Synaptic Dysfunction in Rare Neuroimmune Disorders

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[00:00:04] **Dr. Haiwen Chen:** Thank you so much for having me. Thank you for everyone here for welcoming me into this wonderful community. That's been the best part of this journey so far. I had the great privilege of being one of the James T. Lubin Fellows which actually gave me the opportunity to train as a pediatric neuroimmunologist at Hopkins.

[00:00:27] I came into this field because when I was doing my residency there, we didn't have a pediatric neuroimmunologist. When these kids came in with rare neuroimmune diseases, they need a neurologist, and I became that person, and the training gave me better knowledge and capacity to be the right person for them.

[00:00:47] In addition, as I've gotten into the field, I've been really struck by how much work still needs to be done in understanding what the basic mechanisms of these diseases are. My background is in bench science, and so, I wanted to use that also to contribute to our understanding. And the work I've done in the last year, which I have the opportunity to share with you, so I hope you find something interesting in it.

[00:01:18] So, being on the second day of presentations, I don't have to go into these diseases, nor do I have to tell this very sophisticated group that studying rare neuroimmune diseases are important. In children, in particular, what's interesting is that MOGAD probably takes up about, or comprises about 30 to 40% estimated, but probably even higher now that we have more sensitive tests available, of pediatric demyelinating syndromes.

[00:01:49] This, in contrast to NMOSD, which is 5% or less, or multiple sclerosis, which is about 20% of these cases. So, in children, actually, MOGAD is a large percentage of these demyelinating syndromes, which really makes it even more important for us to understand it in more detail.

[00:02:11] And what I want you to notice from these two MRIs, which are actually from two of my patients, one with MOGAD and one with NMOSD or aquaporin-4 positive NMOSD, is that the lesions, these bright white spots, actually incorporate not just the white matter, which is these darker grey areas of the brain, where a lot of the glial cells largely comprise and surround the neurons, but they actually incorporate a lot of the grey matter, this lighter grey area around the rim where the neurons of the brain largely reside.

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[00:02:43] And so, it always occurred to me looking at these MRIs that these diseases probably not only are demyelinating or affect the glial cells of the brain, but probably also affect the neurons of the brain. This also bears out on a practical level. This is a recent study on 30-some MOGAD patients, these were adult patients.

[00:03:06] But, they found that about 20% of them -- so a fifth of them -- had some form of cognitive impairment, which they measured as scoring less than the 7th percentile of their demographically matched peers in at least two different domains of cognitive function, some of the things you see here. They looked at these measures in relation to volumetric MRI studies that they did on the same patients.

[00:03:32] In the light blue, in these columns, are the patients with MOGAD who had no cognitive impairment, and in the dark blue are the patients with cognitive impairment. And you'll see, actually, the top graph being white matter and the second graph being grey matter, that they actually had more significant decreases in their grey matter than the white matter. And that held true for the deeper grey matter, the structures even lower down in the cortex as well.

[00:03:57] Really making, I think, the argument that understanding more about the neurons are important for understanding some of the disease pathology. And so, zooming in a little more on what comprises these parts of the brain, the neurons in the brain actually form circuits, unlike those of your devices sitting in front of you, and that's how neurons actually communicate with each other.

[00:04:20] These circuits underlie our abilities to do everything, including me standing in front of you talking. And if we look even more in depth at a single one of these neurons, they actually form these very, somewhat odd but intricate structures. This is what we call a dendrite, and on it are these little protrusions called dendritic spines. And actually, on the spines themselves are actually where the connections between neurons form, what we call synapses.

[00:04:50] And a synapse is comprised of a presynaptic neuron, where these bundles of neurotransmitters are released and that communicates with the postsynaptic neuron, the second neuron, through these receptors. And because of ions flowing through the membrane, this actually generates what's similar to an electric current. So, very much like those electric devices that we use.

[00:05:15] And the amount of conduction, the amount of current, how this neurotransmission is modulated actually is believed to underlie the processes by which we learn, by which we have memory, and by which we have cognition. And so, understanding these processes are really important for all of those cognitive aspects.

[00:05:34] Now, how does this tie into our rare neuroimmune diseases that affect the glial cells? Well, neurons don't obviously fire or work well on their own. In fact, when neurons are grown on their own, they actually fire very little. And so, they really depend on the oligodendrocytes, which are the cells that create the myelin, as in the demyelinating diseases, as well as astrocytes, which actually provide a ton of metabolic support for the neurons.

[00:06:02] And in normal function, they live in this very privileged central nervous system brain space that's very distinct from the periphery and has a blood brain barrier to form as a wall. And when neuroimmune diseases happen, parts of these inflammation that would normally be in the periphery enter into the CNS. MOGAD targets a protein on the outer lamellar surface of oligodendrocytes, and aquaporin-4, on the other hand, targets a protein on the foot processes of these astrocytes.

[00:06:35] And so, while we've understood more about these individual cells, about the inflammation, about some of the immunology, what has really been understudied is how all of that actually affects the neuronal

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signalling itself -- those processes that are so important for our cognition. So, that's what I'm interested in studying, in understanding how these neuroimmune diseases actually disrupts that synaptic function.

[00:07:00] And to start that process, I used an in vitro model so that I can grow these cells in a dish, so I can study them in more detail, have more control over them. Currently, I use rat or mice brains that I then use their neurons, their glial cells, and grow them in vitro. Our standard conditions, actually the kind of conditions I've always studied for most of my career, which I'll call these 'new criteria' just for short going forward, contain neurons and astrocytes.

[00:07:32] But actually, if I give factors to differentiate oligodendrocytes from their precursor cells, I can have cultures that can include neurons, astrocytes, and oligodendrocytes. And this is actually what it looks like when I study these cells in a dish. GFAP is a protein that labels astrocytes. MOG, as we know, labels mature oligodendrocytes. So, in these new cultures, they have astrocytes but not oligodendrocytes; and in these all-cultures, they have astrocytes and oligodendrocytes.

[00:08:04] And then, I want to evaluate how the synapses, these neurons differ within these two conditions. Just a little more detail on the all-cultures: in addition to MOG here, I've labelled myelin basic protein, which is a protein that helps us identify where it comes into contact with neurons. This is neurofilament, a protein critical in axons of neurons, and then, VGLUT, which labels the terminals where the neurotransmitters are released.

[00:08:38] And you can see that when we overlie all of these images on each other, there are areas where the oligodendrocyte proteins and the axons overlie that are exclusive of where the axon terminals are. And these are actually segments of myelinated axons. So, we can use this in vitro system to study demyelinating diseases.

[00:08:59] What I've seen already is that just the presence of the oligodendrocytes or in these conditions seems to already influence the synapse composition over development. Looking at the top here, these all-cultures I've looked at, this is a marker of the presynaptic neuron, a marker of the postsynaptic neuron, and you can even see by eye how much brighter it is, and all things given equal amongst the conditions.

[00:09:25] And so, looking at these different neurons and synapses over time, I actually see that the synapses are actually very similar in number but are a lot larger when grown in the all condition. And what does this mean functionally in terms of their signalling? Well, not only can I look at them and look at their size and shape and whatnot, I can also measure their actual signalling. So, as I mentioned before, neurotransmission actually comes across as an electrical signal because of the ions moving across.

[00:09:54] So, if I grow the cells on these specialized plates, which have electrodes at the bottom, they actually allow me to sample the amount of activity amongst the neurons that are grown on top of it, which could be measured as voltage changes reflective of the action potentials or the neurotransmission itself.

[00:10:13] So, if we look at the raster plot that corresponds to one of these wells, each of these rows are a single electrode, each of these little vertical lines are single spike, so some local activity from the neurons firing. And then, because we have multiple electrodes, we can look at how the activity amongst different parts of the wells are related to each other, so that if everything is firing at once, we actually see that it's a network burst -- all of the neurons are firing in a synchronous way.

[00:10:43] This is one of the wells that I grew. You can see all these spots. You can see the electrodes that are really dark, but then you can also make out all of these things growing on top, which are the cells themselves.

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This is a plate in which the left half of the plate, the wells on the left, are all-cultures, and the right half are new cultures. And hopefully, this will play. You can see those bursting of pseudo activity reflects the activity in these different conditions.

[00:11:12] And the left half of the plate, I hope you can tell even just visually, is firing at a much higher rate than the right half. Now I just have to be able to move on. There we go. And if we look at the raster plots for these different conditions, you can see these all-culture neurons are firing at a much higher rate in a much higher network synchrony. You don't see these spare spikes here and there compared to the new cultures.

[00:11:39] And if we look over weeks starting from when they start firing to when they peter out --because, again, I'm growing them in a dish so I can only keep them alive for so long -- we see that the all-cultures actually reach a level of frequency over time that the new cultures never arrive at, and same for the amount of network frequency.

[00:12:02] Then, I wanted to understand: is this increased frequency at an individual cell level, a subset of the population, or the whole population? Because you could think that some of the neurons, if they're perhaps myelinated, that segment of neurons may be more active in these cultures compared to potentially if somehow there is a broader effect -- maybe a secreted protein, maybe some other metabolic support -- that increases the activity on a global scale.

[00:12:29] To do that, I take advantage of calcium imaging, which is a tool we use to measure action potentials or activity on an individual cell basis. It's a genetically encoded protein that can be expressed in the neurons, such that when they fire action potentials -- which allow in that influx of calcium, one of the ions that goes across the membrane -- it binds the sensor and causes an increase in a fluorescent signal that we can see using microscopy.

[00:12:57] So, you can see on the left, this is a neuron that was grown in all-cultures. These flashes of brightness are the neuron actually firing action potentials. You can see a plot of the activity as a function of time, and you can see the neuron in these all-cultures are extremely active. In comparison to neurons grown in the standard conditions without the oligodendrocytes, which are far less active. They still fire, but just not as intensely or as much.

[00:13:31] This is not to say that those neurons aren't capable of firing. When I put them in a condition here now with the addition of a 0-magnesium solution -- which removes a block for a different type of receptor, but in general, just increases activity -- you can see that both the neurons in the all-culture and the new culture are actually capable of firing at very high frequency. It's just that the all-culture neurons are firing that high even at baseline without that extra stimulus.

[00:13:57] And when we look at the group activity altogether, the all-neurons fire at a frequency that is, at baseline, lower. It can be increased if a stimulator adds a condition that increases activity, but that all-neurons actually fire at a rate that is high already and does not substantially increase, even adding more stimulus for activity.

[00:14:25] So, hopefully, I've convinced you or at least suggested to you, some of my early work suggesting that, in these conditions of neurons with oligodendrocytes, they actually may have larger synapses and are far more active in terms of how frequently they fire, but also how frequently they fire together. And that shift is even at the single-neuron level across the population of neurons growing within that condition.

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[00:14:57] Moving forward, I really want to understand the role of oligodendrocytes, how they change neuronal signalling, and how they change synaptic signalling. And then, ultimately, how that's disrupted when we have outside insults, like MOG antibodies or other sources of inflammation. And similarly, moving from oligodendrocytes to also studying how the astrocytes also participate, how that's affected by the aquaporin antibodies as well.

[00:15:23] And so, hopefully, this will give us little more clues as to the fundamental mechanisms that's underlying some of the pathology that we're seeing. I think this is also such a sophisticated crowd that I don't need to explain that we still need better biomarkers for determining relapse, for determining severity of disease, for determining whether treatments are working.

[00:15:46] And without those better biomarkers, it doesn't give us a target for what we test when we come up with clinical trials and new therapeutics. And so, that's why I do basic science. I think that's how I contribute. And with that, I just want to thank so much this organization for giving me the opportunity to have done that through that fellowship.

[00:16:07] I have had great mentors at Hopkins. I'm actually split between the neuroscience department and neurology, so I really bridge that physician-scientist gap. I've had the benefit of having Carlos Pardo as one of my mentors, who you've met today, and wonderful collaborators as well who have been so instrumental in my work. And with that, I'd love to take your questions.

[00:16:48] **Audience Member:** Hi. Thanks very much. Oh, can you hear me? Okay. Just wondering if maybe you can comment a little bit on microglia. I know you mentioned glial cells early on, but just curious, in my limited search, seems that the presence of them can help in the myelin process. But also, I've read that the presence of them can actually help in the damage of the myelin process. So, I was just curious if that factors in here.

[00:17:14] **Dr. Haiwen Chen:** Yeah. So, I think the microglia is a whole other really interesting line of work that I will give the caveat that I don't actively grow microglia in my cultures nor do I have a great amount of expertise in those cells yet. I'll maybe get there at some point.

[00:17:32] I think the microglia are actually also getting their heyday in terms of understanding how much more they contribute also to synapses. Some of the recent work amongst my colleagues suggest that microglia have the ability not just to take up damaged cells, but even to take up parts of damaged cells.

[00:17:51] So, they could potentially actually help distinguish the process of what's a neuron that's too far gone versus something that they could potentially take a part of -- take some of the waste products or parts and actually eat part of the cell or to help save the rest. And so, not only I think are they important in the global role and part of the native immune cells of the CNS, but they actually may have a more finessed role as well.

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