

Commentary

Life of a Neuron: The Mentor–Mentee Backstage Story

Dani Dumitriu¹ and  John H. Morrison²¹Columbia University Irving Medical Center, New York, NY 10132 and ²University of California, Davis, CA 95616

Introduction

In early 2019, a groundbreaking collaboration between Society for Neuroscience (SfN), ARTECHOUSE, and a team of neurobiologists was launched, culminating in the opening of *Life of a Neuron* in Washington, DC, Fall 2021 and New York City, Spring 2022, a digital art installation providing an immersive experience of an actual human neuron (Fig. 1). *Life of a Neuron* holds many stories. At a fundamental level, it is the story of a neuron's life—from birth, through development, adulthood, aging, and ultimately death. But its creation involves other stories. Here, we tell the story of the mentor–mentee relationship and the decade and a half of co-creative growth that gave rise to the science behind *Life of a Neuron*.

The Early Days

D.D.: *I have to be honest with you, John: when I first joined your lab a decade and a half ago, it wasn't because I liked your science. It was because no other lab would take me.*

J.H.M.: *I should be honest with you too, Dani: my lab told me not to accept you. They said you'd be trouble.*

D.D.: *You should have listened to them! I was not an easy graduate student to handle. I was arrogant, entitled, unconventional, often labeled 'difficult' —*

J.H.M.: *and absolutely brilliant! To be honest, I don't mind trouble.... And the thing is, science itself always does the job in humbling the best students.*

those labeled by *in vivo* retrograde injections targeting corticocortical neurons in the macaque prefrontal cortex (PFC) (De Lima et al., 1990). The technique also allowed spacing labeling far enough apart to permit full reconstruction of individual neurons. Importantly, because the labeling was fluorescent, neurons could be reconstructed in 3D using confocal microscopy, giving access to dendritic spines above and below the dendritic plane. Additionally, confocal imaging can be followed by deconvolution, which powerfully enhances imaging access to sub-resolution structures, such as dendritic spines (Radley et al., 2006), and paves the way for automated spine detection algorithms, which remove research bias and improve throughput (Radley et al., 2008). Because slices are from perfused tissue, brains used for microinjections and subsequent cellular morphology can also be processed for other high-resolution techniques, such as immunohistochemical studies at the level of electron microscopy (EM).

J.H.M.: *Suddenly, we could see three times more spines than we had been with Golgi!*

But in 2007, when D.D. joined J.H.M.'s lab without the historical memory of “the days of Golgi,” she was not impressed. First, it was annoying to watch the entire lab frantically rearrange its schedule around the “arrival.” In collaborative work, J.H.M.'s lab tackled questions of cognitive aging in rhesus monkeys. Specifically, the lab was responsible for the neuronal microstructure associated with “successful” versus “pathologic” aging. Approximately once a month, half of a rhesus monkey brain would arrive. Part of it was designated for microinjections and confocal imaging of neurons in area 46 of the PFC, and part of it was processed for synaptic ultrastructural studies using EM.

Received Oct. 25, 2023; accepted Oct. 25, 2023.

The authors declare no competing financial interests.

The authors are deeply grateful to the entire team of teams who worked over a 3-year period to make *Life of a Neuron* a reality. In addition to those mentioned in the text (Marty Saggese, Jenna Kohnke, Melissa Thompson Ayoub, Riki Kim, Sandro Kereselidze, Bill Janssen, Patrick Hof, Corrado Cali, Matt Wimsatt, Marina Varghese, Vimal Gangadharan), we thank Kristen Anderson, Eric J. Nestler, Bob Wurtz, and Bevil Conway for their science communication with various artist teams, and Luke Hammond and Darcy Peterka for their imaging expertise and help in generating the raw dataset of 3D confocal images provided by the neurobiology team to ARTECHOUSE. We are also deeply thankful for the generous support from the National Institutes of Health (NIH) that made the science behind *Life of a Neuron* possible, particularly P01AG016765, R37AG006647, and R01AG010606 to J.H.M. from the National Institute of Aging (NIA), Conte Center MH58911 to J.H.M. from National Institute for Mental Health (NIMH), and NIH National Research Service Award F30MH083402 and R01MH111918 to D.D. from NIMH.

Finally, we are dedicating this Commentary to our dear friend, colleague, collaborator, and mentor, Bruce McEwen (1938–2020). Bruce was one of the giants of mentorship in the last 50 years of neuroscience, always maintaining his humility, humor, and basic goodness with everyone he knew or met. He was intellectually generous, inclusive, never arrogant, and always provided sincere guidance that was invariably on target. Throughout his life, he always maintained that sense of wonder when he considered the beauty and power of the neuron, and he would have loved that we insisted that this be a human neuron.

Correspondence should be addressed to John H. Morrison at jhmorrison@ucdavis.edu.

<https://doi.org/10.1523/JNEUROSCI.2013-23.2023>

Copyright © 2023 the authors

For a century, neuroscientists interested in neuronal structure were limited to Golgi staining, a technique that remains popular despite significant limitations, including its capriciousness. To date, it remains unknown how neurons are selected for the intracellular deposition of silver chromate, the opaque residue of Golgi. There is also limited control over the density of staining, often leading to bushes of overlapping arborizations that do not permit detailed studies of individual neurons. Furthermore, visualization is restricted to 2D because of the optical limits of widefield microscopy and the obscuring of dendritic spines above and below the dendritic plane by the dark metallic precipitate.

In the late 1980s, J.H.M., fascinated by neuronal structure and unhappy with the limitations of Golgi staining, spearheaded a groundbreaking technique: single-cell microinjections in fixed tissue with fluorescent dyes (De Lima et al., 1990). This technique allowed precise targeting of neurons with known projections, such as



Figure 1. Left, J.H.M. speaking at the opening of *Life of a Neuron* Spring 2022 in New York City against a close-up view of the human PFC neuron in its original form. Top right, D.D. and J.H.M. talking inside the *Life of a Neuron* art installation at its opening in Washington, DC, Fall 2021 during a portion of the 20 min loop in which the neuron is copied and rendered at various developmental stages. Bottom right, D.D. microinjecting human tissue that would ultimately provide the neuron serving as blueprint for the entire *Life of a Neuron* exhibit on the microinjection station originally used by J.H.M. in the 1980s and gifted to D.D. after her thesis defense.

D.D.: *No matter what any of us in the lab were working on, we all just had to stop for a week to accommodate the ‘arrival.’*

One of the first problems that D.D. identified was the rapid degradation of the tissue following perfusion.

D.D.: *As the days wore on, tension in the lab would slowly—then not so slowly—increase. The first few days, it was just annoying that all the microinjection stations were monopolized for the new brain. But then, this precious tissue would begin degrading, and there had to be a frantic push against time to get enough good quality neurons microinjected. So, any of us with the microinjection skillset would be pulled into the project even if we weren’t directly involved in it.*

And that was not the only time crunch that frustrated D.D.. The original fluorescent dye used, Lucifer Yellow (LY), would also rapidly degrade, often allowing only one pass through the confocal laser before the neurons faded into the autofluorescence of brain tissue. Even untouched by powerful laser photons, within a few months, LY would slowly fade, especially if the slides were not kept in absolute darkness in a perfectly temperature-controlled cold room.

But what bothered D.D. the most were optical limitations. Even with the best

deconvolution available in the lab at the time, D.D. measured the optical resolution of the system to be 180 nm in the XY plane. This marked improvement above the measured 280 nm resolution before deconvolution, still left many dendritic spines unresolvable, as thin spines tend to have head diameters of no more than 100–200 nm. By this time, J.H.M. and D.D. suspected that thin spines were particularly vulnerable to aging compared with mushroom spines and given the demonstrated functional relevance of spine size and shape, it was critically important to resolve and measure the finest details of this spine subtype.

Even more annoyingly, confocal microscopy leads to a “Z-smearing” effect. When passing through any microscope, photons are detected in accordance with a point spread function (PSF). The PSF of photons passing through the pinhole of a confocal microscope is oblong, leading to an optical resolution 2–3 times lower in the Z plane than the XY plane. In the lab’s system, the measured Z plane resolution was 670 nm before deconvolution and 390 nm after deconvolution. Spherical dendritic spines with head diameters <180 nm would therefore all show up as oblong structures with an XY diameter of 180 nm and a Z diameter of 390 nm, resulting in measured head volumes an order of magnitude larger than their real counterparts (e.g., a thin spine with a 100 nm head diameter has a real volume of $0.00052 \mu\text{m}^3$ but a measured volume of $0.0066 \mu\text{m}^3$).

D.D.: *There **has to** be a better way to do this!*

J.H.M.: *You can’t fight the laws of physics!*

Finding Common Ground

This was the critical moment when J.H.M. learned the best way to unleash D.D.’s creativity in the lab was to tell her something couldn’t be done. At first, J.H.M. instructed D.D. to ‘follow the protocol,’ but after repeatedly ‘battling it out,’ he finally conceded:

J.H.M.: *Ok, Dani—take some time to come up with solutions, then we can discuss.*

That was all the invitation D.D. needed to tackle each problem systematically during a period of over a year, ultimately resulting in a 2011 *Nature Protocols* publication improving all aspects of dendritic spine imaging and analysis (Dumitriu et al., 2011). First on her list: the problem of tissue degradation. Carefully, she tested various combinations of perfusate, post-fixative, and storing solutions, and designed a cocktail that would allow tissue to be stored indefinitely.

D.D.: *No more frantic ‘arrival’ weeks!*

J.H.M.: *I actually liked the excitement of arrival weeks... though I didn’t have to stay up all night loading cells...*

Next, D.D. tackled imaging quality. LY was replaced by newer, brighter, and more stable AlexaFluor dyes. Fluorescent labeling was moved from the green (emission wavelengths 495–570 nm) to the red spectrum (emission wavelengths 620–750 nm), allowing for better signal-to-noise ratios by circumventing brain autofluorescence, which peaks in the green spectrum. A systematic optimization of laser power showed that much higher intensities could be used with the new dyes, further increasing signal-to-noise by imaging at much lower gains. The effect of pinhole sizes below the conventional 1 airy unit was tested and showed to empirically increase resolution by up to 30% in bright samples.

Arguably the most important contribution to the field was the development of a Z-smear correction function. D.D. reasoned that, while the laws of physics prevent manipulations of the PSF during imaging, they do not have jurisdiction over the mathematics performed *post hoc*. Working with the original NeuronStudio team (Rodriguez et al., 2006), a Z-smear correction algorithm was developed. The algorithm first performed a local (per individual 3D image stack) measurement of the PSF along the dendritic shaft, then used the Z to XY diameter ratio of the dendrite to adjust the Z diameters of individual spines before computing their head volumes. Additionally, D.D. found empirically that confocal imaging using nontraditional cubic voxels allowed the deconvolution and Z-smear corrective algorithms to work better. Ultimately, the team showed the resulting spine head volumes were congruent with volumes measured with the gold standard of EM, even for thin spines, but in a fraction of the time (Dumitriu et al., 2011).

J.H.M.: *When I first saw the data, I simply couldn't believe it.*

In the initial publication, the team used male rat hippocampus to show dendritic spine head volume distributions identical to their EM counterparts (Dumitriu et al., 2011). They later confirmed this relationship in monkey hippocampus (Dumitriu et al., 2012a) and rat BLA (unpublished observation).

J.H.M.: *We were resolving sub-resolution structures with EM precision at the speed of light microscopy!*

These scientific breakthroughs have been adopted by dozens of labs around the world and sometimes led to opposing

findings than previously reported using Golgi staining (Dumitriu et al., 2012b). The breakthroughs also propelled the science in J.H.M.'s own lab. In a cornerstone publication, as part of her thesis work, D.D. showed a precise relationship between the head volumes of thin spines from area 46 layer III neurons in dorso-lateral PFC and cognitive aging in the rhesus monkey (Dumitriu et al., 2010).

J.H.M.: *People in my lab refer to your Nature Protocol paper as 'the Bible.'*

What the breakthroughs failed to do was squelch the avid debates between J.H.M. and D.D.. D.D. hypothesized that the thin spine changes seen with aging in the PFC would generalize to the entire neocortex, but J.H.M. was convinced there would be regional specificity. In addition to a new experiment being set up for the next student in the lab, a bet was established. Years later, when J.H.M.'s lab showed primary visual cortex to be resilient to such age-related changes (Young et al., 2014), D.D. had to both concede and deliver an excellent bottle of wine. These early experiences not only humbled D.D., but also infused her with a deep fascination for neuronal microstructure, work that she has continued in her own independent lab (Grossman et al., 2022).

D.D.: *What I liked most about you as a mentor, John, is that you didn't teach, preach, or lecture me. You inspired me. You challenged me. And you gave my scientific curiosity free reigns.*

J.H.M.: *Just as Floyd Bloom did with me...*

When J.H.M. relocated from the Icahn School of Medicine at Mount Sinai to University of California–Davis, he gave D.D. a precious gift: the microinjection station she'd used during her thesis, which was equipped with an old Nikon microscope that D.D. absolutely loved. This was an extraordinarily special gift, given it was also the very first microinjection station developed in J.H.M.'s lab, the one he himself had injected neurons in decades earlier, and the one used in the first paper from his lab using this technique (De Lima et al., 1990).

The Co-Constructive Era

So, what does all of this have to do with *Life of a Neuron*?

J.H.M.: *I always wanted to walk through a neuron. And not just any neuron. It had to be a **human** neuron. And more precisely: a **neocortical** human neuron. Those are the 'thinking' cells; the cells that define our human experience.*

J.H.M.'s idea of developing a virtual neuron had its earliest expression at the 2017 SfN annual meeting in Washington, DC, when he was announcing the launch of the Virtual Brain Project for [BrainFacts.org](https://brainfacts.org). Turning to Marty Saggese, Executive Director of SfN, he exclaimed:

J.H.M.: *The next thing we are going to do, Marty, is develop a neuron that you can walk through and watch it mature, age, get diseased, heal. And it is going to be a **real** human neuron that is **quantitatively** accurate.*

Soon after that spontaneous announcement, the wheels were put in motion with a collaborative effort launched between SfN and ARTECHOUSE, a small firm that specializes in immersive exhibits combining art, science, and technology. A team of teams was established: ARTECHOUSE assembled a team of artists and computer scientists led by Riki Kim and Sandro Kereselidze, SfN provided funding and administrative expertise led by Jenna Kohnke and Melissa Thompson, and J.H.M. assembled a small group of neurobiologists to provide the raw data.

J.H.M.'s first move: contact D.D., now running her own lab at Columbia University. Together, they expanded the core team to include Bill Janssen, Patrick Hof, Corrado Cali, Matt Wimsatt, Marina Varghese, and Vimal Gangadharan. The core members of each group initially met in person in January 2019 at Columbia University, then biweekly on Zoom for over 2 years that spanned a never-ending pandemic.

There were many challenges along the way. New innovations were needed, including the development of protocols to rapidly retrieve and fix human brain tissue from surgical resection cases, tissue that, by definition, could not be perfused. One of the biggest challenges was generating a 3D dataset of EM reconstructed intracellular organelles and synapses from human pyramidal cells, which could then be "placed back" into the "empty" loaded neuron.

As time went by, D.D. assumed two crucial roles: one neurobiological, and one psychological. Just as with their

early work, the now extended team tackled each challenge systematically, reviewing successes and failures on their regularly scheduled conference calls. Inevitably, at some point during these calls, J.H.M. would proclaim:

J.H.M.: *When this meeting started, I felt we had about a 50% chance of succeeding. Now I give us a 20% chance!*

Without missing a beat, D.D. would gently reassure:

D.D.: *No no. We still have a 100% chance of success.*

And so, the team would end the call with just enough optimism to carry on.

In the end, the team succeeded in delivering the neuron to ARTECHOUSE, along with the science behind several other components of the final exhibit. As promised, it was **a human PFC neuron that was quantitatively accurate down to every single spine, mitochondria, and vesicle**. The neurobiologists were not sure what ARTECHOUSE was going to do with the neuron, other than: it would portray the various stages of life, and—critically—J.H.M. would be able to “be inside” it. Once ARTECHOUSE started to work their magic, the neuron took on a reality and a life that was far beyond the

wildest dreams of any individual who took part in the team of teams. It worked.

Ultimately, the neuron that serves as the blueprint for the entire 20 min loop of the *Life of a Neuron* exhibit was microinjected by D.D. on the station J.H.M. had gifted her years before (Fig. 1).

J.H.M.: *When all is said and done, your career as a scientist will be reflected primarily by two things: your publications and your trainees. The greatest part of this whole deal is the multigenerational extended family tree that we all populate. D.D. is my daughter, and Floyd Bloom's granddaughter. How cool is that?*

References

- De Lima AD, Voigt T, Morrison JH (1990) Morphology of the cells within the inferior temporal gyrus that project to the prefrontal cortex in the macaque monkey. *J Comp Neurol* 296:159–172.
- Dumitriu D, Hao J, Hara Y, Kaufmann J, Janssen WG, Lou W, Rapp PR, Morrison JH (2010) Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. *J Neurosci* 30:7507–7515.
- Dumitriu D, Rodriguez A, Morrison JH (2011) High-throughput, detailed, cell-specific neuroanatomy of dendritic spines using microinjection and confocal microscopy. *Nat Protoc* 6:1391–1411.
- Dumitriu D, Berger SI, Hamo C, Hara Y, Bailey M, Hamo A, Grossman YS, Janssen WG, Morrison JH (2012a) Vamping: stereology-based automated quantification of fluorescent puncta size and density. *J Neurosci Methods* 209:97–105.
- Dumitriu D, LaPlant Q, Grossman YS, Dias C, Janssen WG, Russo SJ, Morrison JH, Nestler EJ (2012b) Subregional, dendritic compartment, and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. *J Neurosci* 32:6957–6966.
- Grossman YS, Fillinger C, Manganaro A, Voren G, Waldman R, Zou T, Janssen WG, Kenny PJ, Dumitriu D (2022) Structure and function differences in the prelimbic cortex to basolateral amygdala circuit mediate trait vulnerability in a novel model of acute social defeat stress in male mice. *Neuropsychopharmacology* 47:788–799.
- Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, McEwen BS, Morrison JH (2006) Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cereb Cortex* 16:313–320.
- Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, Morrison JH, Wearne SL, Hof PR (2008) Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *J Comp Neurol* 507:1141–1150.
- Rodriguez A, Ehlenberger DB, Hof PR, Wearne SL (2006) Rayburst sampling, an algorithm for automated three-dimensional shape analysis from laser scanning microscopy images. *Nat Protoc* 1:2152–2161.
- Young ME, Ohm DT, Dumitriu D, Rapp PR, Morrison JH (2014) Differential effects of aging on dendritic spines in visual cortex and prefrontal cortex of the rhesus monkey. *Neuroscience* 274:33–43.