# A Conversation with Edward Boyden

Interviewer: Rebecca Leshan

*Director, Banbury Center, Cold Spring Harbor Laboratory*

Edward Boyden is the Y. Eva Tan Professor in Neurotechnology at the Massachusetts Institute of Technology (MIT), Associate Professor of Biological Engineering and Brain and Cognitive Sciences at MIT's Media Lab, McGovern Institute for Brain Research, and Koch Institute, and has been selected to be an Investigator of the Howard Hughes Medical Institute.

**Rebecca Leshan:** I wonder if you could give a quick snapshot of your research.

**Dr. Boyden:** If we want to understand the brain, we have three technological needs: to see what's going on in the brain with high-speed precision, to map the molecules and the organization of the brain, and to control the high-speed dynamics. We've been working a lot on extending tool sets into these three directions. For control, we've been trying to perfect optogenetic control of neurons and also to develop noninvasive ways to focus the effects of electricity deep in the brain. For mapping the brain, we've been working on ways to physically blow up the brain until it's up to a thousand times bigger in volume so that you can map the very finest connections.

**Rebecca Leshan:** Physically?

**Dr. Boyden:** That's right. So we can take a piece of brain tissue and magnify it physically—it grows before your very eyes—until it could be a thousand times or more larger in volume.

**Rebecca Leshan:** And this is expansion microscopy?

**Dr. Boyden:** That's right. And finally, to watch the high-speed dynamics of the brain, we've been trying to work out, basically, the opposite of optogenetics: to get neurons to report their electrical activity in the form of light. That's a hard problem, so we've been developing robots that could do directed evolution and make these molecules in the laboratory.

**Rebecca Leshan:** What you do feels so different from what a lot of researchers are doing. You don't have a specific disease state that you're focused on. You're really about developing tools and that has such a huge impact on the field. How does that differ in the way that you approach a research topic or the field?

**Dr. Boyden:** We don't focus on a single disease because we want to solve all of them. I've thought a lot about the different diseases and also basic science questions like "What is a memory?" or "How does a decision take place?" And it was very clear that there are so many problems, and so our strategy is to take a step back: What's the underlying problem that, if we solved it, would solve all these other problems. So by building these tool sets and giving them out to thousands of groups—we do some basic science in our group but most of the work we do is either collaborative or through teaching the tools to other people—we think we can help solve all these problems over an extended period of time.

**Rebecca Leshan:** That really fits with your training because you started as an engineer, right? Which I think of as "What can we build?" or "How can we build it?"

**Dr. Boyden:** Well, I started my training in chemistry for two years working on an origins of life project, of all things. Then I switched schools and started studying physics and electrical engineering. By that point I knew a lot of stuff, but I needed a really good problem to work on. It seemed like the brain—I was always very philosophically inclined, ever since I was a kid—had real consequences for understanding the human condition, but there are also lots of practical things we could do if we could help heal the sick or prevent disease.

**Rebecca Leshan:** This not only spans a lot of disease states but you're spanning a lot of model organisms and even, hopefully for the future, to think about humans. Has that been difficult, or has that been an easy transition between different types of models?

**Dr. Boyden:** The basic science we do, thinking like a physicist, is all on very small organisms. I would love to solve a simple organism, like the worm *C. elegans* or a larval zebrafish over the next five to 15, who-knows-how-many years it will take to do that. But as far as building tools that everyone can use, we do our own human experiments. We do our own mouse experiments. We try to really validate these technologies in a wide variety of species so that everybody—as many people as we can [help], anyway—can use our technologies.



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**Rebecca Leshan:** In line with thinking about everybody being able to use your technologies, you have in a remarkable way been very open with all of your protocols and your technologies, getting things out there, even before they're published sometimes. Is that a philosophy you've always had or is that something new, as you became a principal investigator?

**Dr. Boyden:** I think it's kind of the obvious path. If you build a tool and nobody uses it, what's the point? We've always had the policy of giving out everything for free to academics and nonprofit scientists and so forth. That's both charitable, but it's also self-interested. Again, what's the point of our existence if we don't do anything useful?

**Rebecca Leshan:** Is there anything at the symposium that surprised you? Is there anything that has really inspired you here? Or even if not here, in the field today that's really exciting you outside of your own work?

**Dr. Boyden:** Lots of things. I feel like there is a lot of interesting physics that's being discovered about the brain. We're hearing about new forms of energy like ultrasound, and other ways of interfacing to the brain using clever and novel strategies from different parts of science. I also think there's a real interesting connection that's forming between the basic science and the translational side, so hearing about people's studies about how mapping the brain can lead to new targets for treating depression with electrical stimulation—these kinds of topics really show the power, not just of technology, but bridging the science/translational gap through real experiments and real results.

**Rebecca Leshan:** Are you collaborating with clinicians in some of the work that you do?

**Dr. Boyden:** A lot. We've given our tools to literally thousands of groups at this point. We have close collaborations with maybe a hundred groups where we really work side by side with people. For example, there's a way of noninvasively stimulating the human brain through focusing electric fields through some clever tricks that we've stumbled across, and we are working now with a number of people. In fact, we have requests out from dozens of groups to collaborate to try to apply this in different kinds of diseases ranging from Alzheimer's to tinnitus to depression and everything in between.

**Rebecca Leshan:** How much interaction do you have with industry? What you're doing is very innovative and I can imagine a lot of biotech and pharma companies are very interested.

**Dr. Boyden:** We've done technology transfer to a lot of pharmaceutical companies and device companies. Several start-up companies are also licensing technology from us, all the way from discovery to treatments. We also spun out several companies of our own. I co-founded, with Professor Li-Huei Tsai at MIT, a company to try to develop media that you could watch or hear to treat Alzheimer's disease.

We have other projects, too, like this expansion method. If you want to detect diseases early—I think for something like eight out of the top 10 leading causes of death, if you can catch the diseases earlier, you could help people more. So what's the problem with detecting diseases early? Well, it's very subtle, the changes that occur early in a disease. So this expansion method, where we blow up a piece of tissue a thousand times or more, if you could use that to blow up, for example, a cancer biopsy and diagnose it earlier—"Oh, that's not good … oh, that's benign…"—that could really help save a lot of lives. We've spun that out as a company, as well.

**Rebecca Leshan:** Have you thought about tools for other fields like immunology? Or are you squarely staying within the brain?

**Dr. Boyden:** The brain is like a mountain. The climb to the top is a long road, and along the way there's lots of points in time where you can kind of spin out other projects. Last year, for example, we published our first paper in the field of cancer biology. It didn't mention the brain once. We worked with some pathologists from Harvard Medical School, and we showed that if we expand human breast cancer biopsies, for which pathologists disagree about the diagnosis up to half the time, we could actually help train a machine-learning algorithm better to discriminate between these different cases, from benign to something that one might worry about. We're finding that the brain is so complex that if you build a tool that can confront the complexity of the brain, it might be able to help solve a lot of other problems as well.

**Rebecca Leshan:** Your background is quite varied and the way that you approach problems reflects interdisciplinary thinking. Do you also expect that of those you train? Is that an advantage to you? Or if somebody came to you and said, "I've studied neuroscience my entire career and I want to work with you." Is that off-putting?

**Dr. Boyden:** I think to really see if somebody can innovate well you have to watch them in action. We have a grad student in my group who's working on a really cool project. His professional training was as a photographer. He was actually a professional photographer. But if you're a classically trained photographer you know a lot of chemistry, too. You know how to develop pictures and so forth. So he's now leading one of our most out-of-the-field projects.

We have two graduate students who never finished college. They dropped out, but it became pretty clear that they have a lot of problem-solving skills and they're now both leading projects that are quite exciting. So I really think you have to understand how people think, and there's a lot of emotional components to being an innovator as well. A lot of our technologies, once we have figured out how to create them—which requires a lot of failure and a lot of wisdom-gathering through failure—once we understand the problem at a deeper level, the technologies are not so hard to build. It's the understanding of the problem at a deep level that's so difficult.

**Rebecca Leshan:** You mentioned having failures along the way. What has been the biggest hurdle that you've overcome? Or what is still the biggest hurdle for you in the work that you're doing?

**Dr. Boyden:** I've looked back at our group's work over the last 12 years and there's a model of innovation that I think emerges, which really has "failure" as an integral part of it. Step 1 is to pick a really big problem to work on, and I think all the big problems are pretty obvious, like "Let's see what's going on in the brain," or "Let's control everything in the brain." The second step is to think backward from that problem and survey all the different disciplines of science and engineering and try to systematically think of how we would go about solving it. This is actually the approach that Karl Deisseroth and I applied when we started thinking up optogenetics. We just started thinking about mechanical and optical and electrical, magnetic … just started to go through all the laws of physics trying to think of the best way to control neural activity, back in the year 2000 when we were both students. So, I really think that one can be very systematic and having interdisciplinary training can help with that.

Step 3 is what I call "constructive failures." We try things out, and a lot of them will fail, but they'll show us things that nobody's seen before. They'll show us what you might call "wisdom," this kind of elusive appreciation of reality that's hard to get just by thought or by reading.

**Rebecca Leshan:** Experience.

**Dr. Boyden:** Yeah. And Step 4 is "design." So we now know the nature of the reality of it better than before; let's go design the ultimate technology. With a lot of our technologies—like expansion microscopy or automatic patch clamping or optogenetics or voltage imaging, the list goes on—we followed something like that pattern. It allows us to do things that are very orthogonal to what people are doing because we understand the problem for its own sake, at its own level.

**Rebecca Leshan:** That's quite methodological. How much of a component of your process involves a little bit of luck here and there?

**Dr. Boyden:** Well, the "wisdom-gathering"—where we notice things people haven't seen before—I mean, that's obviously serendipitous. When we did the first optogenetics experiments, it basically worked on the first try. We didn't have to improve the molecules; we didn't have to mutate them. They were fast enough, high enough amplitude, and safe enough that they worked on, essentially, the first try. So yeah, there's a lot of luck involved. But I think you can optimize your luck. Sometimes I claim that what we're trying to do is "serendipity engineering": we do on purpose what otherwise might take a long time if it's only accidental.

**Rebecca Leshan:** Your description of the process and the failures sounds very much like the entrepreneurial spirit, where you see start-ups coming through and there's a certain level of failure you need to gain that experience in the world. I know you teach some courses that have an entrepreneurial component, is that right?

**Dr. Boyden:** I teach one class for the MIT business school.

**Rebecca Leshan:** Is this method part of what you're imparting to your students?

**Dr. Boyden:** I think there is an attitude [of] failing fast, and I'm not sure that's exactly what I believe in. I believe more in "you learn through the failure" and that pivots into the real solution. I don't think it's a failure per se, it's just that you have to learn before you can succeed, and the learning requires you to do things that cause you to fail. I don't think it's a failure per se because in the end it is actually a success. And most of the great technologies, you could argue, are failure reboots. You know there are lots of examples where something didn't quite work until somebody went "Hey, computers are faster nowadays," or "Hey, we have better genome sequencing" and then suddenly what was a bad idea has turned into a good one.

**Rebecca Leshan:** I've heard a story that the seminal experiment in the optogenetics work you had started at 1 a.m. Please tell me that's not true.

**Dr. Boyden:** It is true. The way we divvied up the labor was Karl Deisseroth did the transfections of the genes and then I was patch clamping them and shining the light on the cells and, yeah, it more or less worked on the first try.

**Rebecca Leshan:** Do you think he did that on purpose, leaving your part until 1 a.m.?

**Dr. Boyden:** Oh that's just when I had time on the rig; that wasn't him. I was doing the work actually in Richard Tsien's group, my thesis adviser's group, so this is before Karl was a professor.

**Rebecca Leshan:** Do I have it right that this was sort of your side project initially?

**Dr. Boyden:** Yeah, it was sort of an independent side project. We actually published the first optogenetics paper 2 months before I turned in my PhD thesis with Richard Tsien and Jennifer Raymond, which was all about cerebellum-dependent motor learning. Then things kind of took off. I found myself applying for faculty jobs just weeks after turning in my thesis.

**Rebecca Leshan:** I have another "maybe" misconception to clear up. Is it true that first paper was rejected by *Science* and *Nature*?

**Dr. Boyden:** It was, yeah.

**Rebecca Leshan:** At this meeting, are you getting people coming up to you? Trainees and students wanting to talk to you about what's next?

**Dr. Boyden:** Oh, yeah. Well, optogenetics as a tool set has become pretty mature. There're obviously some things that have to be improved but most of the tool set's in a pretty good state right now. But optogenetics by itself doesn't solve the brain. I think we have to also get those really good molecular and wiring maps of the brain. And we also really need to have good high-speed imaging of the dynamics of the brain. So almost all of our effort, on technology anyway, is focused on those two areas. Why can't we see … why can't we evolve all sorts of

fluorescent indicators of every neural signaling pathway? And through these robotic—and, now we're starting to work in artificial intelligence methods to solve this as well—can we actually start generating in a systematic way new kinds of fluorescent reporter. And then for imaging the wiring, we're getting a lot of technologies based on our expansion method that will hopefully allow people to routinely extract diagrams of the wiring of the brain.

**Rebecca Leshan:** I'm glad you mentioned artificial intelligence. Do you think that discovering more about the human brain is able to inform the development of artificial intelligence? How much are you getting from the reverse? Learning from artificial intelligence and processes that are being built on their own, are there things that you're taking away from that?

**Dr. Boyden:** We have 30,000-ish genes in the human genome, and who knows how many hundreds or thousands of cell types in the body. These data sets are obviously going to be very large. If we were to map one human brain, just one, and digitize it to single-molecule resolution and put the data on little hard drives and stacked them up, the tower of hard drives would reach into outer space from the Earth's surface. That's just one brain, too. We're definitely going to need new kinds of algorithmic thinking and we're starting to use quite a bit of that in evaluating the kinds of data we're getting.

# A Conversation with Beth Stevens

Interviewer: Sejal Vyas

Assistant Scientific Editor, *Cell Reports*

Beth Stevens is an Associate Professor in the Department of Neurology at Harvard Medical School, a Research Associate at the F.M. Kirby Neurobiology Center at Boston Children's Hospital, and a member of the Broad Institute.

**Sejal Vyas:** You work on microglia and synaptic pruning, both in normal development and also in various neurological disorders. Can you give a brief overview on what are microglia? What are their developmental origins and basic functions?

**Dr. Stevens:** I'm a developmental neurobiologist by training, and microglia are really the only cells not born in the brain. In many ways, it's one of the reasons why developmental neurobiologists sort of ignore them, at least for the chunk of development where a lot of changes are happening. But we now know from fate mapping studies that these cells actually come from a myeloid progenitor in the yolk sac. They actually enter the brain and become, essentially, brain-resident macrophages as early as embryonic day 8 in a mouse. This was really beautiful work in Miriam Merad's lab by Florent Ginhoux. Once that experiment was done, it really changed the game for thinking about microglia in the context of development.

The other thing that got us really interested in them—and what makes them unique from other cells in the brain—is they're also the brain's resident phagocytes. Not to say other cells like astrocytes can't be phagocytes, but microglia are very good at engulfing things. What we observed is during this early postnatal period when there's a lot of remodeling of synapses and circuits and axons, that microglia are particularly phagocytic and also associating with these structures in these critical periods of remodeling, leading to the hypothesis that they might be actively engaged in pruning and engulfing these synapses. Over the last decade or so, with the advent of new tools and technologies and ways to study them, it's opened up a lot of new questions about what else they're doing besides pruning. Pruning is one of the things that we've been studying, but now we're starting to appreciate it's really just the tip of the iceberg.

**Sejal Vyas:** With the new tools that have been available, how have the questions you're asking from when you started your lab to what you're focusing on now evolved?

**Dr. Stevens:** When we came out, I was interested very specifically on understanding synapse elimination. The work I had done as a postdoc with Ben Barres uncovered a role for a group of immune molecules called complement in pruning. At the time, we didn't know how it was all working because at that time we weren't even thinking about the role of these innate immune molecules in the developing brain. And we started putting these two ideas together and that's what really led us to microglia, in addition to those studies I just mentioned.

We're starting to appreciate that these cells have so many roles—so many day jobs and homeostatic roles in the normal brain. They change states. Probably by virtue of the fact that they're immune cells, they are dynamic and they can change states depending on the environment. That makes them extremely interesting to study, but also quite complex in the sense of trying to understand their functional roles in other contexts. Especially in disease, it's not easy because there's not really, at the moment, ways to label microglia and say, "Okay, this is what microglia are doing at this very moment." There's just a handful of markers for these cells. This is another example where we just haven't had the tools.

What we're excited about in terms of emerging technologies is single-cell sequencing. Drop-seq was initially discovered by Steve McCarroll's lab and Evan Macosko and others; now everybody's using single-cell. We started thinking about applying that, as others are in the field, to understanding microglia. What we're uncovering now is this might be a way of getting a better handle on the different states and developing new markers that will tell us more about how they change in different contexts. If we can look in a more unbiased way in various contexts—development, disease, human, as well—I think that changes the whole game and starts to enable us to look at what else they're doing and how they're changing in the context of disease. Because of the data we've collected already, tons of new projects have emerged because you're looking at data that, without this, we wouldn't have ever hypothesized some of these functions.

**Sejal Vyas:** In these postnatal stages, there's all these molecular subtypes, but in the adults, there's a little more homogeneity. What do you think that tells us about



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functions of these in these different stages for these microglia?

**Dr. Stevens:** What I think the data is suggesting is that during this dynamic period of development where the brain is changing, they are changing with the environment, so they are undergoing these different state changes. Once the brain is more mature, they are generally more homogeneous. However, we haven't yet gone into different brain regions. We basically took the whole brain and profiled them at one time point, say, adult. Probably, if you started looking at different regions you might see some differences, which other labs, as well as the work that we're doing, are starting to uncover. But generally speaking, I think what it's telling us is as these cells mature they are in this sort of homeostatic healthy state and then perturbations—it could be local or it could be global—then you could start to imagine how these cells change and when they change. And in different contexts—and others have also reported this in context of Alzheimer's disease models and other models—you can see shifts in these cells. We still are needing to zone in on understanding what that homogenous cluster really is. But the idea is that they're generally in this homeostatic sort of normal physiological state and that different conditions can shift them.

What we haven't been able to do is figure out: Can we shift them back? We're calling them "state changes," but we haven't exactly tracked these state changes. We don't know how rapidly they change and we don't know exactly how or whether we can shift them back to the homeostatic state. That's one of the things that the field is thinking a lot about, because if you think about therapeutics or think about biomarkers, imagine if we could figure out a way to do that in a very specific way. Ultimately, that's what some of this data could start to lead to: new ideas about how we might think about changing them.

**Sejal Vyas:** Going back to this idea of regionality, you alluded a bit to looking at these molecular markers and seeing the localization patterns in the brain. How does that inform on the regional functions?

**Dr. Stevens:** That's been what's most insightful about the data that we've collected recently. You can look at the single-cell data and see these clusters and these t-SNE [t-distributed stochastic neighbor embedding] plots and say, "Okay that's a cluster that looks interesting." It has all these genes that relate to proliferation or phagocytosis. But what really changes is when you started to look in the brain and ask where they were, and that's what was unveiling populations that were not only localized or changing in different times in development or in different conditions, but they were actually localized in different parts of the brain in interesting places that were telling us a bit about what they might be doing. That is really the key to making sense of this data. Collecting and analyzing all the single-cell data's Step One, but then you need to go into that data and start to understand and map that back onto the brain using single-cell, pooled with multiplex in situ hybridization IHC [immunohistochemistry]-type approaches to be able to ask where they are. The two together are going to unveil some new hypotheses.

**Sejal Vyas:** You talked a bit about the initial findings, about the role of complement and the synaptic pruning in normal development: these weak versus strong synapses and using the visual system as a model for that. Can you talk about those initial approaches before the more advanced single-cell technology came along?

**Dr. Stevens:** We've been using the visual system of a mouse because it's a really beautiful circuit where it's been very well developed and understood in terms of when and where pruning is happening. It's one you can manipulate. It's one you know exactly where the circuit is, and because of that it's where we spent a lot of our time and still continue to do so to really dig into the mechanisms. But at the same time, that's one place, and it's a sensory system. We're now more interested in branching out into other brain regions including the frontal cortex and cortical areas that are more involved in cognitive function, executive function. Then it becomes very tricky, because up there it's much more complex in terms of knowing exactly which circuit's pruning when. We start to then ask, some of the same rules and molecules that govern this in the visual system early in development, are they also doing that in later periods in different parts of the brain? There's a fair amount of work that needs to be done to try to map out that circuit and its critical period. That's what we're trying to do now, and I think that the complement is probably one of many mechanisms by which microglia do this. It's very clear that, even in our own data in the visual system, it's only half the story. There are many other molecules that work in concert with complement to direct or instruct microglia what to do. Others in the field have identified molecules like fractalkine and other molecules that are also both "find me" and "eat me" signals. As we move forward, a combination of unbiased looks at what other molecules the microglia have, both receptors and things they make paired with the neurons and what they're making at that time might open up new opportunities for discovery.

**Sejal Vyas:** Can you draw parallels from other fields like classical immunology or even cancer immunotherapy? A lot of these molecules are considered—for example, the CD47 "don't eat me"—as a checkpoint blockade target. What can other fields where these molecules have been discovered and studied quite a bit in the context of immunology inform on your work?

**Dr. Stevens:** Almost all of our knowledge and insight and ideas from what we've been studying in terms of pruning and these molecules have come from the immunologists. I am not an immunologist; I am what I would call a pseudoimmunologist. I've gotten into this because our science led us there, but I'm collaborating with and interacting with immunologists who have been studying these molecules and the immune system and in different contexts have really opened up new insight and also new tools.

So, the example you just raised—this idea of what tells the microglia which synapses or which parts of the cell not to prune—Emily Lehrman, who was a graduate student in my lab, started thinking about how that works in the immune system. Microglia and macrophages have a lot of parallel receptors and one of them was the receptor for CD47. They have this sort of α-receptor. Actually, there's a whole bunch of these "don't eat me" signals in the immune system that tell a circulating macrophage this is a healthy cell or a "self" cell, so stay away from me, even if complement is all over it. It raised the question of whether a similar group of molecules might be operating in a healthy brain. Of course, we started looking and a whole bunch of these molecules are expressed in the brain but no one's ever really studied them, especially in this context. So we can go back to the knockouts and to the strategies that immunologists use, and we can start manipulating them and ask, using the assays we've developed, could they also be acting in this way? And we're finding evidence that it is, in fact, the case. While there may very well be brain-specific ways this works, a lot of what we've been studying is really coming right out of the immunology playbook. That tells you there's some conserved mechanisms.

**Sejal Vyas:** Flipping the page to pathophysiological development, are these same molecular mechanisms coming out as far as driving things, like too much pruning in Alzheimer's or it's been implicated in autism with too little pruning potentially?

**Dr. Stevens:** We've been thinking a lot about how we might take what we've learned from development and see if there's a way that this could be aberrantly activated to contribute to synaptic loss. We started with Alzheimer's in part because there was already evidence of synapse loss that happens in people and in animal models relatively early in the course of the disease. There's vulnerable parts of the brain like the hippocampus where this is going on, but we don't really understand how that's working. When we looked in animal models, we showed these complement and microglia pruning mechanisms, which were normally down in a healthy brain, became aberrantly activated in these vulnerable circuits. And many of the mechanisms we've been studying in the visual systems of a mouse were showing up early on in these vulnerable brain regions and were contributing to aberrant or pathological synapse loss, at least in a mouse.

What really got us excited right around the time we were starting to think about this, if you start to look at what's coming out of the GWASs [genome-side association studies] and some of the genetics of Alzheimer's, it's both evidence of complement involvement, innate immune molecules, and a lot of genes that are expressed either exclusively or are enriched in myeloid or microglial cells. And so those two converge for us. We started coming after this based on a hypothesis that came from development that may have been wrong. But now that the genetics are also pointing to microglia, we're now in a nice position to start to think about how microglia are contributing, not just to synapse loss, but to other aspects of diseases like Alzheimer's.

That's where the single-cell comes back in again, because without ways to track these cells and know when and where they change, you don't really know how to get at that question. But in addition to that, Alzheimer's is only one of many neurodegenerative diseases and its synapse loss and activation of glial cells is a hallmark of a lot of disorders, including autism, schizophrenia, and other neurodegeneration. Although all of those diseases are incredibly different with respect to their onset, their symptoms, and in some cases, their genetics, could there be a convergence on a pruning-related or a microglia dysfunction pathway that might cut across multiple diseases? We've been going after that as well as others in the field looking at this in glaucoma, in Huntington's disease, in frontal temporal dementia models. And sure enough, evidence is starting to suggest that this pathway may be relevant in a lot of disorders. So we have lots to do to think about translating all this. Fortunately, there are now ways to go into the human brain and also start to think about whether we can start to validate some of the mechanisms in human. The next challenge that lies ahead is to try to take what we've learned from mouse and see if we see similar things going on in the human.

**Sejal Vyas:** Are those your three, five, 10 years into the future big goals you want to establish in your work? And can you also talk a little about the big open questions left in the field?

**Dr. Stevens:** Definitely, we want to move into human and other models that more closely model the human disease. There's an emergence of human stem cell models and organoid models and other approaches to get at this question that weren't available to us before. That's an untapped area. A lot based on emerging technologies is going to enable us to do that, and I think that's exciting: the idea that we might start to generate from the human data—including profiling data from human brain—new models. New animal models, whether that be humanized mouse models, nonhuman primate models, whether that be marmosets or other organisms, and models that might get us closer to understanding things like cognitive impairment, which obviously is a much more challenging thing to think about. Those are the kinds of things that can only be accomplished through collaborative team science. One lab cannot tackle this. That's what I'm most excited about for the next 10 years or five years, taking what we've learned as a field and realizing that the Alzheimer's field and neurodegeneration is a big area, and everyone has their ideas and their camps but take the genetics to help guide us in a way that we can start to work together and say, "Look, it's probably not one gene, one molecule; it's probably pathways." It's probably going to take multiple model systems and multiple approaches. Now, especially, we're really well positioned to start to do that.

I'm excited about that piece, but I also think there's a huge challenge that lies ahead for those that are studying

microglia in the field, and it's one of those unfortunate bottlenecks that I hope we can solve soon. It's one where, now that we have all these single-cell data sets coming out, we want to manipulate these genes in microglia and see what they do, but we don't really have easy ways to genetically modify microglia in the brain in vivo with viruses. You can do this now with neurons and astrocytes and all these other cell types; everybody does it. But for some reason that is probably interesting biologically but frustrating in terms of tools, most viruses—AVs [adenoviruses] and other viral strategies—do not work in microglia, so we need to figure out a way to do that. If we could, then you can imagine starting to screen through and test function versus making all these mice, which could take a very long time. That is where the field could come together to try to work together on this, because it's a common problem. It's been going on for a while and no one's cracked it yet, but hopefully somebody will.

**Sejal Vyas:** You've spent a lot of time finding very interesting things about microglia and glia in general. If someone told you tomorrow you're no longer allowed to work on the brain, what would be the next type of science you'd want to work on?

**Dr. Stevens:** I probably would be an immunologist. I would do the flip and say, "What have we learned about the brain that can be relevant to the immunology side?" I actually think it goes both ways and the brain does also affect the immune system in ways we don't understand. I might think about things that we've learned about as a neuroscientist and apply it to the immune system and work in that field for a bit.

# A Conversation with Huda Zoghbi

Interviewer: Jan Witkowski

*Cold Spring Harbor Laboratory*

Huda Zoghbi is a Professor in the Departments of Pediatrics, Molecular and Human Genetics, Neuroscience, and Neurology at Baylor College of Medicine, the Director of the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, and an Investigator at the Howard Hughes Medical Institute.

**Jan Witkowski:** The subject of your work is enticing: fine-tuning protein…

**Dr. Zoghbi:** Fine-tuning protein levels for neurological health. So, what does it really mean? What does it translate to in scientific terms and in practical terms? This is a principle that—in my experience, having been now in research for 30 years—applies to all of the neurological disorders that I have discovered, from childhood diseases all the way to adult, aging-related disorders: That's the overarching principle: protein levels matter for brain health. Actually, for the first 10—or even 20—years of my career, that was not anywhere on my radar screen, but it was really through the data and the experiments that we've done that it became obvious.

**Jan Witkowski:** When you're talking about protein levels, are you meaning levels of particular proteins?

**Dr. Zoghbi:** Correct. There are examples of proteins that the brain is exquisitely sensitive to their level. For some of these, a small change in the 10%–20% range will make the older brain highly vulnerable.

**Jan Witkowski:** This is a change of level, not a mutation in that protein?

**Dr. Zoghbi:** Well, it can be either. Some examples are pure change in level without any mutation—this is some of the recent data we have—and sometimes a mutation will lead to enhanced level and/or function of the protein, so you can get there in more than one way. You can get there by a mutation in the gene encoding the protein itself, or you can get there by changing the level of a regulator of that protein.

**Jan Witkowski:** An example that comes to my mind is in spinal muscular atrophy where you have multiple copies of *SMN2* [Survival of Motor Neuron 2], and you've got more protein.

**Dr. Zoghbi:** In spinal muscular atrophy you actually have multiple copies of the *SMN2* gene, but there's one copy on each chromosome of *SMN1*—the one that's properly spliced—that makes most of the protein. When the mutations in *SMN1* happen, you inactivate the protein, so you're left with very little protein, and the patients have disease. But their disease is modified by the level of the protein made from paralog, *SMN2*. If you have very little, then it's a very severe phenotype. If you make a little bit more due to more copies of *SMN2*, it is still severe but more moderate. If you have a lot more, you make it milder. That's due to a splicing mutation: If a mutation leads to abnormal splicing, you don't make a full-length protein. So the new therapy—the antisense—allows that exon to be properly spliced, leading to more of the protein to be made, which rescues the symptoms.

I studied a really very rare disease; nobody probably heard of it, an ataxia. It's a balance disorder. It affects coordination and walking and it happens later in life, typically when the person is a mature adult. The reason we studied it is because it's inherited. It's Mendelian, so it was easy to track. My thought was that if we learn something from it, it might inform us about the broader class of neurodegenerative diseases. In 1993, Harry Orr and I identified the gene and found it is due to a repeat expansion: a trinucleotide repeat of CAGs that encode glutamine. In you or I, we may have 30 glutamines. But in the patients, they typically have 39 or more glutamines. So, imagine the difference between 30 and 39: If you just have a little bit more you're going to lose critical cells in the brain that coordinate balance.

So, we created animal models, we studied them, we identified the protein interactions of ataxin-1—the protein mutated in ataxia. I will summarize 20 years of work in the following discovery: We discovered that the glutamine expansion stabilizes the protein, makes it a little bit more stable, a little bit more abundant in the cell, leading to a little bit more enhanced interaction with its known normal native partners. You're basically having a little too much of something. We tested all that genetically and biochemically. We have a readout for ataxin-1 interactions. We can really show that those interactions are enhanced, and if you reduce the interactor by 50%, you can now suppress the disease symptoms in animal models. It told us that we really needed to study what regulates ataxin-1.



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We identified an RNA-binding protein that regulates it: The RNA-binding protein binds the untranslated portion of the RNA and suppresses the level of the RNA keeping the ataxin-1 protein in check. This is where we discovered you don't have to mutate ataxin-1 to cause toxicity.

We found that if we reduce the RNA-binding protein by 50%, then ataxin-1 levels go up by 20% or 30%, and that increase of 20% or 25% of a wild-type ataxin-1 induced Purkinje cell degeneration and balance problems. How are we sure it's that ataxin-1 25% increase? Because we rely on genetics. We can now take these animals that lack one copy of the regulator, and therefore have a slightly higher ataxin-1, and breed them to mice that lack one copy of ataxin-1 to normalize ataxin-1, and you block the degeneration and all the symptoms for balance problems disappear. This pretty much for us nailed the importance of protein levels. Even a healthy protein can be toxic if increased by 20% or 30%.

**Jan Witkowski:** I'm sure this offers therapeutic opportunities, but it also surely expands the number of ways in which one can get a neurodegenerative disease?

**Dr. Zoghbi:** Absolutely. We immediately recognized this offers a therapeutic opportunity, because if we can find the regulators of ataxin-1 that can be inhibited safely and for which you can design a small molecule, then we could perhaps develop a therapeutic. We set out to search for those using genetic screens in human cells and in collaboration with Juan Botas in fruit flies. We identified many candidate regulators that we are pursuing one by one, some of which we're now actually working with a pharmaceutical company to develop a small molecule therapeutic.

If you observe what is reported in the literature for other neurodegenerative diseases, you would find that what we have learned from this ataxia project applies to the more common neurodegenerative diseases? The genetic data tell us yes, because we know that doubling the levels of normal synuclein can cause Parkinson's. Tripling the level of normal synuclein can give you earlier disease onset. Doubling the amyloid precursor protein gene gives you dementia. People with Down syndrome will have an extra copy of amyloid precursor protein gene due to trisomy 21 and have onset of early Alzheimer's. We know for these genes and proteins, levels matter. You don't have to mutate any of them.

**Jan Witkowski:** In Down's…

**Dr. Zoghbi:** It's just extra APP [amyloid precursor protein] and we know it's the APP gene because there are a couple of individuals who have the trisomy but they lack the APP gene and they don't get Alzheimer's. Down individuals may get dementia at 35 or 40, so it's really important for us to understand and figure out how to regulate APP levels.

**Jan Witkowski:** Why hasn't this been thought of before? Have people been so fixed on the idea that neurodegenerative disease, or any inherited disease, you have a mutation in a gene that produces a mutated protein and have ignored the fact that these fairly small differences in protein levels could have profound effects?

**Dr. Zoghbi:** I think people have thought about it, but have not pursued it necessarily as aggressively as needed. Clearly the duplications and the triplications, those were studied genetically and those were Mendelian-inherited disorders, although it's a wild-type protein. I think people are coming around now to realize there is another protein, tau, in which regulatory mutations or mutations that change the isoform ratio can also drive degeneration. This is where the lesson of ataxia got me closer to understanding at least one approach to consider for these common degenerative diseases. Let's find the regulators of these proteins, and let's think of ways we can slightly lower the levels of these disease-driving proteins, and that might protect people. There are many ways we can get neurodegeneration, you're absolutely right. If you're a person with a duplication of APP, you're going to get early Alzheimer's. But you may be a person with healthy APP, a person that doesn't have any duplication but that may have a variant in a regulator of APP that's going to slightly increase APP. That might put someone at risk at 70. Finding the regulator is going to teach us more about the biology of these proteins, but also it's going to help us interpret DNA sequencing data.

Right now, we're hit with so many variants after genome sequencing. We have no idea which ones to study and why. We can put them in animal models and we might or might not get a phenotype. But if we have the framework that these 70 genes are known to regulate synuclein, these 100 regulate APP, and so on and so forth, then at least if you got a sequence variation in them, you have a functional assay that tells you this gene is important.

**Jan Witkowski:** Have people started looking into GWASs [genome-wide association studies] for regulatory genes of the sort that you're talking about?

**Dr. Zoghbi:** We just started, because we just really began to identify a large number of regulators that we're validating, and we are crossing these now with GWASs for Alzheimer's and Parkinson's. We're beginning to find overlap, but we still have to do more. We're just beginning to do that. For some, we're collaborating with Alison Goate and Rudy Tanzi; for others, we're using existing data that are publicly available.

**Jan Witkowski:** Do the sorts of regulators you're looking for have key signatures that enable you to say, "This sequence is for Regulator X?"

**Dr. Zoghbi:** I'll give you an example. We might have a kinase for a protein and if you phosphorylate that protein, you make that protein much more stable. For example, you prevent its degradation. That kinase becomes a candidate where a gain-of-function mutation may put someone at risk for neurodegeneration, whereas a loss-of-function heterozygosity might be neuroprotective.

**Jan Witkowski:** I think of all the kinases and all the phosphatases, and any one of these might be…

**Dr. Zoghbi:** A candidate, absolutely. The majority of people who don't have the family history don't have necessarily the risk to have a duplication of synuclein. But it's

going to be something else, right? Because the pathology incriminates these proteins. These are at least some of the more common. If you took a hundred people with Alzheimer's, the vast majority of them will have abnormal APP accumulation or Aβ [amyloid β] accumulation, abnormal tau phosphorylation, so you know they're effectors at some point in time, but there's no mutation in them. That's why I think the regulators are important.

**Jan Witkowski:** So, you can recognize effectors that are normal but producing clinical phenotypes. So then you say it's the level of the protein—not the mutation.

**Dr. Zoghbi:** Correct. It's what it's affecting. In the case of the ataxia, you remember the regulator I mentioned to you, that if we took one allele we saw elevation of ataxin-1 levels and we show in the mice, degeneration. We then found people with haploinsufficiency—with deletions or inactivating mutations of one allele—and these people have childhood ataxias and they have developmental disability and other neurological problems, but if they have even a milder mutation—not a total null allele in one allele—if they're haploinsufficient due to just a missense modulation, they have a late-onset balance disorder. So, you see the gradation of the phenotype.

**Jan Witkowski:** Certainly, there are therapeutic opportunities provided.

**Dr. Zoghbi:** If you actually knew that this is a big driver of disease, finding such regulators, studying them, and finding ways to elevate them or decrease them is going to be far more valuable.

**Jan Witkowski:** And that's only standard pharmacology to alter levels of things, as opposed to replacing a mutant gene.

**Dr. Zoghbi:** Exactly. So this is all with neurodegeneration. Now if we have a couple more minutes I'll tell you about childhood diseases. As you know, I've always been interested in Rett syndrome, and we discovered it is caused by mutations in the gene methyl-CpG-binding protein 2: *MECP2*. The function of MeCP2 protein is extremely critical for the brain and its levels really matter. If you have a mutation that totally inactivates the protein in a male—because it's on the X chromosome—sadly, these males will be severely affected and they will die early. But if you have a mutation that's milder, that male will survive but will have neurological problems, and depending on the severity of the mutation their phenotype could be early autism, hyperactivity, or a little later if it's a milder mutation where he might present with schizophrenia, juvenile-onset schizophrenia, or other behavioral problems. Here again you see gradation, from death in the first year of life with a null allele, all the way to milder psychiatric symptoms if the mutation is milder. If you have it in a girl, they're mosaic. It's on one of the two X chromosomes, so 50% of the cells are lacking a functional allele and this will cause Rett syndrome, which affects all aspects of brain function. On the other side of the coin, if you double the level of the protein, you get a progressive neurological syndrome. And if you triple it you get an even more severe phenotype. So here again, we're seeing that level/function phenotype relationship.

**Jan Witkowski:** The brain, presumably, is special in this case. Nerve cells are particularly sensitive.

**Dr. Zoghbi:** So far, that's what we're seeing. We're not noticing the effect of these proteins in dividing cells. We're seeing the sensitivity to protein dosage either in adult neurodegenerative diseases or in the childhood diseases like Rett syndrome. It seems that brain cells, once mature and they don't divide, they're probably much more vulnerable to changes in protein homeostasis. You need to get everything right.

**Jan Witkowski:** I seem to remember that the trinucleotide repeat disorders are preferentially brain-related.

**Dr. Zoghbi:** Right. At least the ones where the mutation is in the coding region are brain-specific. There may be some peripheral phenotype in the spinal bulbar muscular atrophy, but that's really not what drives the disease. It's the brain-specific—or nerve cell–specific, because here it's the spinal cord. There's some that affect muscles and other tissues, but these are outside the coding region, so the expansion is not within the protein.

**Jan Witkowski:** Fascinating. And a bit depressing, thinking of all the extra things that could go wrong.

**Dr. Zoghbi:** I actually find it more optimistic if we know now that protein levels matter. That means we have an opportunity to find things that regulate these disease-driving proteins, and any such opportunity could be translated into therapy, whether small-molecule, or antisense oligonucleotides. I think new DNA-encoded libraries can be used to target almost any protein. You don't have to have an enzyme to have a therapeutic anymore. The new libraries that one can use screening billions of compounds could really reveal compounds that might regulate any protein product.