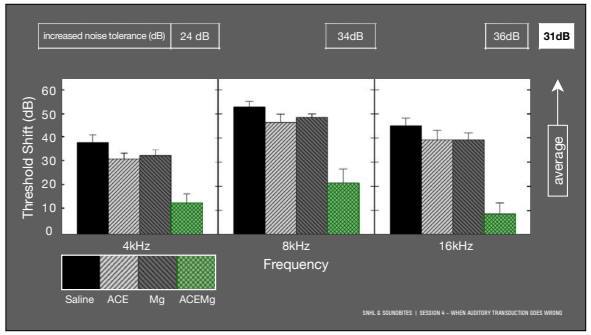
121

When combined, in animal studies the formula demonstrated an unexpected beneficial synergistic effect on noise-exposed cochlear cells, blocking the initiating biological events that trigger SNHL.



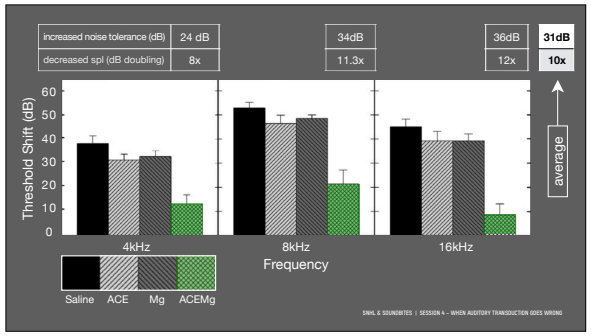
122

Lab research demonstrated that ACEMg, in green, measurably reduced noise-induced hearing loss at 4, 8, and 16 kHz far better than treatment with antioxidants A, C and E together, or treatment with magnesium Mg alone, or saline, the control. The probability is less than one in 10,000 that these results would happen by chance.



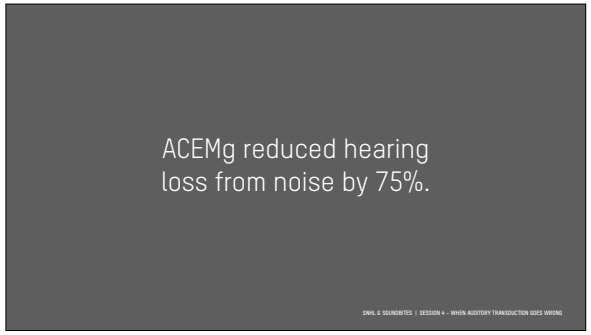
123

Sound pressure level, or spl, is measured in decibels, or dB. The lowest level of human hearing is 0 on the dB scale. ACEMg increased the noise tolerance of cochlear cells by an average of 31 dB over the control.



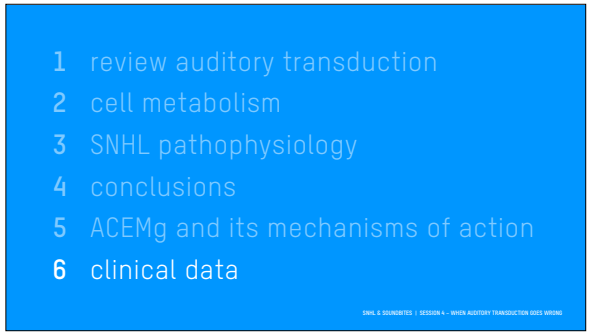
124

The dB scale is logarithmic. Sound pressure doubles every 3dB. ACEMg was demonstrated to maintain normal auditory function when sound pressure level increased by as much as 10 times, roughly the difference between the sound pressure in quiet conversation versus yelling.



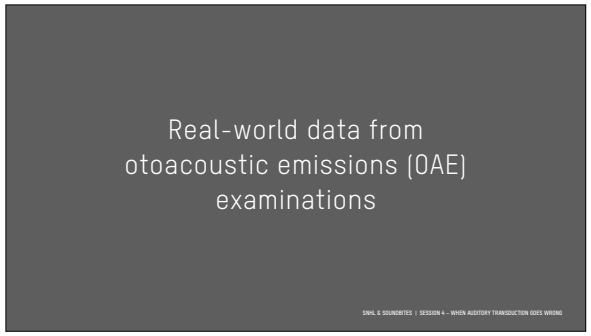
125

ACEMg reduced hearing loss from noise by 75%. These were unprecedented lab findings in hearing research.



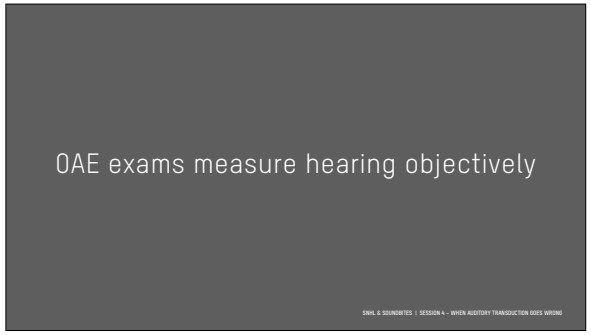
126

To conclude, let’s fast forward sixteen years to findings from real-world clinical data demonstrating that ACEMg, Soundbites, preserves or improves hearing.



127

Otoacoustic emissions (OAE) examination data were collected from patients previously diagnosed with SNHL.

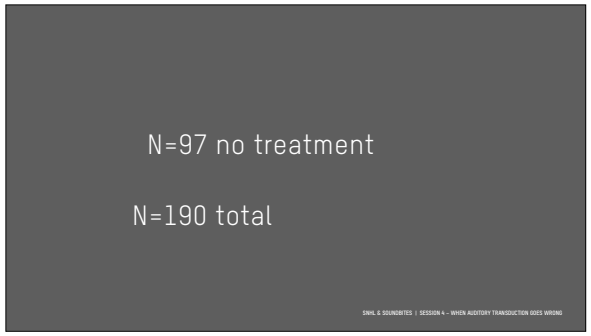


128

OAE examinations objectively measure auditory function of outer hair cells (OHC) in the cochlea.

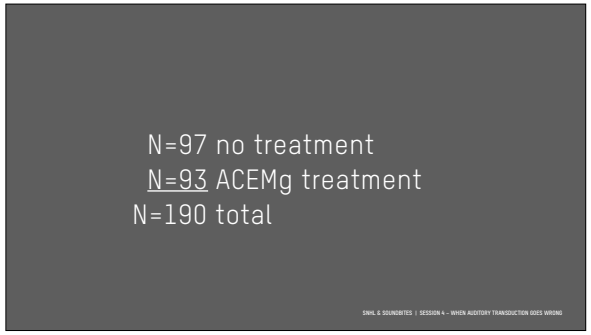
129

The study analyzed data from a total of 190 patients in two groups



130

Five years of no-treatment data on 97 patients, testing the hypothesis that untreated hearing loss gets progressively worse.

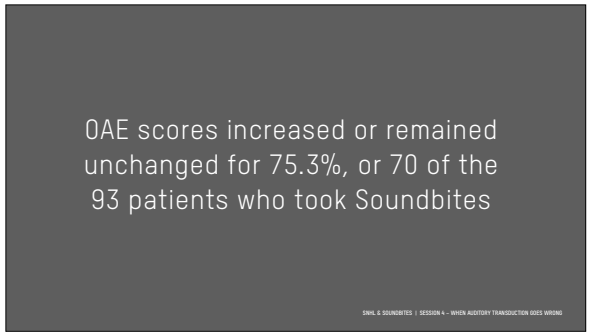


131

And two years of data from 93 patients who had at least two years of previous annual OAE exams and then started taking ACEMg as Soundbites softgel capsules for two years.

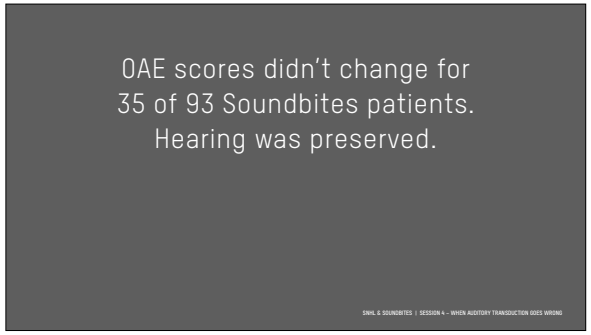
132

Here’s what we found.



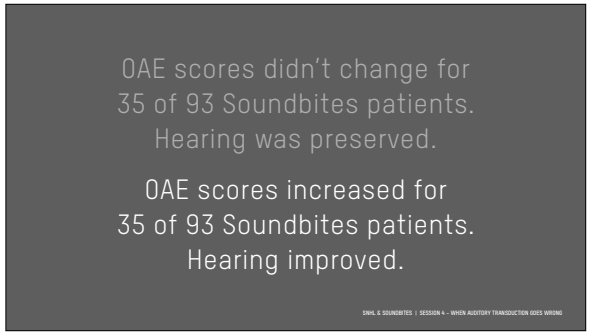
133

OAE scores remained unchanged or improved for 75.3 %, or 70 of the 93 patients who took Soundbites daily for two years.



134

OAE scores didn't change for 35 of the Soundbites patients. Hearing was preserved.



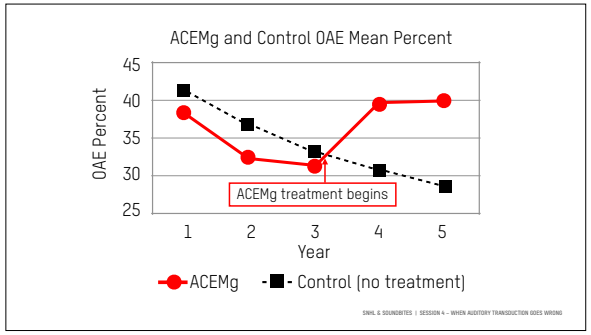
135

OAE scores increased for 35 of the Soundbites patients. Hearing improved.

Most of the improvement happened within the first six months, and continued with daily use.

136

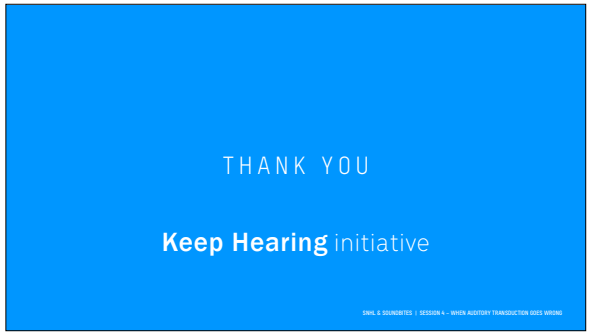
In stark contrast, OAE scores decreased for 73.2%, or 71 of the 97 untreated patients.



137

This chart visualizes the findings. The black line confirms the common belief that hearing loss progresses without intervention.

The red line shows what happened when patients with two previous annual OAE exams starting taking ACEMg as Soundbites immediately following their third annual OAE exam. The research report is available in the extra documents.



138

Thank you. In Session 5 you can learn about the sixteen-year translational research process and ACEMg's journey through it.