Transcript of Cerebrum Podcast—The Brain's Waste-Removal System

Guest: **Helene Benveniste**, M.D., Ph.D., completed her M.D. in 1989 and Ph.D (Doctor of Medicine) in 1991 at the University of Copenhagen, Denmark. She trained in high field magnetic resonance imaging at the Center for In Vivo Microscopy at Duke University Medical Center and went on to take her Family Medicine internship and residency in Anesthesiology, also at Duke University. Benveniste has been actively engaged in translational research for more than 20 years at Brookhaven National Laboratory and Stony Brook University. In 2016, Benveniste moved to Yale University, where she joined the Department of Anesthesiology. Benveniste's research program at Yale is focused on understanding how the brain's glymphatic system (implicated in metabolic waste removal) is affected during different states of arousal (sleep, anesthesia and emergence from anesthesia) and in neurodegenerative diseases.

Host: Bill Glovin serves as editor of *Cerebrum* and the *Cerebrum Anthology: Emerging Issues in Brain Science*. He is also executive editor of the Dana Press and *Brain in the News*. Prior to joining the Dana Foundation, Mr. Glovin was senior editor of *Rutgers Magazine* and editor of *Rutgers Focus*. He has served as managing editor of *New Jersey Success*, editor of *New Jersey Business* magazine, and as a staff writer at *The Record* newspaper in Hackensack, NJ. Mr. Glovin has won 20 writing awards from the Society of Professional Journalists of New Jersey and the Council for Advancement and Support of Education. He has a B.A. in Journalism from George Washington University.

Bill Glovin: Hello out there, and welcome to the *Cerebrum* Podcast. I'm editor Bill Glovin and today's guest on the phone with us is Helene Benveniste, professor of anesthesiology at Yale University, and author of our most recent Cerebrum article, "The Brain's Waste Removal System."

Helene, a native of Denmark, came to the U.S. in the early 1990s and, before moving to Yale, conducted research at Duke and Brookhaven. Her research at Yale is focused on understanding how the brain's glymphatic system is affected during different states of arousal and of neurogenerative diseases.

Welcome, Helene, and thanks for joining us on the phone. I know you recently returned from a late-summer trip to Denmark, how did that go?

Dr. Benveniste: That was wonderful and I got to see all my friends, and spend some time with family. It was really nice.

Bill Glovin: Great. I think just the term, "glymphatic pathway" can intimidate people. Can you explain what the pathway is in simple terms and why it's important?

Dr. Benveniste: Yes, I think it's important to keep in mind that in all body organs, except for the brain, we have what we call "lymphatic vessels." So, these are specialized vasculature, not arteries, not veins, but there's as whole system for example in

our muscles, that take care of transporting fluid and water and take care of all the waste accumulations that we have in the tissue.

However, there are no lymphatic vessels in the brain itself. So, the brain had to, or we think the brain had to, develop another system, and that system is what we call the glymphatic system, which was recently discovered and that everybody is so excited about.

So, in briefly, this system, instead of being a separate system as it is in other vessels, it actually runs along the vessels in the brain, outside the vessels and it helps spinal fluid move into the brain and communicate with the tissue and the fluid around the cells and by communicating with the tissue around the cells, the spinal fluid actually moves and actually facilitates waste clearance. It's a complicated system to grasp and its function is complicated, but I think in simple terms you can think of it as a transit passageway for cerebral spinal fluid through a system that runs along the vasculature.

Bill Glovin: You began your career in MRI, and your title is "Professor of Anesthesiology". That suggests imaging, which seems quite different from waste removal. How do those two specialties relate?

Dr. Benveniste: Yeah, that's a good question. I came to the U.S., and I got to work with magnetic resonance imaging, and being able to image a system using non-invasive technology as magnetic resonance imaging, is a unique, I would say both, it's a tool but it's also enabling to ... you can begin to understand complex systems because you can look at different components of it, non-noninvasively, and in three dimensions.

And when I was approached a couple of years back by Dr. Nedergaard about this new discovery, my interest in visualization using magnetic resonance imaging really came to, I would say, it was really helpful, because it is necessary to have a system like magnetic resonance imaging or an imaging modality that can capture ... let's say the whole brain in real-time. And in order for us to be able to look at this system and understand how it works.

Bill Glovin: When you look at the system, do you look at it on human subjects yet?

Dr. Benveniste: Well we started looking at rodents, both mice and rats, and then the techniques that we developed in the rat has actually been shown to work in the human brain also by Dr. Elda and Dr. Ringstad, especially in Norway. They ... it's a radiologist and a neurosurgeon and they have actually published a couple of papers that are using ... they're basically doing what we are doing in rats, but instead they are injecting it into the ... like when you do a spinal anesthesia, instead of doing anesthesia, they're injecting a contrast agent into the spinal fluid, and then they are waiting for some time and they can see how that contrast agent is moving into the brain and draining out of the brain, and that's another measure of the glymphatic system.

Now, this method is not easy to use. It's not routine. You can't do it on everybody, and it's not something you want to do, because it's invasive. So there's another ... there's many other groups that are working on other ways to capture this system using imaging and different approaches which does not involve injecting a contrast agent, but it's too early to really say whether or not we have complete proof of this system's existence in the human brain.

Bill Glovin: Getting back to the origins of the system in the first place, your article begins by describing how Maiken Nedergaard's research team at Rochester sort of rediscovered that the brain removes waste based on a previous study. It seems so important, yet the discovery went undetected for a long time. Is that something that frequently occurs in research?

Dr. Benveniste: Yes, well, I don't know how frequent it occurs, but I think there's many stories of that nature. For example, with the stem cell story has a similar ring to it because there was an investigator who was reporting that there was indeed cells that could multiply in the central nervous system, and it was not believed for many years, and then suddenly it came to light that indeed this was true, and it was ... you sort of rediscovered, you could say.

And the same thing happened with a lymphatic system. Reynolds and [Pathgrady in, I think it was, 1985, actually did some very elegant studies where they were injecting horseradish peroxidase, it's a molecule that you can track after ... in the brain, after you inject it into the CSF, and they were able to see all these channels that we see today along the vessels. And they did some very elegant measurements on it and reported it. And they measured intracranial pressure, they looked at pulpability of the vessels in relation to the system, and then it was sort of, other people, I think, tried to reproduce it and I think the conclusion from all the discussion with Dr. Nedergaard has ... it is ... the reason I think it was not being ... people were not able to reproduce it is that in order for us to do experiments, often we have to open up the skull either to put in contrast or to look at the brain so that we can see how things move with a sophisticated microscope, etc.

Dr. Benveniste: And so every time you ... this system works, this glymphatic system that we're working on, it's a very low-pressure system, and if you open the skull and expose the brain to atmosphere, all these pressure differentials that we see, that are very small and subtle disappear. And so you simply cannot ... you won't be able to see the movement of let's say, waste particles, along the vessels to the same extent.

So, I think this was a reason for ... that it simply went unnoticed until Dr. Nedergaard started reproducing the old experiments again.

Bill Glovin: For people who haven't read the article, you describe that the research team led by her felt they were really onto something, and they asked you to come to Rochester and work with her research team. Can you describe what your role

was and the impact it had, not only on their research, but on your research, going forward?

Dr. Benveniste: Yes, I mean, I was contacted, I think it was 2012 by Maiken and we knew each other back from our days in medical school in Copenhagen and Maiken knew that I was working with imaging and I was at Brookhaven National Lab and she was in Rochester, so it wasn't that far of a distance.

And I think at that point, he realized that she needed another approach to look at this system in the whole brain. I mean, the very beautiful, two photon microscopy techniques that they are using to look at these small vessels in ... on top of the brain; you can't use that technique in the whole brain. And the ultimate way they were looking at it was to inject something, and then let it circulate, and then they sacrificed the animals and cut off the brain and looked at it after death, and ideally you want to look at it in real time in the live animals.

So, I think she realized she needed something that could do this in three dimensions, and she thought of me because of my work and also I had worked as an anesthesiologist. I had looked at spinal transport in previous studies and had been interested in the brain, and imaging the brain for many years.

So anyway, I went up there and they presented the data and I was truly fascinated. And it took me a long time to grasp the results and sort of get a feel for "What is this system, what is it doing?" And thinking about it, I said, "Well." I came back to my team at Brookhaven National Lab at that time, and I said, "Let's try and start by injecting small amount of contrast into the spinal fluid and look at it in real-time as it moves through the brain of a rat," in this case. Because it's relatively simple, and so we started working with it, and of course you think it's simple and it turned out to be much more complex as we got into it.

But, we managed to come up with, I could say, a test bed, or a diagnostic platform, or at least start probing and quantifying these transport parts, I would say that because the glymphatic system is complicated. It's not the same everywhere in the brain, it's variable, and we are still trying to understand this system as we speak. So, when you ask me, what has it done to my research? I mean, what it did was, it turned me into a ... I would say, almost 100 percent glymphatic system researcher, because that's what I've done for the past, I would say, five years, it to try and refine the techniques so that we can understand how this system works, not only in the rodent brain, but also in the human brain.

And I'm collaborating now with several groups to do these studies in humans, and they are already ... we are not the only ones, obviously, I'm working with Dr. Noravodkum at NIH, we have two studies on the way, and, which only seems to support that this system is existing in the human brain, but we still have a long way to go. But it's certainly changed my research focus.

Bill Glovin: When you Google glymphatic system and you look at the literature, it seems like many of the articles are tied to someone's quality and quantity of sleep, in terms of playing a role in waste removal. What is the role of sleep in this research?

Dr. Benveniste: Yeah, this is the most intriguing part of this system, I must say, and that was when it was discovered, also by Maiken's team, that when you ... The experiments were very complicated that they had to do, but basically what they found is that when you have an animal awake and trained to be awake while they do the experiments of major glymphatic system function, and then you measure glymphatic system function when they're awake and then you wait until the animal goes to sleep, they can see a huge difference in glymphatic transport. So, when you sleep, it seems like the particles or the waste particles are moving much more rapidly through the brain than when you are awake.

And, so, they were trying to understand why that is, and one of the ways to look at it was that the cells are surrounded by fluid, and the cerebral spinal fluid has to move in from the space along the vessels and into that space around the cells, called the interstitial space. So, when you sleep, it seems like the cells shrink a tiny, little bit, and so the fluid can actually move better because the space between the cells are getting a little bit larger. So it's like there's less resistance to fluid movement through the brain when you are asleep.

So, it sounds all very simple, but it's actually very complicated, but because it's not all stages of sleep it seems like. You have to ... we have all kinds of sleep stages, or at least we have four, according to the literature, so we are talking about non-REM sleep, nonrapid-eye-movement sleep, and there's a certain stage that is called "slow-wave sleep", and in slow-wave sleep it seems like the space between the cells is bigger than in the other stages.

And so, it was thought that when you are in this ... when you are in slow-wave sleep, you actually remove, let's say, amyloid beta, which is a compound which has been ... it's a waste compound that is part of the pathogenesis of Alzheimer's Disease. They actually showed that this metabolic waste compound is being cleared faster.

So, we were very fascinated by this. And then we went on to actively also look at different sleep positions. So, we looked at ... and this was done in rats again, so we did glymphatic transport system measurements in different body position doing when an animal were anesthetized, when it was lying with a head up, it's equivalent to you sleeping in a chair and you fall asleep in your chair. And then we did a, when you are lying on your back, and then we did it when the animal was on the side. And we found that when you're sleeping on the side, and on the back, the transport was much, much better than when you are sitting in a position with the head above the heart.

And, so, this was another discovery that we were fascinated about, because we know that from the human literature, we know that actually a favored body

position is on the side. This has been done with video recordings and there are many reports on that, that if you sleep on your side, the human seems to ... even humans and large animals seem to favor that position.

So, it may all be sort of part of, or it has been suggested that the role of sleep ... part of a role ... part of the ... of why we sleep has to do with removing waste in the brain that has accumulated during the day.

Bill Glovin: Where would the waste go?

Dr. Benveniste: Yes, that has been debated, and I think there was just now, I think last month, a very beautiful paper from Kipnis' group that was coming out with a series of studies that suggest that the lymphatic, authentic, or real lymphatic vessels are actually positioned outside the brain. There are membranes that are covering the brain called the leptomeninges, or the meninges ... they're called the dura of the brain. It's a very thick membrane, and there are real lymphatic vessels sitting in that membrane.

This was shown by two different groups at the same time a couple of years ago, and they have also shown that these lymphatic vessels in that membrane, the dura, or at least at the level of the dura, are really functional in the sense that they can actually, if you inject waste into the brain and you wait, you can actually see that waste moving into these authentic lymphatic vessels that are sitting in the membrane covering the brain, but they also cover the nerves, the cranial nerves like the olfactory nerve that goes to the nose cavity, I mean the nasal mucosa, the vagal nerve, there's a lot of ... there are 12 nerves that are actually exiting the brain, and the dura kind of exits or covers them on their way out, and it is thought that there was spinal fluid, or ... and interstitial fluid through the glymphatic pathway is merging with lymphatic vessels at these sites, and that's how it drains.

But this has never been ... I mean, the exact route, I mean visualizing this in real-time, I don't think anybody can say today that everything that is cleared goes through these meningeal lymphatics, but the field, I mean, the field of glymphatics would say that this is a main drainage pathway.

But I think we may see more data in the future that's going to clarify this, so I'm still waiting for more evidence as to exactly how this occurs and the most intriguing thing was that, I think a year or two ago, some, a very beautiful study was done at the NIH showing that these lymphatics in the meninges can also be captured in the live human brain. So, once you are able to do that, new studies will come out, I'm sure, that will probe that drainage pathway better.

And I think, one last think I want to say about this, is that when you look at the normal lymphatic system, or the real lymphatic system, that is in the rest of the body, all the fluid and waste that is drained, let's say from our muscles, actually drains directly into the systemic ... into the blood. But, if the same is not true for

the brain. If the glymphatic pathway is the main waste drainage pathway of the brain, it drains into lymphatic vessels before it drains into blood. So, that's another difference between these two systems.

And I think ... I think it's still a little unclear if that's the only drainage pathway that exists.

Bill Glovin: Well, I'm sure no one knows for sure, but what's your guess, if they system breaks down, you mentioned amyloid, does the amyloid not be carried out, and then it's kind of retained in the brain causing disease?

Dr. Benveniste: Well that was what that new study showed that just was published, and it was where they actually took away those ... they had a way to take away those lymphatic vessels in the meninges and they could see that the glymphatic pathway got dysfunctional doing that. So, I think that was a very strong connection.

But we just don't know the anatomical, exactly how these two systems connect anatomical. That's the problem. It's not completely clear, but they're certainly linked to each other. That I'm not in any doubt about.

Bill Glovin: Has there been any link to diet?

Dr. Benveniste: That's a good question, no, but one paper was published about ... on alcohol, that a certain amount would be breaking down the lymphatic system and a small amount would ... I don't think it enhanced it, but it didn't affect it too much.

So, that's the only thing I can relate to diet, but I'm sure there will be future studies that may come up with more studies in relation to that. We simply do not know.

Bill Glovin: You mentioned the “glymphatics field,” and since this is a discovery that came about as recently as 2012, what is the state of the field? Are there a lot more researchers looking at this? Is it growing? Are there many more studies coming along and many more expected?

Dr. Benveniste: Yes, I mean, it's just a matter of going onto the database, the PubMed.gov database and typing the word "glymphatic." You can see year-by-year how many publications are coming out, but it's clearly risen. I have no doubt that the field will continue to grow.

There are also controversies and ... which is of course necessary, because you have to reproduce data, you have to debate data, you have to look at it in relation to the original concept and the original concept that was introduced in 2012 by Maiken's team has changed somewhat, I mean, we've got the piece of the puzzle with the meningeal lymphatics; now we know a little more about

those in terms of how ... that they are functional and they are involved in clearing amyloid.

And I think now the big thing is going to be to really show how and if it works in the human brain, and how it relates to disease. That, I'm sure, is ongoing, and many, many groups are working on this. And because, if it ... if we can understand how the system works, and if we can understand how we can accelerate its function or at least maintain it across the lifespan, this is a possibility. I mean, it's a possibility of at least postponing or preventing neuro-degeneration. In particular, Alzheimer's Disease, or small vessel disease, which is also a burden in terms of dementia in the world.

So, I think this is why everybody ... I mean, at least the people that I meet at meetings that are in the same field as I am now, that I call the glymphatics field, I think everybody are intrigued and are throwing themselves into studying cerebral spinal fluid movement in the brain to understand it.

Bill Glovin: Well that's a great account of this very important brain function, and I can't thank you enough, especially since you just returned from your long trip and have all the start-chores of the academic year before you.

You can find Helene's article at Dana.org, and it is well worthwhile.

This podcast is brought to you by the Dana Foundation in New York City. Thanks for listening.