Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I've partnered with The Michael J. Fox Foundation for Parkinson's Research to bring you to the forefront of the field of neuroscience to discuss the latest advances in discoveries with leading experts.

The Michael J. Fox Foundation created this podcast for researchers, clinicians, and industry professionals with the hope that these conversations and the resources we share will advance your efforts and partnerships to improve brain health. We are welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

And today we are excited to welcome our guest, Dr. Aryn Gittis. Listeners, Aryn is Professor of Biological Sciences and the Neuroscience Institute at Carnegie Mellon University. And today we are going to talk more about neural circuits, how they are impacted by Parkinson's disease, and how they can be leveraged to develop better therapies. So, Aryn, welcome to the show today. How are you?

Aryn: I'm great. Thank you very much for having me.

Marie: Well, thank you so much for joining us today. And I'd love to start by giving our listeners a little bit of background about you. So, can you tell us more about your academic background and how you found your way to this position there at Carnegie Mellon?

Aryn: So, Carnegie Mellon is in Pittsburgh in Western Pennsylvania, and I'm actually a native of this region. But I did a lot of my education, my graduate and postdoctoral training, in California on the West Coast. So, I was really excited to be able to come back here and start my lab here in Pittsburgh.

As a graduate student, I studied at UCSD, and I worked with Sascha du Lac. And that's really where I kind of first developed my love for neurons, and action potentials, and neural activity, and really studied the mechanics of how neurons generate these electrical signals that essentially are the basis of human thought. And as a graduate student, I studied the vestibular system, which is important for balance. And it was a really fascinating system.

But I really felt drawn to study a system that had more to do with a variety of diseases. If you have problems with your sense of balance, which is a fairly common problem as people age. You're very, very aware of the vestibular system. But if you're just kind of walking down the street and someone says, what do you study? And you say, oh, I studied the vestibular system. People are like, I don't know what you're talking about.

As a graduate student, I started reading about the basal ganglia, which is one of the main brain systems involved in Parkinson's disease. And just learning more about the importance of dopamine, this neurotransmitter that is important for motivation and movement and can play a role in anxiety. And just all of these different symptoms that were kind of a little easier for me to identify with. And I got really interested in trying to understand how this brain system worked. So, as a postdoc, I started to study the mechanics of how cells work, but in the basal ganglia system. And that kind of drew me into the Parkinson's field. And that's kind of where I have been ever since.

Marie: Oh, very interesting. And I know, Aryn, you have a lot of cool research going on in your lab today. So, can you give us a general overview of the research that you do?

Aryn: Yeah, sure. So, I have maintained my love for the basic biology of how the brain functions. And I think the beauty of neuroscience for me is understanding these complicated patterns of activity that are going on in our brain all the time. But they're generated by biological molecules, by proteins, and just kind of understanding how a biological system can generate these complex things that we know of as thoughts and emotions. So, I approach this from a very basic science perspective. And some of the most important questions about how the brain functions is just asking, which parts of the brain talk to what parts of the brain? What does that talking look like? And then in disease, how is that communication in the brain disrupted?

And then how can we repair it? It's sort of the very broad general approach that my research program takes here at Carnegie Mellon. And so, we do a lot of basic circuit work that, because we're studying how these brain systems work with and without dopamine, we sort of are simulating a healthy and disease state. And dopamine is this critical neurotransmitter that is important for very basic neuron function. And I think we are only still scratching the surface, just by decades of research, on this question of exactly how dopamine affects the functioning of neural circuits. So, by trying to get at, just some of the fundamental truths of how these circuits are regulated by dopamine, even though a lot of the work that we do is done in mice, if you can find the basic biology that is conserved across not just mammals, but all species of animals in the animal kingdom. And it's really those fundamental truths that are translatable across systems that we're really interested in in my lab.

Marie: Oh, very cool. And I know today, Aryn, we are going to get into some of the details of the complex circuits involved in Parkinson's disease. So, for listeners who might need a quick refresher before we dive into the details, can you maybe give the 10,000 foot view summary of just some of these brain circuits that are

relevant for Parkinson's disease that we're going to talk about today, including the direct and indirect basal ganglia pathways?

Aryn: So, one of the main anatomical hallmarks of Parkinson's disease is the loss of dopamine neurons. And it's a very, very specific subset of dopamine neurons. The whole brain has like 100 million neurons in it. And we're talking about maybe 20,000 neurons, of that hundreds of millions, that die in Parkinson's disease. And the loss of this very particular subset of neurons is thought to cause dysfunction of many, many different neural circuits across the entire brain. And one of the main circuits that's been the focus of study for decades in Parkinson's disease is the basal ganglia.

It is the main recipient of dopamine from these neurons that die in Parkinson's disease. And that's why it's really been kind of the focus of study. And it turns out that one of the main behavioral features that you elicit when you muck around with circuits in this part of the brain are motor-related. So, experts are still debating the details of exactly all of the things that the basal ganglia do, but they seem to be really important for linking this dopamine signal that carries information about your environment, your past learning, whether things are good or bad, your internal state, like are you hungry, are you sad, or are you happy? And it links signals about those kinds of things with possible movements or behaviors that you could express that's being signaled by your cortex, which is the thinking part of the brain. And the basal ganglia integrate these two signals. So, it's getting input from the cortex about things that you are considering, thinking about, and then this dopamine signal that is providing the motivation, or the incentive, or the cautionary tale about these different movements.

And the basal ganglia acts like a big filter for these things, and then will help to select the appropriate actions for a given environment and then go ahead and act upon those. And at least one branch of the basal ganglia is really important for movement. And if the basal ganglia is not working correctly, you have a really hard time initiating movement. And this is one of the primary motor hallmarks of Parkinson's disease. So, a lot of therapeutic strategies to treat some of these motor symptoms of Parkinson's disease have really focused on trying to understand what's going on inside the basal ganglia.

I think you asked about the direct and the indirect pathways. So, I can elaborate on that just a little bit. So, about 35-40 years ago now, a really influential theoretical model of basal ganglia function was established talking about these two different pathways carrying information through the basal ganglia circuit, the direct pathway, which is thought to carry information about actions that you should do. So, a very simplified way of thinking about it is that it's kind of like a gas pedal, and it says, 'do these actions'. And then an opposing pathway called

the indirect pathway, and it's called that because it kind of makes a couple stops along the way before getting to basal ganglia output.

That is thought to oppose movements and say, 'don't do these things'. So, this theoretical model that's been really influential in the field for a number of years suggests that motor behaviors involve calibration between the stop and the go pathway, so the indirect and the direct pathways. And in Parkinson's disease, the loss of dopamine kind of weights the system towards the indirect or stop pathway. And that's why it's so hard to get movement to be initiated in Parkinson's is because activity along this pathway is the stop pathway is too strong. So, that's the direct and the indirect pathway model of basal ganglia function and specifically some of these ideas that have driven therapeutic development for Parkinson's.

Marie: Absolutely, Aryn. I appreciate you giving us that overview. And as you mentioned, we know there is this loss of dopaminergic neurons in Parkinson's disease. And you recently published a paper in the journal *Neurobiology of Disease*, looking at the effects of dopamine depletion on this direct versus this indirect pathway activity in the basal ganglia. So, can you give us a little background on why you did this particular study and how it was conducted?

Aryn: So, a lot of the research in the basal ganglia field has been focused on a part of the circuit called the striatum. This is the main input part of the basal ganglia. It's the part that gets input from the cortex and the part that gets the major dopamine input from these neurons that die in Parkinson's disease.

So, it's perfectly reasonable that it has received the most attention in Parkinson's disease research. But it's an input structure. And then there are three or four different brain areas that are downstream of the striatum before you get to basal ganglia output. And the contributions of these other nuclei, and how information morphs as it travels from one way station in the basal ganglia to the other, I think is an area that the field still has been a little blind to, historically.

And I think that a lot of people are starting to appreciate that there's a lot of post-processing of information that happens outside of the striatum. So, our study, again, started as a very basic science kind of question. If we look at the output of the basal ganglia, so we turn the system backwards, so instead of just starting at the input, we said, well, let's start with the output. And let's ask how neurons in the output structure of the basal ganglia are affected by activity along the direct and the indirect pathways.

So, using a, I guess I can still say new —— I don't know, 20-year-old technology at this point, I guess, called optogenetics, which I can go into in just a minute. Essentially, it allows us to very, very selectively affect neural activity in a

genetically defined population of cells. So, it used to be, if you want to study what the direct and the indirect pathways are doing, you have to go in with electrical stimulation, which is not very selective. It will sort of activate a volume of tissue.

But because of the way the brains are organized, it's actually going to be activating a whole bunch of different pathways all at once. With optogenetics, you can genetically target neural activators to exactly the kind of cells that you want and nothing else. And so, you can very, very selectively, kind of like with a scalpel, you can go in and activate specific parts of the basal ganglia circuitry. And using this technique, we were able to study how just the direct or just the indirect pathway information to basal ganglia output is affected by dopamine depletion.

And we found, somewhat surprisingly to us, that input from the indirect pathway, the stop pathway, that information transfer is pretty unaffected by dopamine depletion. But what was really weakened by dopamine depletion was input from the direct pathway, the go pathway. So, it seemed like, rather than having an excessive stop signal at the basal ganglia output in Parkinson's disease, the main problem really was the loss of this go signal.

Marie: So then, Aryn, what are the implications or maybe potential impacts of these findings?

Aryn: I think that, again, because a lot of research has focused on the striatum, there are ways that you can try to boost the activity of these direct pathway neurons locally in the striatum. But since we found one of the major problems is that even if you could restore activity of these cells in the striatum, the signal they're carrying is just not getting through to the output nucleus because of changes in information transfer, not in the input nucleus, but rather at the level of this output nucleus.

So, it suggests that we need to not just focus on cellular changes at the input level, perhaps an even more powerful way to restore activity of this go pathway would be to look at plasticity changes. It's almost like the lamppost analogy. We've kind of all been looking for our keys where the light is shining instead of over in the bushes where we dropped the keys in the first place.

And so, I think that if there's problems in the output nucleus, that's not necessarily where we've focused attention in the past. But I'm hoping that the work that we did helps to shine a light in this other part of the circuit that's received a little less attention so that we can dig a little more deeply into some of the molecular changes in that nucleus and try to restore function in a slightly different way than we've been thinking about it in the past.

Marie: I like this. So, Aryn, we mentioned how the striatum has been in the spotlight for some time now, but let's talk next about the role of the globus pallidus externus or GPE. I know this has been a focus of quite a bit of your research. So, can you share your thoughts on why the GPE is so important, or perhaps so interesting, to be studying?

Aryn: The main focus of my lab is the GPE. We actually just published a paper fairly recently showing that the GPE is really important for motivation, maybe not even necessarily motor control, but motivation.

And it also seems to be a major part of the circuit that causes kind of aberrant firing patterns. So, rather than just this go and stop, gas and brake pedal, what's really important is the pattern of activity in which neurons are firing. And the GPE, I think, is actually a really critical site for dysfunctional networks in Parkinson's, but not necessarily because of the go/stop analogy, but because it's interfering with the brain's ability to encode information.

Marie: So, then what maybe was some of the first evidence that you started seeing that got you interested in studying the GPE and maybe hinted to you that this was a structure that was perhaps more complex than it appeared on the surface?

Aryn: When I first started my lab, one of the first sets of experiments that we wanted to do, again, because there'd been so much work in the striatum, and then one of the major recipients of output from the striatum is the GPE. In fact, it's the only way for indirect pathway output of the striatum to reach the output nucleus is through the GPE.

But there really hadn't been a lot of studies testing some of the long-standing assumptions of how the GPE was functioning. So, in some of the first experiments using optogenetics to very selectively target neural activators just to GPE neurons, we found that in our mouse model of Parkinson's disease that we were using, when we applied interventions to the GPE, we were actually able to induce a long-lasting recovery of movement that persisted for hours after intervention, whereas interventions in the striatum are acute, and as long as you're stimulating and driving the circuit, you can get the animals moving. But once you stop stimulating, the animals stop.

But in the GPE, we were able to induce a much longer lasting recovery of movement. And this kind of got us thinking like, we really didn't understand very well what the GPE was doing and its role in the indirect pathway. And so, that's kind of how we got interested in the GPE. And since then, our work has really started to incorporate this idea that one of the main roles of the GPE is in establishing the general patterns of neural activity that are going on in the basal ganglia. So, it's not just how fast neurons are firing, but exactly when they're

firing, and small differences in the timing of two neurons being active, whether they're active at exactly the same time or they're active, shifted a little bit in time, or whether all of their activity happens kind of in a cluster of activity and then they are silent for a little while and then another cluster of activity — all of these things are actually really meaningful at the level of the brain.

And in addition to having neurons be active or not active, the loss of dopamine profoundly changes the way that neurons are talking to each other and the pattern with which they're active. And the GPE seems to be one of the main nodes in the basal ganglia that's setting these normal or aberrant patterns of activity. And so, we hypothesized that the interventions that we were applying at the level of the GPE were actually able to, at least temporarily, train the basal ganglia out of its disease state of neural activity and move it back towards a healthy state where motor commands were able to much more freely move through the system. And that's why we think that interventions at the level of the GPE have a much longer-lasting therapeutic effect than interventions delivered at the level of the striatum.

Marie: Absolutely. And I think this idea of these longer-lasting benefits of treatment is really powerful and potentially very impactful for people with Parkinson's. And I know deep brain stimulation has also been a valuable, as we know, clinical approach for a lot of patients. But it's provided some important insights and generated a lot of questions, frankly, about brain circuitry and motor control and how everything works. So, can you talk about some of your work using DBS and some of the key things that we've learned from DBS research?

Aryn: So, DBS, deep brain stimulation, as you said, it's the use of electrical stimulation to try to affect brain activity. And it's an incredibly powerful technique. The language of the brain is electrical signaling. So, to be able to deliver and shape electrical patterns of activity in the brain in a therapeutic sense is an incredibly powerful way to try to restore brain function in a variety of diseases.

It's not limited just to Parkinson's disease. It's not even just limited to brain diseases. So, spinal cord stimulation is being explored to try to restore movement in patients that are paralyzed. Deep brain stimulation has been used to try to help patients that have eating disorders, addiction. So, it's really an incredibly powerful technique that is being applied to a number of different diseases, not just Parkinson's disease. And I think that really speaks to the power of electrical stimulation, because ultimately the way that our brains are working is electrical signaling between different brain cells. And if we can understand what that language is that the brain circuits are speaking, then we should be able to generate an artificial signal that can replace or retrain neural circuits to have their original functions. So, we can train the circuits out of their disease state and back

into the healthy state. So, I kind of think of deep brain stimulation as something that has the potential to be almost like physical therapy for the brain.

Another analogy that's used is cardiac pacemakers. So, that's an example where if you have heart arrhythmias, you can get a cardiac pacemaker that can force the heart to start following a healthier rhythm. And that's exactly what we're trying to do with deep brain stimulation. In a whole host of disorders, Parkinson's disease is one of the diseases with the longest history of using deep brain stimulation, because it can be incredibly effective.

Deep brain stimulation is still a treatment that is typically only applied to patients at very advanced stages of the disease once all other treatment options have failed. So, these are patients that can be very, very severely motor impaired, and you apply deep brain stimulation, and they're able to get up, and start walking around, and resume daily life functions. So, there's a lot of interest in just understanding how deep brain stimulation works. But the way that we're currently implementing it is it is very much like a cardiac pacemaker approach.

We are kind of forcing the neural circuit into a pattern of activity that's not really biologically inspired. It's just kind of, we found through trial and error, if we use very high electrical stimulation — for reasons we still don't understand — that's able to restore movement. But I'm very interested in trying to leverage the knowledge that we have through many decades of basic research about how very specific patterns of neural activity can actually shape communication between neurons.

You can use electrical stimulation in certain patterns to strengthen communication between some sets of neurons, to weaken communication between other sets of neurons. And the hope that I have, and where I think the field of deep brain stimulation is going, is that if we can use the biology to inform us about how to deliver electrical stimulation, when is delivery of this stimulation most useful? Can we pair stimulation with either body physical therapy or like mental physical therapy? This might be a way of helping the brain to learn how to function without this normal dopamine signal that it's used to and be able to, in a much longer fashion, restore normal brain function. And I think that that's the future of deep brain stimulation treatment. We're still not quite there, but I think we're getting there.

Marie: Absolutely. And I think the field as a whole is shifting in terms of thinking about DBS, or deep brain stimulation, as this circuit treatment approach. Rather than just thinking about, all right, where is the DBS lead placed, and how is that impacting neurons locally? Kind of taking a bigger or broader picture approach in terms of how we think about it. So, can you comment on how you think maybe this shift in thinking is impacting the field, and maybe specifically, on how we can

really target which cells are involved in which circuits in these particularly tiny areas of the brain to be making sure we're optimizing therapy to its fullest potential?

Aryn: I think that the field is progressing along two different paths that at some point are going to come together. So, one path that has really started to show a lot of clinical promise is not quite cell type specific yet, but it's timing specific. So, it's asking, let's try to be really selective about when we're delivering electrical stimulation.

So, how can we do that? Well, we can try to listen to the signals that the brain is generating, and maybe 50% of the time, even in a patient that's in very advanced stages of Parkinson's disease, the signals generated by the brain are going to be pretty normal. And then it's just going to be periods of time where the brain circuits are starting to spit out a pathological or disease kind of signal. We don't want to touch the normal brain function because that's great. We just want to interrupt the pathological brain function. And so, maybe we don't need to stimulate all the time, which is currently how deep brain stimulation is delivered. It's just on constantly.

And then if it gets turned off, the symptoms come back very quickly. So, there's a technique called adaptive deep brain stimulation that tries to use some sort of biological signal. It could be patterns or rhythms of activity in the brain that then can get classified as either healthy or disease-like. If it's disease-like, that will trigger stimulation. So, it will try to disrupt this pathological activity. But when the brain is working normally, stimulation will be off.

There's also some attempts to, instead of just using neural signals, to try to use behavioral signals. So, most Parkinson's patients will have some parts of the day where they're functioning pretty normally. And then they'll have other parts of the day where it's just really, really hard to get going. So, there are attempts to use wearable sensors to try to identify, you know, is this a part of the day where muscles just aren't working that well? So, you kind of need a little extra boost. Or is this a time where you're like actually doing okay? And so, let's back off on the stimulation. So, this is adaptive deep brain stimulation. And I think there's been some really promising advances in that direction.

The other kind of avenue of deep brain stimulation research is asking, how can we apply electrical stimulation so that it is able to better target certain subsets of neurons, but leave other ones alone? So, if you were to kind of look at a tiny piece of brain tissue, like if you were to randomly select 15 neurons from the basal ganglia, you'd probably get 15 different kinds of cells. And that means that they're talking to different parts of the brain. So, some of those cells that you pick are going to be talking to motor circuits. Other cells are going to be talking to

learning circuits. And other cells are going to be talking to motivation circuits. And when you apply electrical stimulation currently the way we're doing it, you just sort of broadly recruit all of those cells. I think it's honestly pretty remarkable that deep brain stimulation works as well as it does, given that it's not necessarily very selective, but it's an incredibly powerful technique.

So, what I think a lot of scientists, especially basic researchers that have kind of been drawn into the disease field, I think a goal that a lot of us have is to try to leverage what we know about some of the unique features of some of these genetically distinct populations of cells. So, what makes the subset of cells in the basal ganglia that facilitates movement — what makes their properties unique from the subset of neurons in the basal ganglia that oppose movement? And we have worked in this area for a number of years now, and we found, going back to the GPE, at the level of GPE, there are cells that are particularly important to activate in order to recruit this long-lasting motor effect. And then at the same time, there are cells that you have to inhibit in order to recruit this long-lasting motor effect. And if you don't do both of those things, if you just globally activate or globally inhibit all of the cells in the GPE, you can get an acute movement effect, but it's not long-lasting.

So, we found that we have to impose a particular pattern of activity at the level of particular cell types in the brain in order to extract this long-lasting motor effect. And we discovered that using optogenetics, which is this research tool to genetically target neural activators, but that wasn't a technique that was going to be rapidly translatable to the clinic because the only tool that we have in the clinic outside of pharmacological agents is deep brain stimulation, which is kind of non-specific electrical stimulation. But we found that if we pattern the electrical stimulation in such a way that capitalizes on some of the different features of these different neural populations, we were able to drive the same kind of cell type specific activity.

We were able to activate the motor facilitating neurons and inhibit the motor inhibiting neurons, more or less, a little more complicated than that, but close enough. And this was able to unmask this long-lasting effect.

Marie: Very cool. And I'd love to dig into some of the detail here in terms of the development of this novel protocol with these different patterns of electrical stimulation. You actually received funding this year from The Michael J. Fox Foundation to investigate a novel DBS protocol with the goal of having these longer-lasting therapeutic effects. And this is still in the preclinical stages, we will emphasize. But can you tell us more about this new study that you're starting?

Aryn: I was incredibly grateful for this funding from The Michael J. Fox Foundation, because it was at a really critical time in this research. So, we had just found

these different cell types using our basic research tool of optogenetics. And at the time, this idea of can we use electrical stimulation to try to also drive cell type specific changes? It wasn't clear it was going to work. So, we were really grateful that The Michael J. Fox Foundation gave us some really critical funding at this early stage of the project.

So, because we're doing these experiments in mice, we're able to gain access to these different populations of cells in a way that we wouldn't be able to gain access to them in a human. And we were able to study how they responded to electrical stimulation. And we found that the kind of continuous high frequency electrical stimulation that's used conventionally in the clinic, there was no cell type specificity. So, all the cells in the GPE were kind of weakly activated by conventional stimulation.

But when we tried just delivering brief pulses of stimulation, which at first there wasn't a particular rhyme or reason to the pattern, we just kind of asked, well, let's look at what's going on if we actually gain electrical access inside of the cells and ask what are the patterns of excitatory and inhibitory signals these cells receive during stimulation. We saw that the cells that we wanted to activate that we knew based on our previous work, they got a lot of excitatory input right at the onset of stimulation.

The cells that we wanted to inhibit got a lot of inhibitory input. So, this is different chemicals get released in the brain, some of them enhance neural activity and some suppress neural activity. And so, we found that the neurons whose activity we wanted to turn off were sort of fortuitously getting a lot of inhibitory input right at the onset of stimulation. But then once stimulation was on for longer than a second, these differences in how these cells are wired up and get input went away.

And given my background as a cellular electrophysiologist, this ended up making a lot of sense because neurons in the brain are designed to release these chemical neurotransmitters at kind of a reasonable pace, maybe every 10 to 30 times a second, they'll release some packet of chemical information. But when you ask them to do this 100 times a second and just like do it forever, they're not built to do that. And the energy that the cell needs in order to sustain these high levels of activity that kind of gets drained, the packets of chemical neurotransmitter that the cells have saved up get depleted.

And if they don't have any time to recover, they can't keep functioning. So, we found that if we deliver electrical stimulation in these very short bursts, we allow the neurons to become active in the way that they're kind of meant to be active. And we don't kind of fatigue them and wear them out. So, we switched our high frequency continuous stimulation to the stimulation with very brief bursts. So,

essentially, once a second, we would deliver electrical stimulation that lasted 200 milliseconds.

So basically, like a fifth of the time, we would be stimulating. And then the rest of the time, we would just let the neurons recover. And we found that when we did this, it allowed us to drive different responses in these two populations of cells that we were trying to differentially modulate. So, the cells that we wanted to activate got excited and the cells we wanted to inhibit got suppressed.

And when we tried this in an animal that was rendered Parkinsonian by depleting its dopamine, this biologically-inspired stimulation protocol was able to elicit a much longer lasting therapeutic response than we got using the conventional stimulation. So, this was really exciting to us. And this now is a protocol that can be translated to the clinic. And in fact, we're starting to work with some neurosurgeons that are trying these protocols in patients.

Marie: I think that is remarkable. And just to maybe put some context around it, you mentioned "longer lasting". What are the lengths of the therapeutic effects that you're seeing?

Aryn: Yeah, so it's been a little bit hard to nail down a very specific answer to that question. But probably on the order of hours, we had some animals, we haven't tried with the electrical stimulation, but from our previous study, where it seemed like effects would last for at least eight hours. So, we are hoping that as we move into studies in humans, this will allow us to get a better sense of just how long the effects last. The hope would be that we can instead of delivering stimulation constantly, we will be able to deliver it for an hour or two once a day, or maybe every like five or six hours, turn the stimulator on, get the circuits working again, and then it just kind of turns off. And so, the hope is that we can reduce stimulation time dramatically by using these cell type specific interventions.

Marie: Well, I think that is really exciting. So Aryn, can you tell us maybe specifically what is the scope of this new funded study from The Michael J. Fox Foundation, and maybe what is the next step that comes afterwards in the timeline moving towards this ideal goal of enhanced therapy?

Aryn: As we have been speaking with neurosurgeons that are very excited to try this in humans, it became clear that the remaining thing that we really need to nail down is exactly where to deliver the stimulation. There are a few different sites within the basal ganglia that are FDA approved to deliver deep brain stimulation.

And these different sites might be able to better or not as well recruit these critical inputs that we need to get these cell type specific responses. So, one of the main targets for deep brain stimulation, the main one that is most often targeted is

called the subthalamic nucleus. That's another part of the basal ganglia circuit adjacent to the globus pallidus where we've been doing most of our work. But where we targeted in our deep brain stimulation study was a slightly different region of the globus pallidus called the globus pallidus internal segment. And that's also an FDA approved target for Parkinson's disease.

It's a little bit less common. The reasons that neurosurgeons choose to target one site or the other is honestly something that I'm not completely clear on myself. But we feel pretty confident that we can get these long lasting effects by targeting the globus pallidus internal segment. It's less clear if we can get them by targeting the subthalamic nucleus.

So, we have funding from The Michael J. Fox Foundation to deliver our electrical stimulation, in mice again, at these two different target sites that would be most likely to be targeted by neurosurgeons. So, we can compare, we can kind of rapidly screen in our animal model, our preclinical animal model. Are both of these sites going to be equivalent for using this biologically inspired stimulation? Will it only work if you target the internal globus pallidus? Will it also work if you target the subthalamic nucleus?

So, that's really kind of what we're working on right now is how critical is the site that you stimulate for determining how well you're going to elicit these long lasting effects.

Marie: Very cool. And then what is, I guess, the project that comes next? Maybe you find it's the GPE, maybe you find it's the STN, maybe you find both are equally good. What do you do with that information?

Aryn: I would say one of the very long term goals that, again as kind of a basic scientist, makes me very excited — definitely gets me out of bed in the morning every day — is the hope that with this electrical stimulation that we're delivering, we're not just kind of imposing some different pattern of activity. We're actually helping to fix the brain. So, we're hoping in kind of the long term where we'd like to go with this is to ask, after we've delivered this biologically-inspired stimulation for some period of time, whether that be a couple hours, a couple days, is there evidence that this is actually helping the circuits to stay in their healthy state longer and longer and longer, the more stimulation goes on.

And could we eventually get the system to a point where we hardly ever need to stimulate or we only need to stimulate once a day or maybe only once a week? So, that's the hope. And we have the tools we need to do these experiments, but they're very, very long and incredibly technically complex. So, we're at early stages of that, but we have lots to work on still.

Marie: Certainly, now a lot to look forward to, Aryn, it sounds like, and you mentioned that you've got some of these really important tools that you're working on. And I think having the right tools, and resources, and collaborators, all of these things are critical for accelerating the pace of research in Parkinson's disease. So, are there particular tools that you're using or resources that you are leveraging that you think are really moving the field forward and really helping you in your own work?

Aryn: Yeah, I mean, I can't overstate the importance of optogenetics in studying the basal ganglia. So, I guess I didn't go into it too much earlier, but this is the ability to take a protein that allows ions to flow across the cell membrane. So, the way that neurons generate their electrical signals is just by the flow of charged molecules across their cell membrane. And scientists discovered that you can take these proteins that are very good at pumping ions across the cell membrane — they were actually discovered in photosensitive algae — and channel rhodopsin is the most ubiquitous form of optogenetics that's used. "Channel" because it is a channel for ions to flow across the membrane. And "opsin," we have rhodopsin molecules in our eyes, and that's what allows us to sense light. So, when photons hit our eyes, the rhodopsin molecules in our eyes convert this light into like a chemical signal, and that's how it enters our nervous system. So, it was discovered that you can use the same general approach that's used in nature, but you can use these proteins that were found in algae, you can put them in mammalian cells, and that allows you to make these neurons light-sensitive.

So, you shine a light on brain tissue, which normally would not respond, but if these proteins are there, you can turn on and activate whatever cells are expressing this protein. And this is what's allowed scientists to really start to dissect the function of neural circuits with much greater precision than they ever have before. You can turn neural circuits on, you can turn them off, and that allows us to really figure out what are the key neurons to target in order to get the therapeutic effects that we're looking for?

What are the neurons you want to avoid because those are the ones that are giving the side effects? And then as we get genetic handholds or physiological markers at these different cell populations, that's what's really going to allow us to translate what we're learning in the research lab into better clinical treatments in patients. So, I think optogenetics has been incredibly transformative in our ability to understand the language of the brain and get better at understanding what circuits are the therapeutic ones to target.

Marie: Certainly, I agree. And I really like that you highlighted just the value of some of these biologically-inspired tools and approaches. I think that's a pattern that we're seeing increasing in the field as well. And in terms of answering some of these

tough questions, we need these sophisticated tools, like you said, to start to tease apart just all the complexity that's there. So, when you think about what remains in terms of the unanswered questions, the areas of opportunity in the field of Parkinson's disease research, what is at the top of your list, Aryn?

Aryn: I think combining these two research directions that I mentioned for deep brain stimulation — identifying when to stimulate and also what neurons to stimulate. So, just like if you were to go to physical therapy, if you break your ankle, and it's a really bad break, and so you're off your feet for a while, you kind of need to go for physical therapy to regain the muscles. Maybe even there's a muscle that's permanently damaged, but you can learn to work the other muscles in order to get by and regain function. And I think that for neurological diseases, and Parkinson's, but also a number of other diseases as well, there's going to be some brain circuits that are just permanently damaged.

But that doesn't mean that we can't still get around that damage and kind of work with what we have left. And I think that finding the key neurons, not affecting neurons that are either working appropriately or the neurons that are going to get the side effects, then being able to specifically target the therapeutic cells in the brain, and then also being able to do that at the right time. And that might mean reinforcing healthy patterns of activity when they're present. It could even be some combination of mental exercises that get your brain in the right mindset, so to speak, and then reinforcing that with the electrical stimulation, with a cell type specific stimulation pattern.

And I think that that is really going to be the future direction. I should also highlight that there's some really exciting work being done in the area of noninvasive stimulation. I can totally appreciate how it might be downright terrifying for a patient to have to undergo neurosurgery to get an electrode implanted into their brain. And I think that that's a barrier for a number of people. Even though I think it works really well, I totally understand the stigma associated with something like that. But there are tools being developed for either things that you can wear on the scalp that can deliver stimulation.

There's even attempts to put electrodes on the periphery, like on your hand or your arms, or maybe using some sort of sensory input like sounds or lights that can drive patterns of activity in your brain that may be reinforcing. And then if you can use that external stimulation that doesn't require brain surgery, coupled with healthy thinking exercises, I think that those are the kinds of directions that are really going to transform the way that we can treat many different kinds of neurological diseases.

Marie: I love it. Well, Aryn, thank you so much for giving me and our listeners a lot to think about today. And I know we talked about a variety of different areas of your

work. And I think your work has tremendous potential for impact. So, perhaps as a closing note here, can you share how you see your work, bringing us closer to finding a cure for Parkinson's or really contributing to improved therapies for people who have Parkinson's today?

Aryn: If nothing else, our work really highlights that it is possible to bridge what's going on in the research lab and translate that into something that can be implemented in humans in a very short period of time. I've been incredibly moved by my interactions with Parkinson's patients. I think many of them recognize that these lofty goals that we have for treating disease, they are achievable, but it is such a long road to get there that the generosity of so many of the patients that I've met and just their willingness to say, I understand that maybe you won't be able to help me, but I think that the research you're doing might help the next generation. I think that evidence that all of this basic research that is being funded can make a difference. And we can use what's going on in the lab to discover the critical cells to target and then generate strategies to actually target those cells that will allow a new kind of clinical trial. I think that that is really what I'm most excited about our research having an impact on.

Marie: Well, I think that is wonderful, Aryn. We appreciate all the work that you and your lab are doing and the contributions that you're making to Parkinson's research. And we really appreciate you joining us on the show today to share your work. So, thank you so much for your time.

Aryn: Thank you very much.

Marie: Well, Aryn, it's been a pleasure to have you here. And listeners, it's been great to have you here with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit michaeljfox.org/researchresources. And you can find new episodes of this show each month on the MJFF website or on your favorite podcast platform. When you have a moment, please subscribe to our show to make sure you don't miss our outstanding lineup of upcoming episodes. We look forward to connecting with you again in our next episode of the Parkinson's Research Podcast.