Liqun Luo

Liqun Luo is interested in how complex circuits in the brain are assembled and in the relationship between brain development, function, and evolution. In an interview with *Neuron*, he describes an influential tool developed in his lab for the dissection of neuronal circuits and shares his passion for teaching.

Liqun Luo grew up in Shanghai, China. He earned his bachelor's degree from the University of Science and Technology of China and PhD from Brandeis University under the guidance of Kalpana White. After postdoctoral training with Yuh-Nung and Lily Jan at the University of California, San Francisco, Dr. Luo started his own lab in the Department of Biology at Stanford University in 1996. With his postdoctoral fellows and graduate students, Dr. Luo studies how neural circuits are assembled during development and how they are organized to process information in adults. They also develop new tools to address these questions with increasing precision. Dr. Luo is currently a Professor of Biology at Stanford and an Investigator of the Howard Hughes Medical Institute. He teaches neurobiology to undergraduate and graduate students, and published a single-author textbook, *Principles of Neurobiology* (Garland Science, 2015). Dr. Luo is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences.

Liqun Luo
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What do you think are the big questions to be answered next in your field?

Our research encompasses a number of fields in neuroscience, but for the purpose of this Q&A I am wearing the hat of a developmental neurobiologist. A central, unresolved question in neuroscience is: "How is the brain wired up during development?" Over the past decades, we have seen significant progress in identifying key molecules that guide axons to their targets. We also know that neuronal activity and experience play important roles in fine-tuning connectivity. However, we only have a rough outline—bits and pieces here and there—about how the brain is wired up. We don't have a systematic understanding of the overall logic. We don't know how any functional neural circuit is assembled from beginning to end. Moreover, our mechanistic understanding of neural circuit assembly comes mostly from invertebrate systems (e.g., *C. elegans* and *Drosophila*) and relatively simple vertebrate circuits (e.g., retina and spinal cord), due to their technical ease. Yet we eventually want to understand how complex circuits in the mammalian brain are assembled. Here we face a daunting task due to the large number of neurons ($\sim 10^8$ in the mouse), their high density, and their complex connection patterns, with each neuron, on average, forming synaptic connections with $\sim 10^3$ other neurons.

Here is a list of specific questions: How does a limited number of wiring specificity molecules (extracellular ligands and cell-surface receptors) determine wiring specificity of a much larger number of neurons and their synaptic connections? What are the mechanisms by which neuronal activity and experience influence wiring specificity? How do molecular determinants and activity-dependent mechanisms coordinate their actions? Do the relative contributions of molecular determinants and activity-dependent mechanisms differ in different circuits?

Answering these questions will not only advance our understanding of how the brain is wired up during development, but also how connectivity in the adult might change by experience, during the process of learning and when memories are formed. Indeed, learning and memory can be considered a replay of development at a microscopic scale. Understanding the rules of brain development will also constrain our answers to a perhaps bigger and more challenging question: How does the brain function? After all, the brain is an evolutionary product that is self-assembled during development. Functions cannot be based on properties that do not have a developmental and evolutionary basis. Indeed, a deep understanding of the relationship between brain development, function, and evolution is another important future challenge.

To tackle your favorite research question, is there a tool that either needs to be developed or is currently available that could be implemented in a novel way?

Sticking to my question above, "How is the brain wired up?," tools that enable researchers to untangle the complexity of the mammalian brain will be enormously important. These include tools that highlight specific neurons and circuits for visualization and functional perturbation during development, and tools that enable higher-throughput examination of the consequences of genetic perturbation. Right now, even in the mouse, the genetic stalwart for mammals, it takes too long and is too expensive to examine

the consequences of a specific genetic perturbation on brain development.

Do you have a favorite anecdote from doing science that you'd like to share (perhaps a key discovery moment)?

I will take this opportunity to introduce the story behind MARCM, a method we published in *Neuron* (Lee and Luo, 1999) that has received the highest number of citations among all the papers I have published thus far.

In the early fall of 1996, I was ready to start my assistant professor position at Stanford, but my lab space was not ready. So I prolonged my postdoc in the Jan Lab at UCSF for three extra months and devoted one of those to sitting in the UCSF library and writing my first NIH R01 grant. The grant was going to focus on Rho GTPase signaling in neuronal morphogenesis. Although I had shown that expression of dominant-negative and constitutively active Rac1 (a member of the Rho GTPases) in neurons disrupted specific aspects of axon and dendrite morphogenesis in flies and mice, I felt unsatisfied that we did not validate Rho GTPase functions using loss-of-function mutants. The problem was that Rho GTPases are key regulators of the actin cytoskeleton, and loss-of-function mutants would likely disrupt the morphogenesis of many, likely all, cells. Even if neuronal morphogenesis was affected, we would not be able to conclude that Rho GTPases specifically act in neurons to regulate axon and dendrite development. We needed to knock them out specifically in neurons to test this formally. This could be done in genetic mosaic flies, which are efficiently produced using FLP/FRT-mediated mitotic recombination, a method developed by Kent Golic and Susan Lindquist and expanded by Tian Xu and Gerry Rubin in the early 1990s. However, in order to actually see what happens to a neuron in which a gene has been knocked out, we needed a way to specifically visualize the mutant neuron in a mosaic animal.

When I was studying Rac1 in transgenic mice, I learned the Golgi staining method and appreciated the power (and necessity) of visualizing individual neurons while studying neuronal morphogenesis. To couple sparse labeling with genetic mosaics, I had used adult sensory neurons in the thorax as a model. Each sensory neuron is associated with a bristle by lineage, and one can create genetic mosaics using loss of a bristle marker to infer the loss of a gene of interest in the associated sensory neuron. One can then pull out the mutant bristle and drop fluorescent dye (such as DiI) onto the empty socket to label the mutant sensory neuron and its axonal process. This way, I was able to create singly labeled mutant neurons in mosaic flies. However, it was very labor intensive, and the phenotypic analysis was restricted to thoracic sensory neurons. As I was proposing that procedure in my grant, I was thinking to myself, there has got to be an easier way to do this in *Drosophila*, a model organism with abundant genetic tools!

One of the most widely used tools in the 1990s was (and still is) the yeast GAL4/UAS-mediated binary expression system introduced into flies by Andrea Brand and Norbert Perrimon. Indeed, I had used GAL4/UAS to express dominant Rho GTPases to show their involvement in neuronal morphogenesis earlier. As I was sitting in the UCSF library one late afternoon, looking through the giant windows at the setting sun over the beautiful Golden Gate Bridge and Marin Headlands, I remembered reading during my graduate school days that yeast GAL4 has a repressor called GAL80. It occurred to me that if GAL80 can repress GAL4 in *Drosophila*, then in flies with GAL4, UAS-marker, and GAL80, none of the cells would express the marker. However, if we coupled loss of the GAL80 transgene with mosaic loss of our gene of interest (via FLP/FRT-mediated mitotic recombination), then the mutant cells would be the only cells that can express the marker. After checking the scheme drawn on a piece of paper, I rushed back to the Jan Lab to grab whoever was there to listen to my plan. Ira Clark, a graduate student known to be thoughtful and highly critical, examined my scheme on the white board and, after a long while, nodded: "I suppose that this could work." Because of its riskiness, I wrote the scheme, later named MARCM (for mosaic analysis with a repressible cell marker), in my R01 proposal as an alternative to my bristle-pulling-and-DiI-filling experiment.

I was very lucky to have a highly talented first postdoctoral fellow, Tzumin Lee, who turned the scheme into reality shortly after we started together at Stanford. In the process, Tzumin made important improvements in the design to ensure that the technique was robust and easy to use. In addition to solving the original problems of analyzing loss-of-function phenotypes for Rho GTPases, MARCM turned out to be a useful tool for lineage tracing and for systematic dissection of neuronal composition in complex circuits with single-cell resolution. It opened a window for exploring the complex CNS of *Drosophila*.

What has been the highlight of your career?

Developing tools that enable us to explore previously uncharted territories.

What aspect of scientific research do you enjoy most?

To work closely with passionate and talented fellow scientists to solve problems of common interest; these include my PhD and postdoctoral advisors, my own graduate students and postdoctoral fellows, and my collaborators.

What's your favorite experiment?

I have many favorite experiments, which I highlighted in the textbook I wrote. Here I describe an experiment that came from my own lab because it changed my view quite drastically about mechanisms of brain wiring. As I discussed earlier, molecular determinants and activity-dependent mechanisms both contribute to brain wiring. In our study of wiring of the fly olfactory system, we have tried quite a few activity manipulations without finding much of an effect. When we developed a genetic mosaic tool in mice called MADM (mosaic analysis with double markers) that allowed us to delete a candidate gene in isolated single neurons and to visualize these mutant neurons with a unique marker conceptually analogous to the fly MARCM discussed above, one of the first genes we tested was a key subunit of the NMDA receptor, NR2B (also called GluN2B). Previous work that examined the role of the NMDA receptor in dendrite development produced conflicting results: some experiments suggested that the NMDA receptor promotes, while

others suggested that it inhibits, dendrite growth. A major limitation was that these experiments could not distinguish between cell-autonomous and network effects of NMDA receptor perturbation. My graduate student Sebastian Espinosa examined sparse knockout of NR2B in stellate cells of the barrel cortex. Each stellate cell normally restricts dendrite growth to one specific barrel center such that it receives thalamocortical input representing one whisker. Sebastian found that NR2B mutant stellate cells had indistinguishable total length and branching number compared to controls, suggesting that the NMDA receptor is not required for growth and branching per se. However, NB2B mutant stellate cells no longer restricted their dendrite branches to a single barrel—rather, they spread across multiple neighboring barrels. Thus, the NMDA receptor is cell-autonomously required for patterning dendritic growth and branching with respect to presynaptic input. This fits well with its molecular function as a coincidence detector of correlated activity in its execution of Hebb's rule.

Since that experiment, I have developed enormous respect for the role of activity-dependent processes in determining wiring specificity in circuit formation. Indeed, the whisker-barrel system is an anatomically exaggerated system where perturbation of wiring specificity can be visualized easily at the light microscopy level. I can imagine that NMDA receptor-dependent mechanisms, and activity-dependent mechanisms in general, can act in many other systems to determine fine-scale connectivity, and that such effects may be more difficult to visualize by anatomical methods at the light microscopy level and are better studied with physiological methods that can measure synaptic strengths.

My Stanford colleagues used to complain to me that students had been brainwashed after taking my class to think that the brain is essentially hardwired. They should no longer complain about that because I now teach with a heavy emphasis on activity-dependent processes.

How do you view the level of crosstalk between disciplines (e.g., physics, mathematics, engineering, humanities, and social sciences)?

I am a big fan of the contributions of physical science and engineering to neuroscience. Over the years, I have seen many such students at Stanford, and I have encouraged them to pursue neuroscience careers, having had solid foundations in quantitative sciences. The brain is complex and requires all kinds of talents to attack it from different angles.

Where do you see the strongest potential for progress and new breakthroughs in neuroscience?

I have written about these in the end-of-chapter summaries of my textbook, *Principles of Neurobiology* (Garland Sciences, 2015). Indeed, the answers to your first question are modified from two of these summaries.

What advice do you find yourself giving to your students and postdocs?

Focus on solving an important problem; if one does well on that, everything else (papers, jobs, grants) will take care of itself.

How do you find inspiration?

From nature, broadly defined. This includes beautiful natural scenery, a beautiful biological specimen that reveals a secret, or a beautiful equation that captures a physical principle.

What question keeps you awake at night?

How is sleep regulated, and why can't I go back to sleep?

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