



The Brain Prize 2023 INFORMATION PACK

THE
BRAIN
PRIZE

Founded by the
Lundbeck Foundation



Pioneering research into the molecular mechanisms that regulate the neuronal proteome during development, plasticity and disease has been recognised with the award of The Brain Prize 2023

A profound aspect of our nervous system is that during development and adulthood our brains are subject to extensive plasticity. Such plasticity requires that the complement of neural proteins- the neural proteome, be dynamically regulated in space and time. An international group of three neuroscientists, Michael Greenberg, Christine Holt, and Erin Schuman have each revealed the fundamental principles of how this is mediated at the molecular level - from activity-dependent gene transcription to the local translation of mRNA into new proteins in dendrites and growing axons. Their findings have provided spectacular new insights into the cellular and molecular mechanisms that guide growing axons during brain development, and that enable the developing and adult brain to be shaped by experience. Theirs is a beautiful discovery story in fundamental neuroscience that also provides clues to the aetiology of neurodevelopmental and neurodegenerative diseases of the brain. For their work, the three neuroscientists are awarded the world's largest prize for brain research - The Brain Prize.

**This year The Brain Prize worth
DKK 10 million (€1.3 million) is awarded to:**

Michael Greenberg (USA)
Christine Holt (UK)
Erin Schuman (Germany/USA)

Professor Richard Morris, Chair of The Brain Prize selection committee explains the reasoning behind this year's award.

“In order to establish appropriate neural connections during development or to adapt to new challenges in adulthood through learning and memory, brain circuits must be remodeled, and the new patterns of connectivity maintained; processes that require the synthesis of new proteins for those connections. The Brain Prize winners of 2023, Michael Greenberg, Christine Holt, and Erin Schuman have revealed the fundamental principles of how this enigmatic feature of brain function is mediated at the molecular level. Together, the Brain Prize 2023 winners have made ground-breaking discoveries by showing how the synthesis of new proteins is triggered in different neuronal compartments, thereby guiding brain development and plasticity in ways that impact our behavior for a lifetime.”

About the Brain Prize

Scope

The Brain Prize is awarded each year by the Lundbeck Foundation. The Lundbeck Foundation is one of Denmark's largest foundations encompassing a comprehensive range of commercial and philanthropic activities – all united by its strong purpose; Bringing Discoveries to Lives. The Foundation's philanthropic grants amount to more than DKK 500m annually, primarily focusing on the brain – including the world's largest personal prize for neuroscience, The Brain Prize. The Brain Prize recognises highly original and influential advances in any area of brain research, from basic neuroscience to applied clinical research. Recipients of The Brain Prize may be of any nationality and work in any country in the world. Since it was first awarded in 2011 The Brain Prize has been awarded to 44 scientists from 9 different countries.

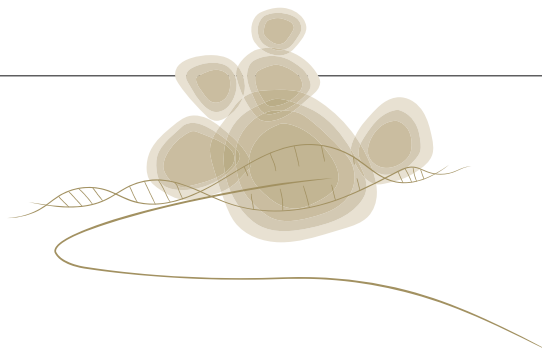
Selection and award

Only candidates who are nominated by others will be considered for The Brain Prize. Each year, the Lundbeck Foundation receives many outstanding nominations from all over the world. Recipients of The Brain Prize are chosen from the pool of nominees by The Brain Prize selection committee which consists of 9 leading neuroscientists from all over the world, and from diverse disciplines within neuroscience. Brain Prize recipients are presented with their medals by His Royal Highness, Crown Prince Frederik of Denmark, at a ceremony in the Danish capital, Copenhagen.

Purpose

The Brain Prize is first and foremost a celebration of outstanding science and outstanding scientists, but it is also an opportunity to raise awareness of the winners, their science and their field. Following the award of The Brain Prize, recipients engage in a series of seminars, lectures, and conferences, organised by the Lundbeck Foundation. These activities celebrate the achievements of The Brain Prize winners and help raise awareness of their work and their field amongst the global neuroscience community. The Brain Prize is also used as a platform to engage with, and educate the public about the importance of brain research, its challenges, and breakthroughs. The Brain Prize also serves to highlight the Lundbeck Foundation's vision of making Denmark a leading neuroscience nation.

More information about The Brain Prize, Brain Prize Laureates and the nomination and selection process can be found [here](#). Here you will also be able to access educational material and documentary films about Brain Prize winners and their science.



Michael Greenberg

Michael Greenberg received a BA in Chemistry from Wesleyan University in 1976, and a Ph.D. in Biochemistry from the Rockefeller University, New York, in 1982. In 1986 he was appointed Assistant Professor in the Department of Microbiology and Genetics at Harvard Medical School, and he was made full Professor in 1994. Since 2008 he has been the Nathan Marsh Pusey Professor of Neurobiology at Harvard Medical School in Boston, MA, USA.



That our sensory experiences shape the structure and function of the brain is one of the profound discoveries in the field of neuroscience in the 20th century. Michael Greenberg's seminal discoveries of activity-dependent gene transcription have revealed how nature and nurture cooperate to shape mammalian brain development and plasticity. Building on his early observation that neurotransmitter reception triggers the rapid induction of new gene expression, his work has focused on elucidating the nature and role of neuronal transcriptional programs induced in response to extracellular stimuli.

Work in the Greenberg laboratory has characterized the signal transduction pathways linking calcium influx at distal synapses to the neuronal nucleus, uncovered an extensive network of neuronal activity-responsive cis-regulatory elements that coordinate these gene expression changes, and demonstrated significant neuronal cell-type- and species-specific diversity in these transcriptional responses. These studies have uncovered an important role for activity-dependent transcriptional responses in dynamically sculpting specific aspects of neuronal connectivity. Current work in his laboratory focuses on how these changes contribute to experience-dependent behavioural plasticity and understanding the basis of neurological diseases that arise when these processes have gone awry.



I am absolutely thrilled to hear the news that Christine Holt, Erin Schuman and I have the great honour to be awarded The Brain Prize for 2023. Thank you very much to the Lundbeck Foundation and the selection committee for their recognition of our work. For me, this is the culmination of a forty-year odyssey aimed at understanding how our sensory experiences impinge on the neuronal genome to orchestrate brain maturation and the plasticity that underlies long-term memory, and how these processes go awry in disorders of the nervous system.

Our successes over the years are without question due to the hard work and creativity of my many laboratory colleagues, including fantastic students, postdoctoral fellows, and research assistants. I think The Brain Prize is in recognition of their many contributions to our current understanding of the brain. I have been captivated for many years by the wonderfully insightful work of my corecipients, Erin and Christine, and I offer them my heartiest congratulations. I'm really excited to share this award with them.



Christine Holt

Christine Holt received a B.Sc. Hons degree in Biological Sciences in 1977 from the University of Sussex and in 1982 was awarded a Ph.D. degree in Zoology from King's College London. She did her postdoctoral training in the Physiology Department at Oxford University and in the Biology Department at the University of California San Diego (UCSD). In 1992, she joined the faculty at UCSD and became a tenured Associate Professor in 1996. In 1997, she moved to the University of Cambridge as a Lecturer in the Anatomy Department and a Fellow of Gonville and Caius College. In 2003 she became the Professor of Developmental Neuroscience in the Department of Physiology, Development and Neuroscience (PDN) at Cambridge.

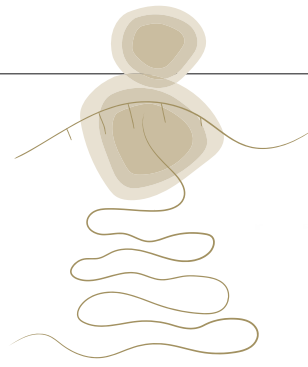


Christine Holt is interested in how connections are first formed in the brain and how they are maintained over the long-term. In the vertebrate visual system, neurons in the eye extend axons over a long distance to find their synaptic targets in the brain. The goal of her research has been to understand the molecular and cellular mechanisms that guide and maintain these axons. Her work led to the demonstration that local protein synthesis and degradation are a required part of growth cone guidance, a highly original step-change in our understanding of axon growth.

More recently, she has shown that local axonal protein synthesis is necessary for axon survival, suggesting that mature axons require a continuous supply of locally synthesized proteins for their maintenance. The ability to make new proteins on-site and on-demand in the most remote cellular compartments of neurons, such as axons, growth cones and presynaptic terminals, provides adaptability and resilience. By studying the cell biology of growing axons and mechanisms of local protein synthesis, her work has provided a better understanding of how neural connections are first established and has made the orderly growth of retinal ganglion cell axons one of the best understood examples of axon navigation anywhere in the brain. Her work has also shed light on how axons are sustained throughout the lifetime of an animal. Fundamental knowledge of this sort is essential for understanding neurodevelopmental and neurodegenerative disorders and for developing clinical therapies in nerve repair

Thank you for selecting me as one of the recipients of The Brain Prize this year. It is an honour beyond my wildest dreams, and I am absolutely delighted. The Prize is an incredible recognition of the work that we have been doing over the last forty years. I have been very fortunate to work with some brilliant scientists - young ones in the lab, more senior collaborators who have each made important contributions to the research, particularly Bill Harris, my lifelong collaborator. So, I see this as a prize for all of us as a team.

It is such a great honour to share the prize with Erin Schuman and Mike Greenberg. Their beautiful work has been an inspiration to me over the years. Erin and I have both explored the role of local protein synthesis in nerve processes, but from different sides of the synapse. It's been an exciting journey of discovery that may eventually lead to advances in therapies for neurodegenerative disease and neural repair. Thank you most sincerely to the Lundbeck Foundation.



Erin Schuman

Erin Schuman was born in 1963 in California. After completing her B.A. in Psychology at the University of Southern California, she received her Ph.D. in Neuroscience from Princeton University. She conducted postdoctoral studies in the Department of Molecular and Cellular Physiology at Stanford University. In 1993, she was appointed to the Biology Faculty at the California Institute of Technology (Caltech). From 1997-2009, Erin Schuman was appointed Investigator at the Howard Hughes Medical Institute (HHMI). In 2009, she moved with her husband Gilles Laurent to Frankfurt, Germany to design and found the new Max Planck Institute for Brain Research.



Erin Schuman has a long-standing interest in molecular and cell biological processes that control protein synthesis and degradation in neurons and their synapses. The complex morphology of neurons, with most synapses located hundreds of microns from the cell body, presents a logistical challenge for the establishment, maintenance and modification of local synaptic proteomes. Erin Schuman's work has been instrumental in demonstrating that neurons have solved this problem by localizing important cell biological machines, including ribosomes and proteasomes, within dendrites and axons. Following on the lab's initial discovery in 1996 that proteins made locally in dendrites are required for synaptic plasticity, Erin Schuman has identified in molecular detail the mRNA and ribosome population present in neuronal dendrites and axons.

In addition, her lab has developed new tools to label, purify, identify and visualize newly synthesized proteins in neurons and other cells using non-canonical amino acid metabolic labelling, click chemistry, and mutation of cell-biological enzymes (the BONCAT and FUNCAT techniques). Taken together, the lab's work has elucidated how gene expression can be regulated in the minute subcellular space of the synapse and how decentralization of cell biological machines allows the single neuron to manage subcellular proteomes in a vast volume. These transformative discoveries have expanded and solidified the field of local translation as a key mediator of synaptic function and Erin Schuman's work has fuelled the development of new technologies that have been widely adopted across neuroscience and non-neuroscience labs around the world.

Being awarded The Brain Prize for our work on the local synthesis of proteins is such an honour for me, the students, the postdocs, the technicians, and the staff that have worked with me over the years both at Caltech and also at the Max Planck Institute for Brain Research. It is wonderful recognition of the entire field and all the labs that have been working hard to understand and visualise the mRNAs, the nascent proteins and the ribosomes that drive the establishment, the maintenance, and the plasticity of synapses, which are very far away from the cell body.

I'm really proud to share The Prize with Mike Greenberg whose work I have admired and been influenced by for decades, and also to share The Prize with my dear friend, Christine Holt, who has been leading the way in local translation. She's been signalling to us from across the synaptic cleft in the presynaptic compartment. I'm very grateful to the Lundbeck Foundation for their extremely generous support of neuroscience research and for this great recognition of our efforts and our discoveries. Thank you.



Brain Prize winners 2023: Commentary

Molecular mechanisms of brain development and plasticity

By **Emily K. Osterweil**

Professor of Molecular Neuroscience,
Wellcome Trust Senior Research Fellow,
University of Edinburgh

The brain has been described in a number of different ways-- from a machine that performs computations to the seat of consciousness. These conceptual descriptors are useful for framing the myriad functions the brain performs to accurately interpret the world and navigate within it. Among these functions, one of the most essential is allowing us to learn and form memories. We must learn to avoid dangerous situations, such as touching a hot stove or a sharp object. We must also learn to navigate towards essential elements of survival such as food and warmth. The way the brain processes sensory information to modify behavior has intrigued scientists for centuries. In the late 19th century, a step change occurred when Nobel laureates Camillo Golgi and Santiago Ramon y Cajal employed a novel silver staining method to perform the first clear microscopy study of brain tissue. The painstaking catalogue of resulting illustrations reveal a complexity of structure and organization that captures the minds of young neuroscientists to this day. Their work led to the conclusion that the brain was neither a machine nor an ephemeral mystery, but rather an organ comprised of individual neuron and glial cells separated by synaptic gaps. This foundational Neuron Doctrine laid the groundwork for mechanistic pursuit of age-old questions about the brain.

Over the next decades, pioneers such as Otto Loewi and Sir Henry Dale discovered that this communication consisted of chemical neurotransmitter release at the presynaptic axon terminal binding to ion channel-linked receptors on the postsynaptic neuron. This information was key for the work of Donald Hebb, who in 1949 proposed that specific patterns of activity

could strengthen a network of neurons by enhancing the synapses connecting them [1]. This long-term synaptic strengthening (LTP) is now considered a cellular model of learning. The challenge for validating this model as the mechanism for memory formation was to determine how a stimulus at one set of presynaptic inputs could alter the biochemical makeup of the postsynaptic neuron in a prolonged manner that was sustained for the lifetime of the memory. This question in particular accelerated interest in the subcellular composition of neurons.

In the 1940s biochemists Holgar Hyden and Ludwick Monne noted that the cytoplasm of neurons was particularly enriched with ribonucleic acid (RNA), the bulk of which is comprised of messenger (m)RNA and the ribosomes that translate these messages into proteins [2,3]. Hyden further showed that RNA levels were synchronized with stimulation, a phenomenon observed in numerous subsequent studies including those performed in squid axons [4] and *Aplysia* giant neurons [5]. These observations were consistent with the first electron microscopy studies performed by anatomists Palay and Palade in 1955, who noted, “the most striking morphological feature of the neuron is the tremendous accumulation within its cytoplasm of small granules (ribosomes) associated with a well-developed endoplasmic reticulum” [6]. By the 1960s, a groundswell of work showed that RNA and protein metabolism increased in brain during learning [7,8]. A molecular theory of memory developed, proposing the enhanced activity of neurons in a memory trace was sustained by production of RNA [9]. Some proposed learning induced a permanent conformational

change in the RNAs or proteins within the stimulated neurons. This theory was tested through a series of “cannibalistic” experiments that involved introduction of brain material from a trained animal into an untrained animal to improve learning. Others proposed that learning produced a new set of RNAs to facilitate activity in a persistent but reversible fashion. This latter idea was strongly supported by the work of Flexner, Flexner and Stellar in 1963, who showed that direct infusion of a protein synthesis inhibitor into rodent brain prevented the transition of learned associations into memories [10]. This powerful evidence was validated by many subsequent studies showing similar results.

A number of critical questions arise from this molecular theory of memory. Is it reasonable to assume a specific set of RNAs and/or proteins are synthesized solely to maintain the activity of neurons of a memory trace? If so, are these produced in a rapid enough fashion to be consistent with synaptic activation? How can a set of RNAs maintain a sustained change in activity at specific synaptic connections? Many of the answers we have to these key questions we owe to the winners of the 2023 Brain Prize.

As a postdoc in the lab of Edward Ziff at New York University, Michael Greenberg was studying the genetic changes that occur in response to external stimulation of a cell. In a foundational 1984 study, he showed that growth factor stimulation of mammalian cells causes the upregulation of the transcription factor Fos on a timescale of minutes [11]. The idea that gene expression changes could be induced on such a rapid timescale was a paradigm shift that ushered in a new era for neuroscience. As an Assistant Professor at Harvard, Greenberg went on to show that neurotransmitter release causes a rise in calcium in the postsynaptic neuron, which travels to the nucleus to activate transcription factors such as Fos and initiate downstream transcription programs [12]. Many of the transcripts produced in response to activity encode gene regulatory factors that shape further activity in a cell-type specific fashion, and proteins that control synaptic maturation and stability. In subsequent decades, these cellular effectors identified by Greenberg have been shown to be essential for many aspects of brain function, from formation of context-dependent memories after exposure to a fearful or rewarding stimulus, to the developmental plasticity of the visual system in response to sensory input [13]. By identifying the key markers of neuronal activity, his work also spurred the development of a critical suite of molecular tools that

remain essential for modern neuroscience research. Beyond this, several of the genetic regulators identified by Greenberg as markers of previous experience are now known to be risk factors for neuropsychiatric and neurodevelopmental disorders, most notably Rett Syndrome [14].

The work of Greenberg was essential for validating the hypothesis that rapid changes in transcription produce sufficient alterations in neuronal RNA expression to sustained changes in synaptic function. However, a key outstanding question was how one of synapse specificity. Given the cellular energy expense, was it reasonable to presume that persistent changes in specific synapses were independently maintained by RNA transport from the nucleus, even if this were millimeters away? This puzzle stimulated Erin Schuman and Christine Holt to examine the possibility that critical changes in synapse formation and function were persistently maintained by local changes in RNA translation.

As a new Assistant Professor at California Institute of Technology in 1996, Erin Schuman performed the first experiments showing local protein synthesis is required for maintaining LTP. The notion that protein synthesis occurs at synapses was supported by the earlier work of Sherry Feig and Peter Lipton who visualized newly synthesized proteins in dendrites, and the 1982 electron microscopy study by Oswald Steward and William Levy, which showed that polyribosomes localize to postsynaptic sites [15-17]. However, whether local protein synthesis was relevant for synaptic function remained unknown. Schuman had recently shown that stimulation with growth factor BDNF caused LTP to occur at hippocampal synapses in rodent brain slices. To determine whether this plasticity could be maintained by local protein synthesis, Schuman performed the same recordings in dendrites physically separated from their cell bodies [18]. Her results showed that not only is LTP persistent in isolated dendrites, it is eliminated with protein synthesis inhibitors. This crucial evidence proved that local protein synthesis supports changes in synaptic strength. Indeed, subsequent work by Kimberly Huber and Mark Bear showed that long-term synaptic depression (LTD) downstream of metabotropic glutamate receptors is also maintained in isolated dendrites [19]. These experiments provided the long-awaited answer to whether protein synthesis was a biochemical mechanism for supporting long-lasting changes at individual synapses.

Schuman's work has since shown that local protein synthesis is a pervasive and necessary participant in the regulation of synaptic function throughout the brain. In the early 2000s, her studies using newly-developed imaging methods established the extent of translation in dendrites during plasticity and in response to different synaptic stimulations [20]. Indeed, through her investigations of mRNA translation in synaptic activity, she has created a number of cell biological tools that are widely used by neuroscientists to probe mechanisms of synaptic function [21]. Schuman has also defined a role for protein synthesis in the modulation of spontaneous activity at the synapse, and in restructuring the neuronal proteome during homeostatic plasticity [22,23]. In her more recent work, she has employed powerful methods in ribosome profiling to interrogate the translational landscape of the neuropil, identifying thousands of mRNAs, many of which are regulators of synaptic function [24,25]. Alongside this, she has revealed a complex interplay between protein synthesis and degradation that reorganizes the synaptic proteome in response to activity on an impressively fast timescale [26]. Together, her work over the past few decades has provided a comprehensive and impressive amount of information about the way that local protein synthesis supports plasticity in the brain. Her work is also increasingly relevant for the understanding of neurodevelopmental disorders such as Fragile X Syndrome and Tuberous Sclerosis, which have been shown by Mark Bear and others to be disorders of synaptic translation [27].

At the same time that Schuman was performing foundational work on local protein synthesis in synaptic function, Christine Holt was performing foundational work on the mechanisms that guide synapse formation. As an Assistant Professor at the University of California San Diego, Holt's work focused on understanding how axons are guided over long distances to eventually form the correct synaptic connections [28]. She discovered the key cell adhesion molecules that recognize external cues to guide the growing tips of axons to their appropriate targets during the development of the visual system in *Xenopus* tadpoles [29]. Her work also revealed that stimulus-dependent local remodelling of the growth cone critical for axon guidance. Previous ultrastructural studies had localized polyribosomes to developing axonal growth cones, prompting Holt to speculate that the local translation of RNA could support the local restructuring needed for growth cone guidance. In 2001, her seminal study was the first to show that local translation supports axonal function, by revealing that protein synthesis inhibitors prevent stimulated growth cone turning in axons separated from the cell body [30].

Similar to the experiments performed by Schuman, Holt found that growth cones could steer even when separated from the cell body, and this is eliminated in the presence of protein synthesis inhibitors. Her subsequent studies provided rich and detailed analyses of the mechanisms of translation that control axon guidance. Most recently, Holt used advanced molecular tools to show that 1000s of mRNAs are translated in axons of both developing and mature circuits of the mammalian visual system [31,32]. As part of this research, she has established that local translation is necessary for the maintenance of axons in the mature brain, with implications for neurodegeneration [33]. This pioneering collection of work has defined a critical role for local RNA translation in the development and maintenance of synaptic connections.

The early biochemical work showing a striking enrichment of RNA in brain tissue was an indication that the gene expression is utilized on a different scale in neurons versus somatic cells. What is clear from the work of the 2023 Brain Prize winners is that RNA transcription and translation is the medium used by neurons to maintain a persistently altered state, a fundamental process for allowing previous experience to direct future action. The mechanistic insights generated from their work continue to generate new avenues of investigation and illuminate aspects of learning critical for understanding brain development and function.

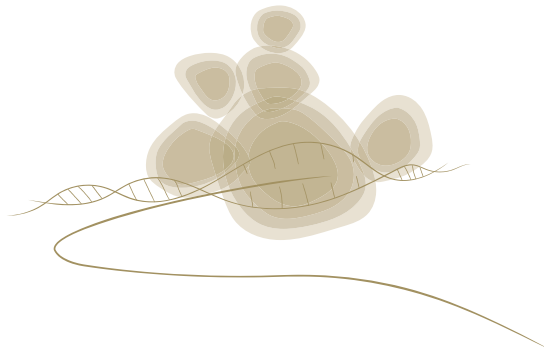
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Autobiographies of the 2023 Brain Prize winners

Michael Greenberg

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Early life and a rough beginning

My early life was complicated but understanding how I got to where I am now requires that I tell you a little about it.

I was born in Miami Beach Florida shortly after my father was discharged from the Navy. Our family soon migrated north to upstate New York where my parents pursued their interests in art and pottery, and my older sister and I took our first steps.

Just before my fourth birthday our family fragmented, and my mother moved to New Orleans with her new husband, while my father went back to Brooklyn where he had been born and raised. My sister and I spent the next four years between New Orleans and New York. During the school year we were in New Orleans, and we spent our vacations in Brooklyn.

These four years were very difficult. My mother seemingly lacked the nurturing gene (something I later came to study), and her husband, perhaps suffering from PTSD due to World War II, was abusive. I spent these years filled with anxiety and uncertainty. However, during this time, I learned to look out for myself, and developed a strong independence streak. I also developed an ability to climb over obstacles that were in my way, a trait that has served me well as a scientist.

Just before my eighth birthday I made my first major life decision. I cajoled my father into letting me move to Brooklyn to live with him. Leaving our possessions behind, my sister and I boarded the plane from New Orleans to New York for the last time. This was perhaps the most consequential decision I have made

in my life. As one of the famous New York baseball players of my youth Yogi Berra said – “when you come to a fork in the road -- take it”. I took a path that had not been charted for me, and I have not for a moment regretted it.

I could have started this autobiography beginning in the 1960s when I arrived in NYC and became a part of an exceptionally supportive blended Jewish family in the Flatbush section of Brooklyn. There were six of us, my father Ben, my step-mother Nancy, my three siblings and me. Two sets of grandparents lived within blocks of us in one direction, and our aunt, uncle, and cousins lived two blocks away in the other direction. Our family was exceedingly close. All holidays and adventures were shared with the extended family. We spent many idyllic summers in Bucks County Pennsylvania swimming, reading, and relaxing.



Me at age 12

Our family had already been in Brooklyn for many generations as my great-great-grandparents had fled anti-semitism in Russia and Poland in the middle of the nineteenth century. By the time my generation came along our family had set down deep roots in Brooklyn. My siblings and I were part of the post World War II baby boom. Our family provided us with an enormous amount of love and support. We were educated in the NYC public schools which instilled in us mid- 20th century American values. Academic achievement was encouraged, while doing good in the world was an expectation. President Kennedy has told us “ask not what your country can do for you ask what you can do for your country”. My friends and I took this very seriously.

My father was a Madison Avenue film producer and my step-mother an elementary school teacher. With two working parents, and four children to raise, we kids were given a lot of independence. School was a serious part of our lives but was not particularly demanding. This allowed us a lot of time for outside activities which included biking, hiking, camping and music.



My stepmother and father, Nancy and Ben Greenberg in 1982

The making of a scientist

I am not sure if my family and friends would have predicted that I would become a scientist. There had never been a scientist in my family before. My parents were interested in the arts and politics. Current events and family matters were topics of discussion around our dinner table, but never science.

It’s been said that the NYC public schools were breeding grounds for world class scientists. I was educated in the Sputnik Era as the U.S. was racing to put a man on the moon. Throughout that era public school kids like me, who had an aptitude for science, were provided opportunities to delve into science. Perhaps because of my difficult life in New Orleans I grabbed every opportunity that was placed in front of me. One of these opportunities was participation in a National Science Foundation sponsored summer program for high school students at the Roswell Park Memorial Institute (RPMI) in Buffalo.

I worked in the laboratory of Dr. Jake Bello, a biophysicist studying the structure and function of ribonuclease. What I did there was less important than what I learned. I was exposed for the first time to the recent discoveries of DNA, RNA, and proteins in a laboratory setting, and became totally hooked almost obsessed with the prospects for discovery in the burgeoning field of biochemistry. Dr. Bello spent way more time than he should have answering my seemingly endless questions about the experimental details, but also about what the big unanswered questions were.

The laboratory environment was thrilling. It was much more social than I expected with its constant flow of ideas and hypotheses. And the requirements for slow meticulous work, the studious exploration of the literature, the solving of difficult puzzles, and the pursuit of the unknown suited me well. My previous work experiences packing boxes in my uncle’s ribbon and fabric factory, delivering films for my father across Manhattan, and washing dishes and making donuts in the school cafeteria were tedious by comparison.

I think because of my strong work ethic I was invited to return to the lab for three subsequent summers. This proved to be an exceptional opportunity because I was being paid to do something I loved at a time when my family’s resources were very limited, and I needed to earn money to cover my tuition and living expenses.

I entered Wesleyan University in Connecticut in 1972. The school’s emphasis on a broad education with few requirements was a strong attraction. By immersing myself in literature, philosophy, and history as well as science I broadened my perspective and I learned to express myself well both in writing and speaking. I think that these skills are under-valued during scientific training, and that a lot of my later success is due my having developed these skills while in college.

Biochemistry fascinated me, but because my course work emphasized chemistry and physics, when I finally sought admission to graduate school in biology, I was rejected by most of the schools I applied to. However, I was eventually admitted to the Rockefeller University off the waiting list, and started there in 1976.

If you had stood at the Rockefeller gates in 1976, and asked people leaving the campus which lab was the most difficult to navigate I think ten out of ten people would have said “the Edelman lab”. Nevertheless, attracted by the brilliance of the lab head Gerry Edelman, who had received a Nobel Prize for his work on the sequence of immunoglobins, and the exciting current work going on there on virtually every important problem in biology, I joined the lab within weeks of my arrival at Rockefeller thinking that some of Edelman’s brilliance might rub off on me.

The lab proved to be quite intense. Edelman, while brilliant, was also intimidating, and demanding. Collaborations with outsiders were strongly discouraged. Edelman demanded hard work and provided very little day to day guidance. A student was forced to chart their own course within the narrow confines of what was considered permissible, while extracting guidance from an assortment of excellent postdoctoral fellows and assistant professors within the lab. The bar for success was very high, and the likelihood of failure was ever present.

As a PhD student I learned the basics of being a scientist by endeavoring to figure out the function of Src the first tyrosine kinase discovered. My job was to purify the enzyme and discover its substrates. While I had a modicum of success, and eventually published several papers, my contributions were sufficiently mundane that as I was nearing the completion of my thesis work. Dr. Edelman barked at me more than once – “Greenberg you’ve learned to carry out experiments, you can even complete a project and write papers, but we have to get you out of the practice room and into the concert hall”. His words were severe and at least momentarily devastating. What Edelman meant was that I was now a journeyman scientist, but one who lacked creativity. His harsh words reverberated and stuck with me as I ventured out of the lab with my PhD in hand.

A career defining discovery

Prodded by Edelman, I was in search of an important unanswered question to dig my teeth into – a question that was big enough that it might take a lifetime to answer. What I decided to study was how extracellular

stimuli such as growth factors, and neurotransmitters send signals within mammalian cells to elicit adaptive responses.

To address these questions, I joined the laboratory of Ed Ziff at NYU. What attracted me to Ed’s lab was his expertise in molecular biology and also my sense that he would give me freedom to pursue my own ideas and would help me to develop my creative side. This turned out to be the best decision I could have made.



My postdoctoral advisor Ed Ziff and me at my Harvey Lecture 2007

When I began my postdoctoral work, it was known that processes such as growth factor-stimulated cell cycle re-entry required new gene expression. There was also intriguing evidence that new gene transcription must somehow be required for experience-dependent brain plasticity. However, none of the genes that might mediate these processes had been identified. In addition, the mechanisms by which growth factors and other extra-cellular stimuli send signals to the nucleus to activate genes were unknown.

With respect to cell cycle re-entry, Ed and I reasoned that proto-oncogenes, whose mutation leads to oncogenesis, might mediate cell cycle progression. In the best of all worlds, perhaps the transcription of one or more of the proto-oncogenes would be induced by growth factors, and their encoded proteins might control cell cycle re-entry. To test this idea, we wrote to labs across the U.S. requesting plasmid DNAs that carried the sequences of 20 known proto-oncogenes. To assess the transcription of the 20 genes as fibroblast cells were stimulated by serum to re-enter the cell cycle, I spotted the plasmids onto filters and hybridized to ³²P labeled RNA produced in an “in vitro nuclear run-on assay”.

The results of this experiment couldn't have been better. Ed and I discovered that growth factors trigger the rapid and transient induction of Fos transcription thus identifying the first of the immediate early genes. The induction of Fos occurred much faster than anyone would have expected, as soon as five minutes after growth factor addition. This observation, together with the finding of Tom Curran that the Fos gene encodes a nuclear protein, led us to speculate that Fos might function in the nucleus to promote cell cycle re-entry.

The results were stunning in that the Fos protein was absent from the cell prior to the stimulus, and upon growth factor exposure the Fos protein was increased several hundred-fold.

We found that the Fos gene is tightly regulated at multiple levels. Almost immediately after its activation Fos transcription is shut off again. Because the Fos mRNA and protein have extremely short half-lives the Fos induction event occurs only very briefly, consistent with the idea that Fos must have a critical regulatory role when cells were stimulated to re-enter the cell cycle.

With these results in hand, I knew I had stumbled upon a fundamental biological process that was likely to take me many years to understand. Key questions were how widespread is the Fos phenomena, is Fos just one of many inducible genes, how is the growth factor signal conveyed to the nucleus to activate Fos transcription, what is the function of Fos in the nucleus, and what are the cellular and organismal consequences of Fos induction?

In determining if the induction of Fos is specific to cell cycle re-entry, or a more general response that occurs when many different cell types are exposed to extra-cellular stimuli, we enlisted the help of Lloyd Greene a faculty member at NYU, and whose lab was upstairs from ours. Lloyd had pioneered the use of PC12 cells, a neuronal cell line that is responsive to both nerve growth factor and agonists of the acetylcholine receptor.

With these cells we asked whether NGF, which stimulates PC12 cells to differentiate into sympathetic neuron-like cells, induces Fos, and do agonists of neurotransmitter receptors also induce Fos. The answer to both questions was a resounding yes, and it soon became clear that many different extra-cellular stimuli can induce Fos transcription in a wide range of cell types.

The ubiquitous nature of the Fos response led many investigators to conclude that the response was non-specific, and not likely to give much insight into how cells respond to a stimulus. I felt differently. Perhaps Fos acts as a sort of 3rd messenger in the nucleus functioning downstream of second messengers such as Ca²⁺ and cAMP. If we could understand Fos function perhaps we could uncover a vast array of transcriptional programs that control cell growth, differentiation and plasticity.

Based on this work, and Ed's support, I obtained a faculty position as an assistant professor in the Department of Microbiology and Molecular Genetics at Harvard Medical School (HMS). The department chair Bernie Fields gave me maximum freedom to follow my curiosity, and no one seemed to mind that I was about to become a neurobiologist in a microbiology department. My initial findings on Fos had garnered the attention of many well-funded laboratories that were way better equipped than I was to study Fos during cell cycle re-entry. I therefore left the fibroblasts behind and turned my attention entirely to the nervous system. While I had little training in neuroscience, I had been exposed to the burning questions in the field while a student in the Edelman lab and was excited to steer my lab in a new direction.

It was known from the work of David Hubel and Torsten Weisel that sensory experience in early life is essential for brain maturation and plasticity. As these processes unfold in humans over decades, I reasoned that they might require experience-dependent transcription and that Fos (and genes like it) might be a key to understanding these experience-dependent processes. Even more exciting was the prospect that Fos might be a regulator of long-term memory. These ideas formed the basis of my lab's work for several decades, and while wildly speculative at the time, we and others in the field have obtained evidence in support of these concepts.

Essential to the success of my foray into the field of neuroscience were the many fantastic students and postdoctoral fellows that I have attracted to my laboratory. Harvard has proved to be a lightning rod for talent. Our work has also benefitted immeasurably from many collaborations with the amazing faculty that make up the neuroscience community at Harvard. Unfortunately, given space limitations I am not able to acknowledge each of my wonderful colleagues by name.

Life in the Fos lane

My early findings demonstrated that ion channels have functions beyond the control of neuronal excitability and neurotransmitter release, as calcium channels signal to the nucleus to activate gene expression. My colleagues and I initially focused our attention on deciphering the mechanism by which channels at synapses signal to the nucleus to activate genes in neurons. Along the way, we discovered the importance of Ras signaling and CREB phosphorylation as key mediators of activity-dependent gene induction. This led to a flurry of studies in other labs suggesting that CREB is a mediator of long-term memory.

Around the same time, we and others demonstrated that Fos is a transcription factor that binds DNA as a heterodimer with the product of another inducible gene called Jun. And a model was formulated that the Fos/Jun dimer binds to the promoters of target genes, and by activating these genes mediates the cells response to the initial stimulus. We also identified a host of additional genes that are activated by sensory stimuli. These include so called immediate early genes (e.g Jun, Egrs, Nr4a1, and Npas4) that like Fos encode transcription factors that are rapidly and transiently transcribed in response to the stimulus. We now know, after many years of work, that the immediate early gene (IEG) encoded TFs activate late response genes that control experience dependent brain maturation and plasticity.

Our work benefitted greatly from the development of new technologies that have come into existence along the way – some of which we pioneered ourselves (the development of phospho-specific antibodies, the first methods for transfecting neurons). By the 1990s we had become experts at defining the signaling pathways that govern the distinct steps of nervous system development, and had discovered pathways and mechanisms of neuronal and glia cell fate (STATs and Ngns), axon guidance (Ephs and ephexins), synapse development (EphBs and NMDA receptors), neuronal survival (Akt, Bad, and Foxo3A), and neuronal death (JNK).

Nevertheless, my scientific obsession was still to understand how activity-dependent gene transcription shapes brain maturation and plasticity. In 1994 when we disrupted the function of the closest relative of Fos, FosB, in mice we observed a dramatic phenotype. The mice appeared to be normal but displayed a profound defect in parental nurturing. This provocative finding was enthralling at the time. It suggested that there is a genetic basis for parental nurturing and provided evidence that activity-dependent genes regulate brain plasticity. How this comes about required a much deeper dive into the function of Fos and other IEGs. However, it seemed clear that we had made a major advance in understanding how nature and nurture are intertwined in the orchestration of brain plasticity.

*Greenberg lab members
at my 50th birthday celebration*





My wife Roz and me in front of Harvard Medical School in 2017 at the "Science March"

Assessing Fos function in learning and memory proved to be challenging given the heterogeneity of the brain, and the complexity of the Fos family. However, with the dawning of the genomics era, the advent of single cell sequencing approaches, and the CUT&RUN approach to identify TF binding sites across the genome, after 25 years of staggering around in the dark we finally began to identify Fos binding sites in each type of neuron, and bona fide Fos targets in the brain. We found that in response to sensory stimuli, Fos and other IEGs are activated in diverse neuronal subtypes and in non-neuronal cells. However, Fos stimulates distinct subsets of LRGs in each cell type, and the LRGs are tailored to the specific functions of the individual cell.

My siblings, mother, and me several years ago at our house in Woods Hole, MA Left to right: me, my sister DeDe, my mother Nancy, my sister Penny, and my brother Bill

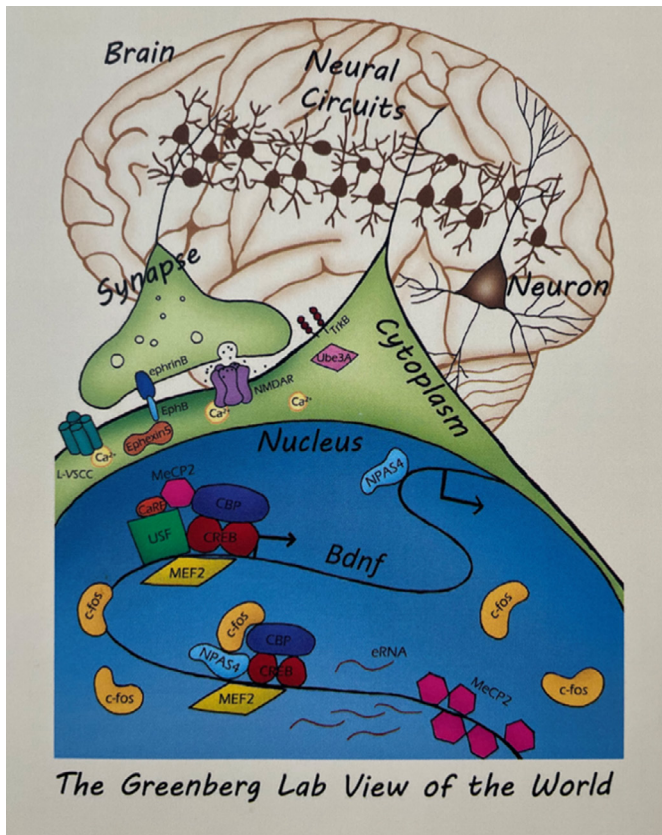


Fos activates gene transcription by binding to the consensus 5'TGA(G/C)TCA3' in DNA. While there are over 600,000 potential Fos binding sites in the human genome, we discovered that the Fos/Jun heterodimer binds to a unique subset of these sites in each cell type. In addition, we found that the early model that Fos/Jun heterodimers activate genes by binding to their promoters is incorrect. Rather Fos/Jun heterodimers bind almost exclusively to enhancers, sequences that regulate gene expression often from a great distance from the promoter. The discovery that Fos binds to enhancers was important because it explained how Fos is able to confer neuronal subtype-specific responses since enhancers are specified in a cell type-specific manner.

Remarkably, we found that many Fos target genes encode secreted proteins that regulate neural connectivity. And perhaps it's not surprising that as we identified these Fos targets, we were able to show in a series of studies that the activity-dependent gene program regulates each aspect of experience-dependent brain maturation including neuronal survival, dendritic growth, synaptic pruning, the recruitment of inhibitory synapses to excitatory neurons, and the plasticity that underlies learning, memory, and behavior.

Our most recent progress was made with conditional knockout mice where we injected Cre into the brain and eliminated Fos family TFs or their target genes in a highly specific manner. This allowed us to define the importance of the activity-dependent gene program for somatic inhibition, a form of neural plasticity that is likely critical for the formation of ensembles of neurons that encode long-term memories. Finally, in a collaborative study we discovered that Fos is critical for spatial navigation and has a profound effect on place cells in the hippocampus. When Fos function is disrupted the stability, reliability, and selectivity of place cells is impaired providing further evidence that activity-dependent gene programs regulate learning and memory.

A gratifying aspect of our work has been the insight it has provided into disorders of the nervous system. The signaling networks that activate IEGs are literally studded with proteins that when mutated can lead to intellectual disability, autism, and epilepsy. Likewise, we discovered that the IEGs themselves bind multi-subunit chromatin regulatory complexes that encompass many proteins that are mutated in still other human brain disorders. These findings provide evidence that the activity-dependent gene program is critical to human brain development and plasticity and



give us hope that our studies over the years will yield new approaches for treating some of these devastating disorders of the brain.

Eventually our studies of activity-regulated gene expression and its role in learning, memory, and behavior gained traction. Over 1,000 different stimuli have been identified that induce Fos in the brain, and this induction occurs selectively in the regions of the brain that mediate the organism's response to the stimulus. The development of "Fos traps" has made it possible to capture the Fos expressing cells for opto- or chemo-genetic control allowing the neural circuits that mediate long-term memory to be identified. Given the widespread use of these approaches by neuroscientists it has become hard to deny the importance of Fos for systems neuroscience. However, it is still not fully appreciated that Fos is much more than a just marker of active neurons. In line with this idea, our recent findings provide clear evidence that Fos is in fact a mediator of plasticity. And I am optimistic that the continued use of genomic approaches to identify Fos targets, and the application of spectacular new technologies for studying gene function within neural circuits will yet provide unparalleled insight into the molecular and circuit basis of long-term memory.

A few final points

My career in science has been immensely satisfying. In addition to the thrill of discovery that has accompanied my relentless pursuit of the mechanisms by which nature (genes) and nurture (environment) conspire to shape brain maturation and plasticity, I have been gratified to have the opportunity to train many of the molecular and cellular neuroscientists of the next generation. After a number of years in the HMS Microbiology Department, I was recruited to be the leader of neuroscience at Boston Children's Hospital (1994-2008), and later the Chair of Neurobiology at HMS (2008-2022). In these roles I have been able to recruit many new faculty and help them launch their careers. This too has been a truly rewarding experience.

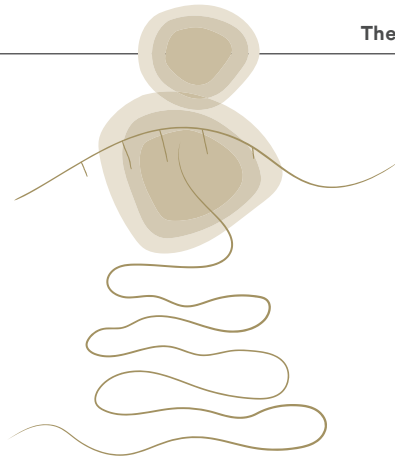
While I was a graduate student in the Edelman lab I met, and eventually married Rosalind Segal who went onto become a physician scientist and Professor of Neurobiology and is now the Dean for Graduate Education at HMS. Roz has provided steadfast emotional support throughout my career, and her scientific input has been critical to my thinking at every stage of my career. Roz and I have raised two children, Rachel and Daniel who have become fine human beings with careers of their own. They too have supported me immeasurably as I pursued my obsession with Fos and the brain. Roz, Rachel, and Daniel, along with our parents and siblings, have made it possible for a kid who got off to a rough start to have a particularly fulfilling personal and professional life.

My family Rachel, Roz, Daniel, and me



Christine Holt

Department of Physiology, Development and Neuroscience, University of Cambridge, UK



My upbringing, family and education

My interest in science stemmed from my rural roots in Northumberland, northern England. I grew up in a village in the Tyne valley where I spent my days roaming in the fields, streams and woods surrounding the house. I loved exploring nature. I was the youngest of three children. My sister, Jennifer, was 6 years older



My parents getting married in 1947, or thereabouts



Siblings: me with my older sister Jennifer and brother (1956)

and my brother, Stephen, 2 years older. Stephen also loved nature he and I had all sorts of adventures together, such as badger-watching which meant settling into position before the sun set, waiting patiently for a glimpse of a badger as the birds and sounds of the woods settled down for the night around us. In many ways it was an idyllic childhood. My father was a Royal Naval officer during the war and afterwards he worked as a director in a shipping company. He also loved wildlife and he had a great sense of humour, as did my brother, so there was lots of laughter in the house. My mother served in the Women's Royal Naval Service during the war and, when she married my father, became a home-maker. She loved literature and animals, there was always a menagerie of dogs and cats living in the house. My parents were caring and full of fun, they supported and encouraged me in whatever I wanted to do. They gave me freedom to explore.

I went to a small primary school in a nearby village (Stocksfield) with just two teachers and two classrooms for children from aged four to twelve. The head teacher was scary, and I was not one of her favourites. I remember she refused to teach me Latin despite teaching it to younger pupils! I was sent away to an all-girls boarding school, aged 10, joining my sister at Harrogate College in Yorkshire. This was considered 'normal' at that time, and I happily packed my trunk and boarded the train every term. My memories of Harrogate College are of chilly mornings, frost inside the windows, chilblains, morning runs, strict teachers and fun friendships.

I enjoyed Nature Studies and Art and I remember being inspired by a teacher, Mrs. Smales, who decorated the classroom with wonderful pictures of flora and fauna and took us for walks in the woods to make observations of nature. I did reasonably well academically. I also learned piano and ballet, joined the choir and particularly enjoyed arts, crafts and sports. At age 16, I moved to a sixth form college, St. Clare's College, in Oxford. This exposed me to an international set of pupils and Oxford University, and indeed to the possibility of going to university which up until then I had not considered.



Me aged 21 at Sussex University

The subject I most loved was Biology, but my A-level Biology teacher was abysmal. He often forgot to show up for lessons and he did not realise that the syllabus had changed until just before we took the national exams! I did well enough to get into Newcastle University to study Zoology, however, I was not keen on what I viewed as the ‘old-fashioned’ biology curriculum based largely, it seemed, on the learning of Latin classifications. After one year, I switched to Sussex University which offered a much more exciting modern biology course. The Biological Sciences department at Sussex was filled with energetic and inspirational teachers such as the distinguished evolutionary biologist, John Maynard Smith, who used to join us in the students’ Common room for coffee sitting cross-legged on the floor after his Lectures. The final year involved a practical lab project and writing a grant proposal which kindled my interest in doing research.

As a final year undergraduate, I took courses in developmental biology and neuroscience and became particularly interested in how nerves make connections in the brain. I simply could not understand how the retina could make such an accurate ‘map’ of the outside world in the brain. At the suggestion of Mike Land, my personal tutor and a vision neurobiologist, I applied to do a PhD with John Scholes in the Biophysics Unit at King’s College, London. Scholes worked on the map-

ping of connections in the cichlid fish and was doing absolutely beautiful work on how retinal-topographic order is precisely arrayed in the ribbon-shaped optic nerves of these animals.

My scientific career

My PhD work was on the development of the visual system of the frog. My supervisor, John Scholes, and I often had lunch together in the pub on Drury Lane. He was incredibly articulate with a beautiful use of language and was always full of ideas. He encouraged me to do the work with very little interference and direction, but he was always happy to discuss everything. I wanted to understand how the embryonic eye transforms into the mature eye during development. John suggested that I might be able to trace the cell movements by surgically removing tiny pieces of embryonic eye tissue, incubating them for a few minutes in a tiny droplet of radioactive nucleotide to label DNA (H3-thymidine), and then replacing them in the eye. This technique worked well enabling me to discover the cell migrations occurring during eye formation, and this helped to explain a major controversy in the field about eye specification.

Considering how well the radioactive-labelling technique worked for tracing cell movements, I next used a radioactive-amino acid to label the axons. This allowed me to label different parts of the retina and map their nerve projections to the brain. At that time, a prominent theory was that nerve connections are initially disordered and only sort out to become ordered by ‘use’. My work, however, showed that the axons from the eye are ordered right from the start and suggested that a high degree of specificity underlies the navigation of axons to their targets in the brain.



*John Scholes,
my PhD supervisor*

Towards the end of my PhD, I applied to Torsten Wiesel (Harvard) for a postdoc. He replied that he did not have space for me but suggested Bill Harris (my future husband as it turned out). Bill had trained with Wiesel and recently set up his own lab in University of California San Diego (UCSD). I visited Bill's lab in 1981 and together in about a week we were able to gather electrophysiological data from tiny tadpole brains that supported the anatomical work I had done on labelling retinal axons. I was impressed! I deferred a postdoctoral fellowship I'd been awarded back in the UK for a year while I continued to work with Bill at UCSD. There, I explored the question of whether the relative order of axon growth has a role in setting up the topographic order of connections. I then went back to the UK to work in the Physiology Department at Oxford University with the Colin Blakemore and Ian Thompson, both inspirational neuroscientists who made doing science enjoyable and fun.

I married Bill Harris in 1983 in Hexham, Northumberland, and we returned to UCSD together to live in La Jolla. In the next few years, we had two beautiful children, Julia and Jake, who have both gone on to become young scientists. For a long time, I found it hard to make good progress in the lab given the combined pressures of motherhood, teaching and having to do all the research with my own hands as I did not have a lab and could not take PhD students or postdocs. Then, in 1986, we spent 9 months at the Max Planck Institute in Tübingen, Germany, working with Friedrich Bonhoeffer. Friedrich was a major influence on my research. Together, we made live-movies of axons growing in the vertebrate brain using his new fluorescent-dye tracing methods and state-of-the-art time-lapse microscopes. This was a most exciting and memorable time, being able to see the tip of a growing axon, the growth cone, moving in the living brain for the first time, a dream come true!

In 1997, after fifteen wonderful years at UCSD, during which time I eventually secured a tenured appointment in the Biology Department, we moved to the University of Cambridge, UK. It was a marvellously supportive environment academically and scientifically. My Cambridge colleagues were always willing to help with new technical challenges and with exchanging ideas. I think it is fair to say that my science thrived in Cambridge! I shared my lab with Bill's lab - we had lab meetings together and we became the "H/H lab". Developmental Neurobiology as a field was strongly represented in the UK in the late 1990s/early 2000s with many labs doing ground-breaking work, such as



Bill Harris, my husband and life-long collaborator

Andrew Lumsden and colleagues at King's, London, so it was an exciting era.

My Science

My lab research has largely focused on investigating how neurons wire-up properly in the early brain. In 1990, we developed techniques for introducing genetically engineered molecules into developing neurons and, with the help of many collaborators over many years we were able to identify some of the key steps involved in the navigation of growing axons in the visual system. For example, we found that a molecule, Ephrin-B, pops-up at the optic chiasm where it helps to direct the divergence of axons to the correct side of the brain.

In 2000, a Science journal landed on my desk with a paper from Mark Bear's lab reporting that synaptic activation triggers *rapid* (within minutes) protein

*My PhD student,
Doug Campbell,
and me*



synthesis that modifies synaptic transmission in dendrites. Erin Schuman's pioneering 1996 work had previously shown that local protein synthesis is required for immediate synaptic plasticity. This surprisingly fast synthesis gave me the idea that new protein synthesis might also be involved in the directional guidance of axons. A simple experiment to test this was to add an inhibitor of protein synthesis to a growth cone guidance assay. Several of my group members were doing guidance assays and I tried, in vain, for a long time to interest them in doing this experiment. Finally,

PhD-student Douglas Campbell tried the experiment and I remember how excited we were when he found that the inhibitor completely blocked the response of axons to a guidance cue! One of the assays was only 10 minutes so we thought it unlikely the effect was via the distant cell body. To test this, Doug cut the axons and conducted guidance assays on sawn-off axons. Amazingly these sawn-off growth cones showed normal guidance unless in the presence of a protein synthesis inhibitor demonstrating that the protein synthesis critical for axon guidance occurs locally in the growing axon itself. One of the remarkable features of the growing tip of an axon that Bill, Friedrich and I had discovered serendipitously in 1987, is that when it is severed from the cell body, it continues to migrate and navigate correctly in the brain. This highly autonomous behaviour jived well with the new finding that axons have their own machinery for translating mRNAs and for supplying new proteins.

In 2001, we published our local protein synthesis results but I was surprised by the scepticism which greeted our work, especially since there was already evidence for protein synthesis in axons in the literature stretching back to the 1960s (e.g. Koenig, 1967). Our work then focussed on devising ways to isolate and identify the mRNAs present in axons and to investigate the role of local translation in growth cone

*Family selfie:
Julia, me Bill and Jake*



guidance. We also found ways to image mRNAs and translation in axons which revealed the extraordinary dynamics and spatial precision of these processes. For example, new proteins are made in growth cones within minutes precisely in the places where they are 'tickled' with a guidance cue, and different cues trigger the synthesis of signature sets of proteins. In 2012, using Erin Schuman's BONCAT technique, Jason Yoon, Hosung Jung and Asha Dwivedy and other colleagues in my lab found a locally synthesised protein that was unexpectedly required to keep axons alive in young *Xenopus* brains. In 2016, Hosung together with Toshiaki Shigeoka used a genetic trick in mouse to label the translating ribosomes inside retinal axon terminals and were able to show mRNAs being translated in mature axon terminals as well as developing axons. Electron microscopy revealed tagged ribosomes in myelinated axons and presynaptic terminals, indicative of protein synthesis occurring in mature axons. This, together with our earlier data linking translation dysregulation to axon degeneration, took our research into a new direction and I received an ERC Advanced Grant to study axonal translation and ribosome biology with respect to axon survival.

My journey from a child who was fascinated by nature to a life-scientist driven by curiosity would not have been possible without the many talented co-workers and collaborators along the way and the support I received for my blue-sky science from agencies like the Pew Charitable Trusts, Mcknight Foundation, the Wellcome Trust, MRC, NIH and the ERC. I've been very fortunate to share this journey with Bill whose clarity of thought, deep scientific knowledge and extraordinary positivity have guided every project. My advice to young scientists would be to always keep an open mind to new ideas, listen to what the data are telling you, and do not give up asking questions and looking for answers!



Me walking in the Lake District



H/H Lab reunion 2018

Erin Schuman

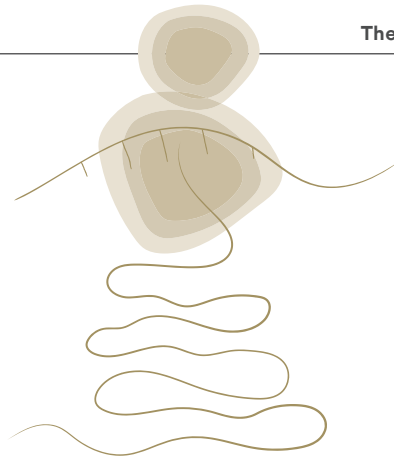
Max Planck Institute for Brain Research,
Germany

I grew up in Southern California and was mostly educated in local Catholic Schools that were known more for their athletic prowess than their academics. My mother, Susan Arant, was a school teacher for over 35 years. I was her student in 8th grade, and she was an enthusiastic supporter of my learning for those formative early years. She divorced my biological father, Bruce Chandler, when I was very young, and I've had little contact with him since then. I was raised with my sister and brother in the house of my stepfather, whom my mother later divorced. My grandfather, an engineer who designed some of the pumps for the Hoover Dam on the Colorado River, was my earliest



With my grandfather Perry Arant

mentor. My grandparents lived down the street from my childhood home and my grandfather and I worked on math and physics problems together sometimes. As an undergraduate at the University of Southern California, originally enrolled in a pre-medical course, I quickly became disenchanted with the intense competition between the pre-meds- and what seemed to me an all-encompassing focus on optimizing future medical school admissions. I turned to the Psychology department and was lucky to have as a mentor a



new Assistant Professor, Dr. Laura Baker. Laura is a behavioral geneticist who studied cognitive abilities in human twins at the time. As my time at USC progressed, I became fascinated with the brain- and how information is stored when we form memories. For my senior honors thesis, I designed my own study to measure memory abilities in 7-12 year-old identical and fraternal twins, recruiting subjects from a Southern California school district.

At the end of my time at USC I was convinced of two things: that I wanted to do research on the brain substrates of learning and memory and that humans, as subjects, were not so easy to work with. At that time, simple learning had been demonstrated in several invertebrates and their brains could be studied directly with electrophysiological recordings. I joined a lab at Princeton University and began to study associative learning in the marine snail *Hermissenda crassicornis*. I became an electrophysiologist and discovered that the enduring nature of a *Hermissenda* memory was due to the persistent activity of a protein kinase, acting on two different brain potassium channels. Midway through my PhD studies, my advisor did not get tenure and moved the lab (just me and him at that point) to the Midwest. I loved recording from neurons but my relationship with my supervisor was very dis-

With Dan Madison on a visit to Stanford in 2019



piriting and isolating. I thought about quitting science frequently. Luckily, in the summer the lab moved I took the Neural Systems course at the Marine Biological Labs in Woods Hole, Massachusetts. The course directors were Tom Carew and Darcy Kelly- and there was an incredible line-up of faculty who all re-affirmed my love of science. They must have recognized something promising in me and they encouraged me to continue. Later, Tom Carew was instrumental in my moving back to Princeton to write up my PhD thesis and helped me find a great postdoctoral environment- working with Dan Madison at Stanford.

Working in the Madison lab as a post-doc, I felt like I was on a scientific joy-ride in comparison to my dismal graduate school experience. The Molecular and Cellular Physiology department at Stanford was newly formed, chaired by Dick Tsien, and there were lots of smart, interesting and colleagues who became life-long friends. I joined Dan's lab because I was still interested in plasticity and keen to discover mechanisms using electrophysiological recordings in hippocampal brain slices. The concept of a retrograde messenger had been invoked in the long-term potentiation (LTP) field- to coordinate plasticity that was induced in the

postsynaptic cell with eventual presynaptic changes. I showed that the gas nitric oxide could serve as such a retrograde messenger during LTP in hippocampal brain slices. Jane Haley, Paul Chapman, Tom O'Dell and Eric Kandel published similar data around the same time. In a follow-up study, that involved paired intracellular recordings from neighboring neurons, I found that LTP induced in one cell could spread to nearby, but not distant, synapses, consistent with the actions of a diffusible messenger. Dan was an extremely supportive and smart boss- and he was a great teacher too. I remember those days at Stanford as some of the best times in my career- I discovered how much I love doing experiments- and how exhilarating science can be in the right environment.

Around the time I was in the middle of my second set of experiments, Dan went to give a seminar at Caltech, and he came back and told me that some Caltech faculty had asked that I apply for an Assistant Professor position, that had been open for some time, in the Division of Biology. I was not thinking about starting my own lab at all. I was actually wondering if I might hit Chuck Stevens up for a second postdoc. I went and interviewed for the Caltech job and was surprised when it was offered to me. I began my faculty position in 1993, feeling a bit plucked-from-the-nest too early, but I soldiered on. This was a time when today's concept of mentoring did not yet exist, I initially found the Caltech environment rather isolating- I missed the camaraderie of my Stanford buddies doing experiments together. A daily cry in my office after screwing amplifiers into racks and unpacking boxes kept me going. I bought a used VW convertible and I have vivid memories of driving home late from the lab on paradoxically warm Pasadena winter nights and inhaling the intense smells of citrus blooming. It was during these early years that I met my brilliant life-long partner and husband, Gilles Laurent, a systems Neuroscientist, who was already on the Caltech faculty. Our (rather nerdy) courtship began when Gilles asked if he could build an electrophysiological rig in my lab over the summer. Amongst the many friends and colleagues I eventually made, I found a fabulous mentor in the late Norman Davidson, a chemist by training, who turned to neurobiology later in life.

I was extremely lucky to have a fantastic scientist, Hyejin Kang, as my first graduate student. She was super smart and very hard-working. We set out to explore whether some of the same molecules that sculpt neurons and their connections during development might also participate in changing synapses in adult animals.

*With my husband Gilles Laurent
strolling on the Caltech campus*



We applied brain-derived neurotrophic factor (BDNF) to brain slices and found that it caused a rapid and long-lasting enhancement of synaptic transmission. We knew from other behavioral and synaptic plasticity studies that long-term memories require newly synthesized proteins. So, we did a simple experiment- we added a chemical inhibitor to block protein synthesis together with BDNF. We got a surprising result. The enhancement of synaptic strength by BDNF was immediately blocked- not just the long-term plasticity. The synapses we were recording from were a few hundred microns away from the cell body, too far away to allow for the transport of the new proteins so quickly - suggesting that the protein synthesis source was not the cell body, but local, near the synapses. We went on to show this directly by isolating the synapses from the cell bodies and doing the experiment again. In this “soma-free” slice preparation, the plasticity could still be elicited and still showed the early requirement for protein synthesis. The conclusion that proteins made near synapses are required for plasticity surprised nearly everyone. More than a decade earlier, Os Steward and colleagues had detected ribosomes near synapses, but this observation did not change the over-riding view that all proteins were made in neuronal cell bodies and then transported to synapses. Indeed, when I first shared the data, the idea was called “crazy” by more than one of my colleagues. Luckily, around the same time, Kelsey Martin was working in Eric Kandel’s lab, and she found that local protein synthesis was required for plasticity at the sensory to motoneuron synapse in *Aplysia*. Meanwhile, Christine Holt was working on the mechanisms that allow axonal growth cones to turn towards their targets- she found that local translation and degradation of proteins was essential for the appropriate turning decision.

At that time, I recognized the importance of visualizing protein synthesis directly in the dendrites. I felt with conviction that if we could see new proteins emerge in the dendrites, we would really convince ourselves and others that proteins can be made locally. I had the idea of creating a GFP-based reporter- where the mRNA could be targeted to the dendrites. We would then visualize GFP fluorescence pop up in the dendrites- providing proof for local synthesis. I remember sharing this idea with a senior colleague at Caltech and she said, “That will never work!”. The GFP reporter in fact worked beautifully and we coupled it with delicate “neurosurgery”- delivering a small cut to isolate the dendrites from the cell body. With this, we provided direct evidence for local translation.



With graduate student Hyejin Kang at Caltech

Around that time, Daniela Dieterich joined my lab as a postdoctoral fellow. She forged a collaboration with my colleague Dave Tirrell in Caltech’s chemistry department. Dave had been developing non-canonical amino acids, mostly methionine derivatives (e.g. azidohomoalanine, AHA), which were functionalized with azide or alkyne groups and could be charged onto methionyl tRNAs by the cellular methionyl tRNA synthetase. Dani showed first in living cells treated with AHA that newly synthesized (AHA-bearing) proteins could be isolated and purified using click chemistry and then identified using mass spectrometry. We called this method biorthogonal non-canonical amino acid tagging (BONCAT) and it has been invaluable to us and many others. Using azide or alkyne-bearing fluorescent tags, Dani then showed that nascent proteins could also be directly visualized (FUNCAT). Later, with Dave, Dani and Beatriz Alvarez-Castelao, we modified the tRNA synthetase and its cognate amino acid to make the system work in a cell type-specific manner and now there are platforms available in mouse, fly, worm and zebrafish.

In 2008, Gilles and I went on sabbatical to Paris. There I worked in Antoine Triller’s lab to learn single particle tracking and using FUNCAT successfully visualized the movements of newly synthesized proteins within living synapses. During that time, we were approached by colleagues from the Max Planck Society to gauge our interest in building and directing a new Max Planck Institute for Brain Research in Frankfurt. In 2009, after ~35 years of joint experience on the faculty at Caltech, with differing levels of discontent about



Schuman lab in the Spring of 2022

doing science in the U.S. and with a big spirit of adventure, Gilles and I decided to move to Germany with two of our daughters. Together with the architects we built (with the usual delays and headaches) a beautiful space that is now a high-energy, interactive neuroscience institute.

The first experiment we did after the lab moved to Frankfurt was to use emerging next generation sequencing technology to directly sequence the mRNAs present in the neuropil- this was spearheaded by Ivan Cajigas and Georgi Tushev. At that time, the potential impact of local translation was severely hampered by the very limited number (~25) of mRNAs that had been detected in dendrites. The laminar nature of the hippocampus was perfect for the isolation of the neuropil (enriched in axons and dendrites) and the somata from brain slices. Together we microdissected hundreds of slices and discovered that mRNA population was not in the 10s or even 100s, but rather in the thousands. Our first RNA-seq experiments identified ~2500 mRNAs- and as the technology improved this number climbed to ~5000 species. There was convergence on this big number of localized mRNAs from all of the groups who were sequencing axonal and dendritic transcripts at the time, including Kelsey Martin's and Christine Holt's lab. This huge number surprised us- and strongly suggested that local translation is used for the constitutive function of synapses, not just invoked during special cases of plasticity. Anne Biever and Caspar Glock's later work describing the local translatoome (all mRNAs in the process of active translation) confirmed this- all the mRNAs detected were also translated under basal conditions. In addition, by comparing the somatic and local translatoomes, we learned that the neuropil is the primary site of synthesis for over 800 synaptic proteins. We are

now in a perfect position to return to my initial goal, i.e., to examine how memories are formed, by adjusting protein levels and states at synapses. We are pursuing these questions using new tools for molecular imaging and sequencing and by monitoring synaptic changes

in living animals. Taken together, our work and that of our colleagues have changed the way we think about how individual neurons can control thousands of synapses with specificity: we identified how the protein composition of individual synapses can be modified using local translation machinery.

I'll close with this: I believe it is an enormous privilege to be a scientist. I feel very lucky to have been able to devote my life's work to understanding how brain synapses work and change to store information. One of the greatest pleasures of my life in science has been to work alongside and discover new things with the many, very clever, driven, interesting and fun people that made my lab what it is. Without them, none of the work would have been possible. In addition, I have to also acknowledge how important it has been to share this life journey with my fabulous partner, Gilles Laurent, and our wonderfully talented, smart, sensitive and funny daughters, Emma, Charlotte and Camille, who are also currently pursuing careers in science.



With my husband Gilles Laurent and our daughters, Emma, Charlotte and Camille Laurent in 2022

