

Hearing Health Hour Webinar Transcript

Central Auditory Processing Disorders | Monday, July 25, 5pm ET | 4pm CT

Presenter: Elizabeth McCullagh, Ph.D.

Host: Anil K. Lalwani, M.D.

ANIL LALWANI - Well, it is now 5:02 here in New York city. And hello and welcome to our Hearing Health Hour Webinar. I am Dr. Anil Lalwani and I appreciate you joining Hearing Health Foundation for this Hearing Health Hour Webinar. Today's topic is central auditory processing disorders known by the acronym, CAPD, and also sometimes referred to as auditory processing disorders.

Now this event has a live captioner and is being recorded. You can enable close captions by clicking the CC button in the toolbar at the bottom of your screen. And if you need any other assistance using Zoom, please follow the link to the technical guide shared in the chat.

By way of introduction, my name is Dr. Anil Lalwani. I'm a professor and vice chairman for research in the Department of Otolaryngology, Head and Neck Surgery, or more affectionately known as ENT, as well as the associate dean for student research at Columbia University Vagelos College of Physicians and Surgeons in New York. I'm also a board member of Hearing Health Foundation, where I oversee the Emerging Research Grants program, also affectionately known as ERG.

ERG provides critical funds, just critical, to researchers studying hearing and balance conditions. These grants supported many leaders in our field to become successful scientists, including our illustrious speaker today. The ERG program that provides seed money to scientists just starting in the field is only possible through the generosity of supporters like you. So if you'd like to support our work on hearing loss, tinnitus, or related conditions, as well as today's topic of central auditory processing disorders, you can do so at hhf.org/donate.

Our presenter today is Elizabeth McCullagh, a 2016 ERG recipient, who's generously funded by Royal Arch Research Assistance. Now she will describe her work on auditory difficulties and Fragile X syndrome, which is the most common single gene form of autism spectrum disorder, and also will give us an overview on CAPD, what we currently know about it in our field. Dr. McCullagh is an assistant professor in the department of integrative biology at Oklahoma State University.

And again, we at HHF are so grateful for the support of Royal Arch Research Assistance who have been tremendously committed to funding research on CAPD. This portfolio of research is an important part of Hearing Health Foundation's work. Let's move to our guest today, Dr. McCullagh, who will begin our presentation. Please do ask your question to the Q&A box linked at the bottom of the screen again, where the CC button is. And we'll try to answer those questions following Dr. McCullagh's presentation. Dr. McCullagh.

ELIZABETH MCCULLAGH - Hello everybody. Let me just start my presentation. So yes, I am Liz McCullagh. I'm an assistant professor in integrative biology as Anil said, and a Hearing Health Foundation ERG grant recipient.

I wanted to share briefly that I am an assistant professor, so I study a lot of basic science research, which is super important to help us understand mechanisms behind complex disorders like CAPD or autism. So I'm not a clinician, but the research that I do is super important and is important for things that we may not be able to study on humans.

I'm going to go through and briefly describe CAPD. And make sure my slides are advancing. So again, I'm going to talk about CAPD, central auditory processing disorders, and then spend most of my talk actually talking about my research on Fragile X syndrome.

Here's the intro part. One thing that is clear about CAPD is that it is often difficult for people with this issue to handle situations that are acoustically noisy or acoustically difficult. People without any sort of auditory issues tend to also struggle in these environments, but it's particularly difficult for those who are suffering.

I want to warn you, I'm going to show a video next that essentially shows this complex acoustic environment and gives kind of a flavor for how this might be difficult. If you want to mute or do not want to listen to all the noise, feel free to do so now. And full disclosure, this is not my video. This is from Widex, so it's specifically about speech understanding and noisy environments. We don't have to watch the whole video, but hopefully you have a flavor for how complex that environment is. [Video plays]

So what is a definition for CAPD? Well, that is part of the issue with this disorder is that it's actually difficult to define, but here is a very nice broad description and I'll read it aloud. And then I'll share kind of some of the areas that I think pop out to me at least. CAPD describes a heterogeneous group of disorders of central auditory processing, identified in an ever-increasing population, spanning infancy through the elderly, and of diverse etiology, due to various underlying pathology, all leading to difficulties in making sense of the sounds that one hears.

These include challenges in recognizing which sounds are important and which are background noise, telling one sound apart from another, locating where sounds are coming from, remembering sounds in the order they are heard, and experiencing additional difficulties in understanding after exposure to loud noises. The hearing difficulties associated with CAPD occur despite normal hearing thresholds, thus audibility of sounds per se is not the cause. Consequently, it remains challenging to diagnose,

manage, and treat given the wide variety of symptoms grouped under the label, the complex relationship between CAPD and other disorders and disabilities, and uncertainties about its causes.

That's a long paragraph to describe a very complex issue. Here are the things that kind of stand out to me. CAPD is heterogeneous, so essentially it's a group of disorders that aren't always the same. And importantly, it affects a large group of people, anybody spanning from infancy all the way to the elderly. So that's a large age group. It includes challenges in recognizing which sounds are important, which are background. Similarly to what we just described in that video, hopefully, and then telling one sound apart from another, locating where sounds are coming from, and importantly, this occurs despite normal hearing thresholds. This is not an issue that is necessarily due to changes in the ear, but tend to happen more centrally. So hence why the "central" part gets added often.

That was a very brief, very very brief introduction to CAPD, because like I said, I want to focus most of the talk on my research on Fragile X syndrome. And hopefully you'll see some similarities here between CAPD and some of the issues in Fragile X syndrome and autism. Here is another video, again, it could be loud, depending on your speakers, etc. So if you'd prefer not to listen, feel free to mute, but it's basically trying to describe how somebody with autism may be perceiving their environment and how some of these sounds can be distracting, can be difficult when there's multiple sounds together, etc.

And also I like this video lot because it includes some other sensory components, which of course we're not going to talk about today, but we know are likely also happening in people with autism. I'll let it play out so it gets credit for the makers of the video, since I did not again make this video, the National Autistic Society did.

Okay, so let me tell you a little bit more about Fragile X syndrome. So as Anil said, it's a genetic model for autism. It's the most common genetic form of autism. And it occurs in about one in 4,000 males and one in 4,000 to 6,000 females. It's an X-linked disorder. Clinically, it presents generally as hypersensitivity to sensory stimuli and lots of other clinical manifestations as well.

But this sensitivity to sensory stimuli is really important in my research, specifically, the auditory sensitivity, there is often comorbidity with epilepsy and susceptibility to actually autogenic seizures. These are seizures that can happen as a result of exposure to loud sounds. Fragile X itself is caused actually by a CGG expansion on the Fmr1 gene on the X chromosome. It's a point mutation mouse model, I use a point mutation mouse model to study the disease. It's not the same expansion phenotype as we see in humans. And I forgot to change the definition of the protein, but they've now changed it to not have mental retardation on the name just this year. So I apologize that I didn't get that into my slides.

But this protein regulates mRNA expression and its targets include neurons, the cell body, dendrites, binds, and presynaptic terminals, as well as other parts of the body as well. But I focus on the brain, so we'll focus on those aspects.

So what do I study? I study the auditory brainstem, which is an area really important for sound location information. As you saw in that definition of CAPD, that I believe Hearing Health Foundation and Royal Arch Masons put together, this area is really important for encoding timing differences between the two ears, to figure out where sounds are coming from, as well as level differences. These are intensity differences. Essentially the difference between these two is we can use two different types of information, if a sound hits one ear first and then the other, we can actually use the differential timing between when that sound hit that one ear, and then the other to figure out where it's coming from. This is typically for low frequencies.

Or we can actually use intensity changes. So is the sound really loud at one ear and softer at the other due to the shadow of our head? And these are typically for frequencies that are a little bit higher. And I'm going to focus on this ILD or interaural difference circuit, because I work with mice and they typically hear at relatively high frequencies. This is the ILD circuit that I'll cover next. So this is the brain, over here are these two things on the sides, are the ears. And then inside there, the little squiggle is the cochlea. The information obviously travels from the ear into the brain, through the auditory nerve, and then gets processed in a few different areas, the lateral superior olive and a different area called the medial nucleus of the trapezoid body.

Essentially it's using these level differences as a comparison between the two ears to again compute where sounds come from. And there's a nice balance of excitation and inhibition. It's tonotopic, so meaning that different areas of the brain respond to different frequencies. So the really important and nice features of this pathway that I like is that this frequency information is encoded, it's really highly myelinated, because again, these have to be super fast signals to integrate this information and then allow us to attend to or change our behavior based on where sound cues are coming from. It's really temporally precise, right? It has to encode that timing information. And again, it's a great model for excitation, inhibition, balance because it's all about excitation-inhibition that allows the brain to do this.

I'm going to take you through a couple of different studies that we've done, looking at anatomy, physiology, and behavior in a mouse model, again, of Fragile X syndrome and focusing on this auditory brainstem pathway. What are the anatomical changes that we see? We recently published a study looking at myelination changes in the brain in this Fragile X mouse model. I'll refer to it as either a Fragile X knockout or an Fmr1 knockout down here in the bottom. And we were able to look at all this cyan color here is myelination within the brainstem. So we quantified that and again, myelination's super important in the brain to encode these fast signals. This little GIF up here is just showing a myelinated fiber on the right versus one that's unmyelinated and how quickly the signals can propagate.

What we saw is actually that there was a decreased diameter of myelinated fibers near one of these areas in the brainstem, the MNTB, in Fragile X animals compared to controls. So that might show us that there's going to be changes in signal processing time, etc., in

these axons. We also then looked at the glial cells that are important for actually making this myelin called oligodendrocytes. And we looked at their number in the Fragile X versus the wild-type mice. And what we saw is the Fragile X is on the right, we actually saw a decrease in the total number of mature oligodendrocytes in this model, as well as progenitor cells for oligodendrocytes. Oh, sorry, increased number, these are flipped, it's confusing.

So over here is the knockout on the left side and the quantification and the wild type on the right. These are the things that myelinate the glia. It's interesting that there's in fact more of them in the Fragile X animals. Then we also looked at physiology and we performed what's called an auditory brainstem response, and we can do this in humans as well. So essentially what happens is some electrodes go under the skin of the animal over the brainstem, and we play clicks or tones to the animal, and try to see how their brain responds.

We get these nice stereotyped waves, as we see here, one through four, and they correspond to areas of the ascending auditory pathway, specifically into the brainstem. We look at responses again, to different stimuli. We can also compute something called the binaural interaction component, which gets us a really nice readout of the binaural capability of animals.

And so what happens basically is we play sound into one ear or to both ears simultaneously, and we can sum those responses from the two single ear manipulations and compare it to when we play sound of both ears and we get this thing, this nice wave called the DN1 or BIC. And we can look at the amplitude or latency, when does it occur or how big is it in our Fragile X animals versus our wild type?

What we found, very briefly summarized, is we saw sex-specific differences in hearing. So it wasn't just that the Fragile X mice were different than the wild type, but that there was actually a sex effect. And the female Fragile X mice had a decrease in wave four of the ABR, so over here, this purplish wave, which actually corresponds to a lot of the binaural or sound localization type pathways.

Then interestingly, we also saw an increased latency in males at zero ITD. We played timing differences in this BIC. It occurred a little bit later in males specifically, which again, may be due to actually to that myelination phenotype that I just talked about in my previous slide. So behavioral changes also, this may still have startling stimuli present, so I apologize if that's true. Essentially what we do is we play a loud sound to an animal, and when we do that, they startle. Okay, good, I think I removed them.

And then if we play a sound prior to that loud sound, they will actually startle less. We can use this startle amount as a proxy for did they detect the first cue or not? Essentially, as you can see in this graph here, in the control, that's a really large accelerometer response, which is what we're measuring in blue or red, where when a pre-pulse is played, you can see the animals doesn't startle nearly as much. Then we can calculate this thing called pre-pulse inhibition, which is essentially how much did this pre-pulse allow the animal to

inhibit their startle response? So larger numbers of PPI means that they detected this pre-pulse signal more or were able to inhibit their startle response.

I'm going to briefly go through what we saw. We did a bunch of different tasks to measure the animals' sound localization ability using different pre-pulse cues. In the initial experiment, we basically just played sound, white noise from a speaker directly in front of the animal, and then startled them to see at what level, what intensity does it take to startle animals? And when we did this, we found that the Fragile X mice do actually startle slightly less than wild type.

They don't actually have as large of a startle response. We also can present this white noise now, but with small gaps in that noise that precede the startle speaker. And this actually may not be as brainstem-mediated of a response, which is interesting, because that gets us kind of more along the ascending pathway and maybe into some cortical areas as well.

What we saw is actually animals had a longer latency to respond, the Fragile X animals, and they inhibited their startle less than the wild-type animals, suggesting maybe they're having trouble perceiving these gaps. We also then were able to swap speakers in space as that pre-pulse cue. So now the cue is essentially a speaker moving from one side of the arena to the other side, this broadband noise, and then startling the animal. If they detect that speaker swap, we can then measure their pre-pulse inhibition as a result thereof.

And what we saw again was longer latencies to respond and less inhibition of their startle, specifically only at 90 degrees, so actually the easiest part of the task, which was a bit surprising. Then we can also do what's called signal release for masking. We play a masking sound directly in front of the animal and then move the signal further and further away and then see if the animal can discriminate the signal from the background. So we can change both how far away it gets, but also how loud that signal is compared to the masker. And I hope you're seeing a theme here.

We saw longer latencies to respond and we saw less inhibition of their startle at 90 degrees specifically with a loud masker. So again, this latency to respond thing seems to be a really consistent phenotype and actually is what led us to want to study those myelination changes since we know that myelination is so important for timing of responses.

In summary, I very briefly told you about CAPD and that it's a very heterogeneous group of disorders, and happens from infancy to adulthood. It results often in having background noise issues, telling sounds apart, sound location information. Often folks with these issues have normal hearing thresholds. And then I also hopefully told you a bit about my research on Fragile X syndrome.

And I spoke through some auditory-specific anatomy, physiology, and behavior that are altered in mice with Fragile X. These changes tend to be really subtle, right? There wasn't anything that kind of hit me over the head, but that it's clear that they have some auditory

issues. And my next research, I'm really hoping to focus more on these mechanistic-type studies underlying these issues including, for example, studying myelination, which is actually research that I was recently funded on through NIH, and funded through NIH on.

Then I also wanted to kind of summarize at the end, to talk about Hearing Health Foundation and the ERG specifically, and the types of impact it's had on my career. A little bit of background on me. I know it's the end of the talk, but I figured this is a good time to do it. I did my master's and undergraduate degree at Virginia Tech with John Phillips. I then went and did a PhD at University of Illinois Chicago with Dr. David Featherstone. I then had the fortune of going to do a postdoc at University of Colorado Anschutz Medical Campus with Dr. Achim Klug.

And this is where I really started focusing on Fragile X syndrome and the auditory system and when I was able to receive the Hearing Health Foundation ERG award. And so since that award it's really set me up nicely to be able to continue doing my research and to get additional funding. I was able to get a grant from FRAXA, which funds basic science research and clinical translation research in Fragile X syndrome specifically. I was able to get an NIDCD T32 postdoctoral fellowship to help me continue my research as well.

Then recently in 2020, I became an assistant professor at Oklahoma State University. And since then, I've also received awards from the NIH, including the NIDCD and NSF. And since I received my funding, I also published six papers, they're specifically on Fragile X and auditory issues therein. We can post these papers, if you're more interested in reading the actual paper behind it, I gave a very brief summary of them all, so there's a lot more detail in those as well.

Then I just want to thank, these are folks I worked with either during my PhD or postdoc, and a lot of them helped actually conduct this research or helped me do this, perform the actual research, and then my current lab. These are a lot of the students that actually have done the more recent work on myelination and some of the physiology as well. So thank you very much for your time. And then of course my current funding sources.

ANIL LALWANI - Well, Dr. McCullagh, thank you so much for that talk. It clearly is apparent that it takes a large community to do this work. And you've really had a wonderful experience working with a lot of them, it seems, some of the leaders in our field. And also thank you for highlighting the contribution of the funding from Hearing Health Foundation and the Arch Masons. It was impactful in my own career and is clearly impactful in so many others. Many in the audience have similarly contributed to supporting this work and us again, I want to thank the Royal Arch Research Assistance program.

I think what we might want to do, I know you mentioned that you're not a clinician, but we might just ask some clinical questions, and maybe you can help us in terms of how it either impedes your work or helps your work, or in fact, maybe motivates your work. Is there a way to diagnose CAPD, whether it's in mice or whether it's in humans, I'm going to start there.

ELIZABETH MCCULLAGH - Yeah, that's a great question. And that's really why I wanted to highlight the heterogeneity of CAPD, in that it makes it incredibly difficult sometimes to diagnose, because depending on the audiologist's experience with different aspects of the disorder, they may go with a more specific issue as opposed to CAPD, or use CAPD as an overall classifier, much like we do with autism spectrum disorder now. But I do think that's one of the areas in which a lot more can happen. So we need to build better diagnostic tools that can really discriminate CAPD from other maybe similar or different conditions. Yeah, I think that's really one of the things that research could move into in the future, that would be a really great help I would guess for clinicians.

ANIL LALWANI - And we've also had several questions about treatment of CAPD. Can you comment about that?

ELIZABETH MCCULLAGH - Yeah, so again, I don't actually see patients, but I did actually get to see some Fragile X kids when I was in Colorado, just rotate through the clinic and hang out. And I think that is also an area in which the more we understand about the basic science, we can help to build better pipelines for treatments.

But currently my understanding is that there's multiple aspects of treatment that folks can try, often working with kind of a team of physicians who can come up with a strategy that incorporates speech pathologists, and other folks who can really work with kids, I think especially if you can do it as young as possible. Typically, these types of interventions help when you get them as early as possible. So personally, this motivated me a little bit to do this, because I had preemie, she was nine weeks early and I would say that the team of physicians in the NICU were just fantastic and having that support of early intervention was critical for us to be able to just help in those early ages. The one thing I can advocate for is, if you notice something about your child in particular or somebody that you care about or want to help, to advocate for getting them help from a variety of sources and using any resources that you maybe have available to you.

ANIL LALWANI - Clearly your talk highlights and for me anyway is the clarity between how there's really two parts to hearing. The one is the sensory part, which is the ear, the peripheral ear that we always so much focus on, but the processing part is equally important. And that's where the whole rubric of central auditory processing occurs and disorders occur. Now I noticed that you spent some time on myelin. Would you just take a few minutes to talk about myelin and its importance? I know we have a very heterogeneous audience as well. And why is it so obvious that if you have myelin problems, that you might have delays in the startle response or the ABR wave, maybe a good place for us to start. And there's some specific questions about your research that I'll get to, but I think those are much more complex, I'll start with the simple ones first.

ELIZABETH MCCULLAGH - Sure, so myelin is essentially, if you can think of an electrical cord, right, it's that sheath, that black sheath, that essentially protects that electrical signal to be able to continue along its path and not just go out into the environment. So it's really, really important for the way that a lot of our brain processes anything. Myelin's a pretty

common substance throughout the brain. And how it works is it's actually cells, called oligodendrocytes that wrap around the neuron and around the axon to actually create that sheath. And so it's a fatty substance that kind of wraps around and it helps those signals propagate in the brain. You can imagine if there's less myelin or changes to the thickness of the myelin or to the diameter of those axons, that maybe those signals aren't moving as quickly, so increased latency to respond, and issues with auditory, because all of our auditory system, specifically in the brainstem, it's all about speed. We have to be able to compute those timing or those level differences between the ears, basically has one of the fastest synapses in the brain. And it does that by being really, really myelinated. So the more myelin the better usually in those instances.

ANIL LALWANI - And also looking at the Fragile X, it seems like these children have lots of other issues going on, they may either reflect direct influence of the auditory system or a primary problem with the other system. Along those lines, Dave from our audience asks, given the substantial intellectual difficulties of most individuals with FXS, and the critical role of cognition in CAPD, how do we manage to disentangle hearing from the more general abilities? I think it's something along those same lines as how do you disentangle the contribution of hearing to the variety of things you see in these people versus a primary issue? I think it's all the same question, if you could comment about those.

ELIZABETH MCCULLAGH - Yeah, that's a really great question. That's partly why I try to do things that don't involve cognition. So the auditory brainstem response specifically measures the auditory brainstem, which is nice. That pre-pulse inhibition task that I showed you also is an innate response so it doesn't require learning. And I do this in part so that I can try to tease those things apart because it is so complex, like nothing in the brain is in isolation.

But if we can try to focus on just the brainstem, then we can learn one of the really earliest parts of the ascending auditory system, that's going to affect everything else upstream, right? Because if things start to change at the basic level, of course there's going to be changes in cognitive processing of those auditory signals too because the signal's already degraded or changed in some way. So that's kind of how my research tries to get around that complex part of the issue, and why I think actually it's very helpful to be able to disentangle these things as much as possible. But then I think the next step, right, so maybe we learn these things about specifically the brainstem but who cares, right? Does it matter, does it change those higher level changes as well? And so I think, when we can try to build systems and tools that can look across the entire brain, we can try to connect these things in a meaningful way.

ANIL LALWANI - So as a series of interrelated questions having to do with Fragile X, somebody asks, Katrina asks, how can someone know if they're carriers of Fragile X? And somebody else asked, asked whether you study only carriers or the full-fledged Fragile X? And finally, can it be inherited from one generation to the next? Sort of interrelated, maybe a broad overview about Fragile X would be really helpful.

ELIZABETH MCCULLAGH - Yep, so I, for the sake of time, didn't go into too much detail. So I'm glad these questions are asked so I can expand on them a little bit more. As I said, it's repeat expansion, so when we're talking about carriers, as this one person asked, really they're saying this person has an expansion, but it hasn't reached the full expansion yet. So essentially there's these CGG, if that expands beyond 200 repeats, we then say that that's a full mutation.

And to that other question, that happens with each subsequent generation, it tends to expand. So it is heritable from one generation to the next, and it's often somebody who's a pre-mutation carrier, meaning they don't have that full expansion, whose then children have the larger expansion and have what we consider full-blown Fragile X syndrome.

So now they're above 200 repeats and that's usually what we find is the amount to change that protein that I talked about, so that it's no longer expressed or expressed differently, or not as much as it should be. It all comes down to how much protein you have. And typically those with over 200 repeats, there's either no more protein or very little, or it's changed the way that it's functioning.

When I'm studying the mouse model, it's actually a different way that the mutation has arose. So the nice thing about mouse models and genetic modules is that we can basically recapitulate the disorder by no protein, right, that's what we're trying to get at, by just changing the mutation and just not allowing that gene to express. That's what's happening in the mice, they're full mutants, there's no pre-mutation, and so I also then have male and female full-mutation animals as well. We can do heterozygote females, which essentially means that they have one X that has the Fragile X syndrome, and one that does not, because this is an X-linked disorder, it's females who are passing it down to their children. And then that gives us a really interesting perspective maybe on some of the heterogeneity in Fragile X syndrome as well, which starts to get really complex. So hopefully that broke it down a little bit in terms of those questions.

ANIL LALWANI - That's really very helpful. And so I think David wants to know, and this is sort of a very common question people ask when somebody does work in an animal model, is how translatable is this to humans and what are your thoughts about that?

ELIZABETH MCCULLAGH - Yeah, I love and hate that question as well. I think anything we do in animals, we have to put a little asterisk, to say this is mouse Fragile X. And so I think that has been an issue in the Fragile X field and in all science, is this translatability problem, of things that we do in animals, how much do they actually inform human disease? And fortunately for the most part, things are fairly translatable, right? We can study vaccines in animals and we get a product that works in humans.

With more complex neuroscience-type questions, it does get a little bit difficult, and with the auditory system specifically, as I told you, I was just focusing on this ILD circuit and it's really because mice probably don't do too much ITD processing, whereas humans do a ton. So that's another big caveat to this research is that that makes it difficult, but there's

hope. We have other models that we can use. So there's also a drosophila model and a zebrafish model for

Fragile X syndrome. There's, in development, a gerbil Fragile X model, which actually those animals hear in both ITD and ILD, there's a rat model, two different rat models in Fragile X, there's several different mouse models.

What I'm getting at here is that the more research we do and the more we see commonalities across what we're finding in one model and can apply it to other models, the more likely it is to be able to apply to human issues as well. Because in the end, we're all vertebrates and do share a lot of similarities across our systems. But it is important to note the caveats to any research that's being done and recognize that there's probably some issues with that as well.

ANIL LALWANI - Just seems like there's a great ability to dissect some of the various issues and these different mouse models. You can use those differences to your advantage to answer very specific questions.

ELIZABETH MCCULLAGH - Yeah, there are things I can look at a mouse brain in ways that we can't look in humans as well. So there are definitely value to our model organisms too.

ANIL LALWANI - So there's a very specific question, asked from Akshay. Were the groups normalized for startle given there was a difference in startle between the two groups to begin with?

ELIZABETH MCCULLAGH - Yeah, that's another good one. So we chose...

ANIL LALWANI - Do you mind restating that question in a different way for the larger audience before you go into the answer itself?

ELIZABETH MCCULLAGH - Yes, thank you. We saw a difference in overall startle ability of the animals, between the Fragile X and the wild-type animals. The Fragile X animals tended to startle less is what we saw. There's a lot of variability in what different people see across the same paradigm, but that's what we saw.

But what we chose for our startle stimulus going forward, for all those other experiments that I showed you, gap, speaker swap, etc., we chose a value of startle in which the wild type and the knockout animal startled the same. At the loudest sounds, there was no difference in their startle ability. So we chose a loud enough sound that that difference hopefully was no longer apparent and could be translated than across the rest of the experiments that we performed.

ANIL LALWANI - A very broad question, I think this is a good one. Alan asked is CAPD only associated with autism spectrum disorders?

ELIZABETH MCCULLAGH - No. So CAPD is associated with a lot of other issues, ADHD, autism. And again, anytime we talk about these issues, they're definitely not a one-to-one either, right? Everyone with autism doesn't have CAPD, everyone with CAPD not have autism, but there is some overlap. We like to consider and try to think about how these things interact. So I study autism in a way through Fragile X syndrome, but that's just one aspect of this very complex issue.

ANIL LALWANI - Yeah, I was going to bring it back to the whole general concept of how you have a peripheral auditory system, the sensory organ, the ear, and then the central auditory pathway. And really, tell me if you agree or not, but broadly speaking, anything that happens in the central auditory pathway could be a processing issue. So for example, hidden hearing loss is a type of a central auditory processing issue. Auditory neuropathy is sort of a central auditory processing issue. People that might have multiple sclerosis, specifically the myelination, but they also have inflammation stuff, central auditory processing issue. A tumor of the hearing and balance nerve, all the way up to brain tumors may lead to auditory processing issues. And then certainly of course, any age-related consequences. So it's a very broad term with many different manifestations and therefore CAPD may be part of autism, but it may stand alone as part of itself or other things. So can you expand on that a little bit on CAPD and exactly, not necessarily going back to the original definition, but kind of bring it to how broad it could be.

ELIZABETH MCCULLAGH - Yeah, so I think you hit the nail on the head in that, it is something in and of itself, right? So you can have CAPD and no other comorbidities, right? So this could just be something that you struggle with or have as your diagnosis. And then it may just be that there's a lot of other mechanisms involved that maybe contribute to your CAPD or that the CAPD contributes to. It is super broad in the sense of, things that are happening along the auditory pathway, as Anil said, could all be auditory processing difficulties in some way or another.

ANIL LALWANI - Mary Lou asks a very interesting question about CAPD changes over time. So do those occurring in early childhood continue into adulthood? Does it change over time? And is there a difference in the mouse models that you have as they age as well?

ELIZABETH MCCULLAGH - So the first part of that question, I do know a lot less about, and I would guess it's possible, right. As humans develop, then things change as well. I know certainly my 9-week-old preemie had a lot of difficulties early on, and now you wouldn't necessarily be able to differentiate her for any other 5 year old out on the street. So development is a complex process.

And so things definitely can change as kids develop or as people develop. Now, interestingly, it's like you've read my NIH proposal, what we're trying to do as part of that myelination phenotype is now see when during development do these issues arise? Including in that physiological response, and in that behavioral response, because in mouse, they're different than humans, they don't hear when they're born. And so it's only

around 14 days old or so that they start to hear. So what we want to look at is are there shifts in that timing? How does the brainstem, how is it set up? Are there ways that this developmental shift is altered maybe in our Fragile X mice? So, yeah, it's a great question, and something that we're trying to focus our research on now is a lot of these issues are developmental in origin. And so if we can pinpoint those time points, then it gives us a better idea of how we can intervene or have treatments at different time points.

ANIL LALWANI - A curious audience member named Anil wants to know if gene therapy's been tried or reducing the protein at some point, does it make a difference or not? And is there hope that this may be an intervention in the future?

ELIZABETH MCCULLAGH - Yeah, again, you're reading my proposal. There have been some adeno-associated viral reintroduction of FRMP into different brain areas. And they have seen that reintroduction of FRMP may ameliorate some of these issues. I think, the jump to the clinic may be a little bit difficult, but they're definitely in the process of trying to test those out in monkey models and other more human-like models to see if it works. We really want to see specific to the brainstem because again we can tease apart those cognitive issues.

If we introduction of FRMP specifically into our areas, it tells us two things, is it brainstem-specific? Because if we only put FRMP back in our area, we can then differentiate from other potential interactions with other areas. And then two, what is FRMP doing in that brain area? But with any science, it's complicated. And we have to try to figure out the right amount of FRMP and when to introduce it. So we have to know those developmental time points and if they're different and kind of the optimal window in which we want expression to happen.

ANIL LALWANI - This is really an interesting question. Victoria asked, can you say something about the differences and/or similarities between developmental versus acquired CAPD such as through brain trauma or something?

ELIZABETH MCCULLAGH - Yeah, so again, I don't see patients, so it's a little bit hard for me to say, but I think that's part of the beauty and the difficulty of CAPD is that the same outcome, so a CAPD diagnosis likely, comes from a lot of different origins. And the different mechanisms behind those are likely to have similarities but also have some really drastic differences in terms of how the brain, how it leads to auditory processing difficulty. So I think that's where it's really helpful to have kind of a team of scientists and physicians who are working with you to try and tease apart what your APD is coming from and how then your treatments may differ from somebody else.

ANIL LALWANI - One of the things you said about these mice is they have audiogenic seizures. So can you talk about that in terms of either auditory processing or how sound plays a role in overactivity or hyperactivity in the central nervous system, anything of interest there, is this helpful to us in understanding the auditory system?

ELIZABETH MCCULLAGH - Yeah, so it's also something that's common between humans and mice which again is the beauty of Fragile X syndrome, but also when there are more difficult things is that actually a lot of the research that we found in mice does seem to recapitulate the things that humans are experiencing, and these autogenic seizures are one of them. If you play a loud sound to these animals, they will have an autogenic seizure. In fact, so severe that if the sound continues, they may even die.

They go through a tonic or clonic seizure. And interestingly, these audiogenic seizures are also not cortical, so they're not happening in really high processing areas. They're happening early on in the auditory pathway. So they're either midbrain or brainstem, my area. It's really interesting that there are one of these kind of commonalities in the disorder.

Again we don't know when humans necessarily where in the brain these are happening, but it could be, similarly these issues are occurring across the auditory pathway and could be related to overall hyper-excitability, as you said. And yeah, that part is really a cutting-edge part of research at the moment, is kind of understanding these seizures, where they come from, what they're due to. And obviously in mice, they're really debilitating. We want to make sure that if this is in any way, what people are experiencing, that we can try to come up with treatment options.

ANIL LALWANI - I think we're just coming up to our last question, as we come to the end of the hour. Your mice have some myelin issues. Is there a difference in terms of sound being transmitted by the slower neurons versus the faster neurons or low frequency, I don't know, high frequency, is there a difference in how these mice react to it? And is there any clue for us to humans in terms of other aspects of auditory processing disorders?

ELIZABETH MCCULLAGH - Yeah, so interestingly, this myelin phenotype also is not specific just to Fragile X syndrome. So just in the past two years, there's actually been a lot of emerging research on autism specifically and myelination as maybe kind of an underlying mechanism. Now, whether myelination is an underlying mechanism for other auditory, I think is a really open area for research and a really interesting one.

I think your question is one that we're still really in even trying to understand the most basic science in a neurotypical brain or in a brain without any auditory difficulties, how is myelin conducted, etc.? There's a couple papers on this, but even that part, we're still kind of in the early stages, except that we know it's important. We know that there's some differential speed between faster or slower neurons. But again, in my mice too, unfortunately we have that ILD/ITD problem. So there are mostly going to be fairly high frequency-sensitive cells that we're looking at too. So if we can get those other models working, then we can really learn a lot more about those differences in processing between those.

ANIL LALWANI - Well you've clearly identified an area of research that can be funded for years to come, so you have a good sense of job security going forward. We want to thank first of all, all the attendees for coming to the webinar today. Thank you, Dr. McCullagh for this very informative and exciting presentation.

We are so, really so grateful to Royal Arch Research Assistance for their generous and longstanding support of CAPD research through the Emerging Research Grants program. Remember that you too can donate to our efforts to advance better treatments and cures for hearing and balance disorders, central auditory processing disorders, by donating at hhf.org/donate. Again, thank you, and please do enjoy the rest of the day. Stay cool wherever you are, or warm if you're in the southern hemisphere. Thanks so much.

