

# The need to connect: on the cell biology of synapses, behaviors, and networks in science

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**ABSTRACT** My laboratory is interested in the cell biology of the synapse. Synapses, which are points of cellular communication between neurons, were first described by Santiago Ramón y Cajal as “protoplasmic kisses that appear to constitute the final ecstasy of an epic love story.” Who would not want to work on that?! My lab examines the biological mechanisms neurons use to find and connect to each other. How are synapses formed during development, maintained during growth, and modified during learning? In this essay, I reflect about my scientific journey to the synapse, the cell biological one, but also a metaphorical synapse—my role as a point of contact between the production of knowledge and its dissemination. In particular, I discuss how the architecture of scientific networks propels knowledge production but can also exclude certain groups in science.

## NEUROGENESIS

In second grade, I almost failed science class. I was not a bad student. I was a nerd and I loved science—but I was bored. The books we used were filled with concepts that I found foreign and irrelevant. There was a picture of a kid, with really straight hair, whose hair was standing on end after being rubbed with a balloon. It was used as an example for static electricity. It looked fun, so I tried it. It did not work.

I was born and raised in the archipelago of Puerto Rico. In the tropics, where there is ~80% humidity year-round, you do not need to rub a balloon for your hair to stick straight up. Your hair is always sticking up.

Most of the scientific examples I learned in school were similarly irrelevant to my reality growing up in Puerto Rico.



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The books we used were written for kids from Europe and North America. I had to memorize examples that seemed fictional to me. For seed dispersal, I learned about the helicopter-shaped maple tree seeds, something I had never seen, because maple trees do not grow in the Caribbean. There is nothing wrong with using examples from elsewhere to illustrate scientific concepts. But when only examples from elsewhere are used, one learns that science is a distant, irrelevant thing done elsewhere.

Outside the classroom, my experiences were different. I was stimulated by the richness of the biological diversity around me, and my mind was constantly churning out questions. If plants can't feel, then why does the *morivivi* (*Mimosa pudica*) close when touched? How does a *tinglar* hatchling (leatherback turtle, *Dermochelys cori-*

*acea*), know to go to the ocean when it comes out of its egg? How does it remember, many years later, to come back to the same beach on which it was born to lay its eggs?

Scientific curiosity is a shared human instinct. Regardless of where we are born on planet Earth, we wonder and marvel about the world around us. While the knowledge produced by science is of universal importance, not everyone has equal access to scientific networks that produce knowledge. How we communicate and make scientific discoveries relevant to others—a strategy known in

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education as “contextualization”—contributes, by design or by accident, to who sees themselves as belonging in science.

## DIFFERENTIATION

I did not fail science class in second grade. Instead, I became that annoying kid who is constantly interrupting and asking questions, many of them largely irrelevant to what is being taught in class. By the time I graduated from elementary school, I had earned the nickname “*el estudiante de las mil preguntas*” (the student with one thousand questions). I was fortunate that my parents and many of my teachers in Mater Salvatoris elementary school and, later, in Colegio San Ignacio high school had the patience and the disposition to answer my endless barrage of questions.

There is such a thing as a stupid question. I know, because I have asked many of them. But asking questions, even questions that might seem stupid, is a critically important skill, particularly for a scientist. I admit it can be daunting—a question can expose one’s ignorance. But in scientific research, as in any form of learning, what one knows is a starting point toward the unknown. A question is the first step in a journey seeking to connect our brains to a broader network of knowledge.

Some questions lack answers. In research, knowledge serves as a platform on which one stands and, through questioning, stares at and recognizes the dark boundaries where our collective human knowledge ends. Those boundaries are the special places where scientific discoveries become most impactful, extending new paths toward the unknown. But to find those boundaries that haven’t been mapped, one cannot be afraid to ask questions and stare at the precipice of one’s own ignorance.

## OUTGROWTH

In high school, some of my questions turned into scientific projects. For example, after learning that plantain sap, considered largely useless in Puerto Rico, was used in the Dominican Republic for treating tuberculosis, I developed a project that demonstrated the antimicrobial properties of the plantain sap. The project, rooted in my experience and surroundings, felt relevant and helped me see science as a tool of discovery and learning. I was hooked.

By the time I went to college at Harvard University, I was convinced that I wanted to be a scientist. I majored in biology, attended scholarly lectures by world-class scientists, and received well-meaning mentorship. Yet, in college, science again felt foreign and distant, and I struggled to connect.

In the enormous lecture halls where the basic science courses were taught, I found little space for what I enjoyed most in science: asking questions. My questions became casualties to the fast-paced, meat-grinder courses structured as filters for pre-med students. I joined a lab to gain research experience but felt lost in the concepts and intimidated by the environment. By the end of my sophomore year, I had performed mediocrity in my science courses, gotten fired from my job as a lab tech, and started seriously wondering whether science was really for me.

The search for my place in science was an intellectual journey that eventually took me thousands of miles away from Cambridge, into the jungles of Central America. Working in collaboration with the Smithsonian Tropical Research Institute, I traveled in dugout canoes to remote Tawahka villages in Honduras and Emberá communities in Panamá. I lived among indigenous groups and documented their use of medicinal plants and the rain forest. These experiences allowed me once again the flexibility to develop and ask my own questions. In the remote villages, I met many individuals without formal scientific training who were asking critical questions about

the world around them. Science felt relevant again. These were important experiences that greatly influenced my development as a scientist and as a person, and they resulted in my first publication (Godoy *et al.*, 1998). There, far away from any lab, in villages that lay completely off the grid and went entirely dark after sunset, I started to clearly see my path toward becoming a scientist.

## SYNAPTOGENESIS

In the rain forests of Central America, I had the flexibility to formulate my own questions but lacked the training to push them forward. Science is an apprenticeship, and I needed a mentor to teach me how to transition from being a consumer of knowledge to being a producer of knowledge.

I also needed a role model. At a time of much self-doubt, I needed to see that people like me could contribute to science. So I reached out to the only Puerto Rican scientist I knew, Mariano García-Blanco. As a postbaccalaureate student in his lab at Duke University, I studied the nuclear architecture of cells undergoing organelle regeneration. I established a collaboration with Robert Singer’s lab at Albert Einstein College of Medicine and developed a protocol for fluorescent in situ hybridization in the alga *Chlamydomonas reinhardtii* (Uniacke *et al.*, 2011). I discovered changes in the nuclear architecture of *C. reinhardtii* corresponding to transcriptional changes occurring during flagellar regeneration (Colón-Ramos *et al.*, 2003b). I also discovered my interest in cell biology and decided to go to graduate school.

I joined the University Program in Genetics at Duke University and the lab of Sally Kornbluth. In Sally’s lab, I worked on the molecular mechanisms of programmed cell death and identified a viral family of proteins similar to *Drosophila* Reaper that induce apoptosis (Holley *et al.*, 2002; Colón-Ramos *et al.*, 2003a; Olson *et al.*, 2003). I also made the surprising discovery that the proapoptotic protein Reaper and the viral proteins I identified regulate translation by directly binding to ribosomes and modulating ribosomal subunit assembly (Colón-Ramos *et al.*, 2006). To answer these questions, I had to leave the comfort zone of the techniques I used regularly and set up collaborations that allowed me to establish in vitro systems to study ribosomal profiles and protein synthesis. It was a journey that resulted in internships in labs in Oklahoma and California. A critical skill I learned during this period was how to seek and establish meaningful collaborations that open up new areas of discovery.

By the end of my PhD, I decided to switch fields and do a postdoc in developmental neuroscience. I became interested in how the complex but organized architecture of neural circuits emerges during development to regulate behavior. Neuroscience had never been part of my formal training, but science is less about what you know and more about what you are willing to learn. I reached out to the network of mentors and peers I built during graduate school and used them as sounding boards to refine my interests, identify potential postdoctoral mentors, and learn about the outstanding questions in the neuroscience field. I then systematically approached (some would say pestered) labs working in model organisms and using approaches that linked cell biology and genetics with circuit connectivity and behavior.

## MATURATION AND PRUNING

In breezy summer nights in the archipelago of Puerto Rico, leatherback turtle hatchlings can be seen racing across the moonlit beaches of the island of Culebra toward the surf. These turtles are born with an ancestral memory: they instinctively know to go toward the ocean. This behavior is wired into the leatherback turtle’s nervous system and was selected for by evolution. Once born, the hatchling



**FIGURE 1:** Colón-Ramos lab in New Haven, CT.

forms a new memory, one that it will carry throughout its lifetime. It will remember the beach on which it was born, and many years later, after traveling the world's oceans, it will return to that same beach to nest. How does the leatherback turtle know to go to the sea upon hatching? How does it remember where it was born?

These were questions that I asked myself as a child, and they are directly related to the fundamental questions in neuroscience that my lab examines today. I joined Kang Shen's lab at Stanford University to establish a system in the nematode *Caenorhabditis elegans* to study these questions. "A worm is only a worm," said Diderot. "But that only means that the marvelous complexity of its organization is hidden from us by its extreme smallness." *C. elegans* forms memories. For example, *C. elegans* does not have an innate preferred temperature and can instead remember the temperature at which it is cultivated (Hedgecock and Russell, 1975). The neurons that control this behavior and their connectivity are known (White *et al.*, 1986; Mori and Ohshima, 1995), but how synapses are established and modified to form these memories is not. During my postdoc, I adapted markers that allowed me to inspect the cell biology of synapses during development. Using these cellular markers, I discovered a role for glial cells in specifying synaptic connections in vivo through Netrin signaling (Colón-Ramos *et al.*, 2007).

Now, in my own lab at Yale (Figure 1), we are interested in understanding how synapses are assembled and maintained to build the neuronal architecture underlying thermotactic behavior, and how they are modified to store memories. We established collaborations to develop and use new instrumentation and approaches for better visualization of the events leading to correct synaptogenesis (Rankin *et al.*, 2011; Wu *et al.*, 2011, 2013a,b; Kumar *et al.*, 2014; Christensen *et al.*, 2015; Santella *et al.*, 2015). We also collaborate to visualize neuronal activity and thermotactic behavior in *C. elegans* (Ha *et al.*, 2010; Luo *et al.*, 2014a,b).

Using these tools, we identified conserved mechanisms of neurodevelopment and synaptic assembly (Christensen *et al.*, 2011; Smith *et al.*, 2012; Stavoe and Colón-Ramos, 2012; Stavoe *et al.*, 2012; Nelson and Colón-Ramos, 2013; Zhang *et al.*, 2014) and unexpected roles for glia in establishing and maintaining synaptic

positions (Shao *et al.*, 2013). We also discovered a role for autophagy in synapse formation (Stavoe *et al.*, 2016) and documented a metabolic subcompartment that powers synaptic function and animal behavior (Jang *et al.*, 2016). All of our projects started by asking simple, fundamental questions, understanding how to break them down into solvable experimental problems and pioneering new approaches, by forging collaborations, that provide tractable ways of addressing our questions.

## CIRCUITS AND NETWORKS

My journey into science has been one of searching for connections. Now, as part of the scientific network, I see connections everywhere.

Scientific ideas do not sprout in isolation. Scientific knowledge results from a robust network of influences and cross-pollination of ideas. Scientists influence one another through their publications, research talks, collaborations, and, of course, through teaching, training, and mentoring. How these networks of knowledge are wired influences who is connected to the world of science. It also influences who is kept out.

When I trained with scientists like Mariano García-Blanco, Sally Kornbluth, and Kang Shen, I synapsed onto this larger network of knowledge, one that extends back through time and links me to a global community of scientists. These networks were key in my education and training as a scientist and in my ability to do science today.

The scientific community produces knowledge with the aspiration that it will be consequential and influence the way that we understand the world around us. For science to fulfill this aspiration, the ideas and knowledge produced by scientists need to be accessible. Yet our networks are not necessarily representative of our aspirations, as they remain inaccessible to most. They are instead representative of the history of science, one that until recently, exclusively served a very narrow demographic. It is a history that affected E. E. Just as an African-American scientist (Manning, 1983). It is also a heavy-handed legacy that today influences who belongs in science, who benefits from the scientific enterprise, and who does not.

I frequently reflect on my role as a scientist in these networks of knowledge. These interests led me to found an organization called Ciencia Puerto Rico (CienciaPR; [www.cienciapr.org](http://www.cienciapr.org)). It is an online network of more than 7500 scientists, students, and educators who are geographically dispersed across 50 countries, but who are connected in their commitment to promoting scientific research and education in Puerto Rico (Guerrero-Medina *et al.*, 2013).

Wonderful things happen when you connect people's minds. By crowd-sourcing essays that contextualize science for kids growing up in the tropical Caribbean, the CienciaPR community was able to produce a book that communicates to children in Puerto Rico that science is relevant to them (González-Espada *et al.*, 2011). The book discusses the discovery of giant fossilized sharks in the Puerto Rican karst country. It talks about how parts of the Puerto Rican archipelago were "born" in the Pacific Ocean and shifted to the Caribbean basin through tectonic plate movements. It describes the resilient microbial communities that paint many colors in the salt flats of the Puerto Rican southwestern coast. The book is now being used in elementary schools in Puerto Rico.

My hope is that networks like CienciaPR sprout elsewhere. When developed carefully and deliberately, networks have the potential of extending the impact of our knowledge and discoveries. In doing so, they bring down structural and geographical barriers that have historically limited access to science. They also help scientists like me fulfill our responsibilities as points of contact between the production of knowledge and its dissemination, not to a privileged few, but to all.

For further descriptions of my research interests in cell biology, please see Colón-Ramos (2013). For a video description of the value of collaborations in our work, please see [www.ibiology.org/ibiomagazine/issue-11/daniel-colon-ramos-networks-and-the-nervous-system.html](http://www.ibiology.org/ibiomagazine/issue-11/daniel-colon-ramos-networks-and-the-nervous-system.html). For a video description of the CienciaPR network, please see [www.ibiology.org/ibiomagazine/issue-10/daniel-colon-ramos-making-science-relevant.html](http://www.ibiology.org/ibiomagazine/issue-10/daniel-colon-ramos-making-science-relevant.html).

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