Published in final edited form as:

Curr Opin Neurobiol. 2019 August ; 57: iii–vi. doi:10.1016/j.conb.2019.06.002.

Editorial overview: Molecular neuroscience

Timothy A Ryan,

Department of Biochemistry, Weill Cornell Medical College, USA

Yishi Jin

Neurobiology Section, University of California San Diego, USA

Neuroscience as a field tries to understand the mechanistic principles by which the nervous system can carry out its complex sets of tasks. Individual neurons, of which ~80 billion collectively make up the central nervous system in a human, are unlike most cells of the body. Most live for the lifespan of the animal without dividing, extend their axons over distances from 50 to 1000 times the diameter of the cell body where they will form synaptic contacts with between 1000 and 10 000 other neurons and receive inputs from as many other cells that form synaptic contacts along their dendritic tree, cell soma and axon. Additionally, neurons interact closely with glial cells and form an essential partnership with these cells throughout the lifespan of the animal. The molecular basis of how the cellular biochemistry is harnessed to allow neurons to grow, communicate, renew their machinery, respond to appropriate cues and dispose of the machinery at the end of its lifespan are all frontier topics in molecular neuroscience. Molecular neuroscience got its start with traditional biochemical purification approaches, but over fifty years ago molecular genetics vastly expanded the repertoire of genes identified to be critical for nervous system function. Today new clues often emerge from human genetics as links between neurological disorders and genetic mutations reveal our many gaps in fundamental understanding of biological processes within neurons and glia.

This issue brings together reviews that span a gamut of cutting-edge topics in molecular neuroscience. Details of the functioning of critical machinery at synapses, how synapses are guided to form at the correct time and place, how proteins are locally synthesized and when they turnover, how entire organelles are maintained are a few of the topics that are crucial to how the nervous system survives and are discussed in the reviews below.

taryan@med.cornell.edu.

Timothy Ryan is a Rockefeller/Sloan-Kettering/Cornell Tri-Institutional Professor in the department of Biochemistry at Weill Cornell Medical College and a Senior fellow at the Howard Hughes Medical Institute Janelia Research Campus. He received his BSc with honors in Physics at McGill University and his PhD in Physics at Cornell University. He carried out postdoctoral work at Stanford University in the department of Molecular and Cellular Physiology. Dr Ryan's lab has pioneered the development and use of quantitative optical tools to interrogate nerve terminal function. He was an Alfred P. Sloan Fellow, a two-time recipient of the McKnight Technological Innovations in Neuroscience Award, a recipient of the NINDS Javits Award and a recipient of the Siegel Family Award for Outstanding Biomedical Research.

Yishi Jin is a Distinguished Professor in Neurobiology Section, Division of Biological Sciences and Department of Cellular and Molecular Medicine, the School of Medicine, at the University of California, San Diego. She is the inaugural holder of the Junior Seau Foundation Endowed Chair in Traumatic Brain Injury. She received her BS with honors in Cell Biology at Peking University and her PhD in Molecular Biology at University of California, Berkeley. She carried out postdoctoral work at Massachusetts Institute of Technology. Dr Jin's lab has pioneered the use of *in vivo* labeling of synaptic components and employed the powerful genetic analysis in *C. elegans* to elucidate conserved molecular pathways regulating synapse formation. Her lab has also established *C. elegans* as a model to interrogate axon response to traumatic injury. She was an Alfred P. Sloan Fellow and a recipient of the NSF-PECASE award and the NINDA Javits Award.

How neurons form synapses with the correct partners and how synapses are maintained throughout lifetime of an organism remain the long-standing questions. Synaptogenesis is a multi-step process, leading to the establishment of synaptic vesicle release machinery on the presynaptic side, clustering of neurotransmitter receptors and signal transduction machinery on the postsynaptic side, and precise registration of the two subcellular specifications across the synaptic cleft, a specialized extracellular matrix made of molecules secreted from glia, neurons or other cells. In the absence of synaptic partners, each specialization has the intrinsic capacity to assemble morphological rudimentary structures. Which side initiates synaptic cross-talk *in vivo* depends on the neuronal types and specific synapses. Cell-surface proteins, such as neurexin and neuroligins, and secreted proteins, such as laminins, have long been studied for their roles in specifying synaptic connectivity. Four reviews in this series cover recent advance in understanding the molecular complexity of synaptogenesis and synapse maintenance, and present complementary insights on the same molecule in distinct neuronal circuits and from different model organisms. Kurshan and Shen emphasize the progress on the molecular composition and dynamics in pre-synaptic and post-synaptic compartments, and highlight the roles of γ -neurexin, a newly discovered short and conserved isoform of neurexin, in synapse formation in *Caenorhabditis elegans*. This γ -neurexin lacks trans-synaptic binding domains, and is capable to amplify presynaptic assembly pathways through its intracellular PDZ-binding motif. Apóstolo and de Wit cover new findings revealing the compartmentalized distribution and function of various cell surface molecules in mammalian brain. They further discuss how numerous glial-derive factors interact with neuronal cell surface proteins, and how secreted proteins, such as complement proteins C1ql2 and C1ql3, modulate excitatory synapse connections and function. Sakers and Eroglu focus on the neuroligin protein family, which are ligands for neurexins and were previously thought to be only produced from neurons and reside in postsynaptic compartment. However, a series of recent papers have demonstrated that astrocytes produce neuroligin 2 and that the glial produced neuroligins not only regulate synaptogenesis and synapse transmission but also contribute to disease pathogenesis and glioma. Lastly, Connor et al. provide a comprehensive review on how the vertebrate-specific immunoglobulin superfamily proteins known as MDGAs interact with neuroligins to suppress synapse development. MDGAs are tethered to the membrane through a GPI anchor, and MDGA–neuroligin interactions occlude neuroligin–neurexin binding. As accumulating evidence links mutations in these molecules to various neurodevelopmental and neuropsychiatric disorders, these reviews are timely and point to many questions for future investigation.

Among all types of cells within an organism, neurons display most exquisite complexity not only in cellular morphology but also compartmentalized proteomes. The microtubule cytoskeleton sculpts neuronal structure and mediates the transport of vesicles, proteins, RNAs, and organelles that sustain neuronal activity. While the molecular constituents of microtubules are seemingly simple and many *in vitro* reconstitution assays are well established for quantitative measurement of microtubule-mediated functions, a central challenge remains to translate the biochemically defined activity of many microtubule-associated-proteins (MAPs), such as motor proteins, into understanding their function and regulation in neurons *in vivo*. For example, microtubules in axons and dendrites are

distinguished by their polarity, organization, posttranslational modifications (PTM), and microtubule-binding proteins. Kelliher et al. review how microtubule function in neuronal activity is locally controlled by different PTM patterns, such as the acetylation of α -tubulin lysine 40 in mechanosensation and axono-morphogenesis and dendrito-morphogenesis. Vanhauwaert et al. cover recent findings on mitochondria transport in neurons, which is dependent on microtubules. Falk et al. highlight many roles of the GTP-binding Septin proteins. Originally discovered in yeast for their roles in cytokinesis, Septins can oligomerize in proto-filaments and can associate with membranes and also with microtubules and actin cytoskeletons. In higher mammals Septins include >10 genes, many of which are now shown to be important for neuronal development, maintenance and function, leading to the proposal that combinations of different Septin complexes, or 'dynamic Septin code', could enable simultaneous morphological remodelling at distinct locations within a single neuron. Moreover, mis-regulation of Septins is likely linked to autism and schizophrenia (also see Caldeira et al.) Thus, understanding of how Septin codes could switch from long-lasting structures to plastic state will be an important to the diversity of neuronal morphologies.

The vast majority of neurons and their synapses must sustain their function over the lifetime of an animal, therefore, neuronal and synaptic proteins need to be continually replaced. A quantitative understanding of neuronal proteome, especially those providing compartmental specificity, is necessary for understanding the metabolic load imposed on neurons to sustain synaptic protein homeostasis. Cohen and Ziv review the approaches that have been used to measure neuronal and synaptic protein lifetimes. Metabolic labeling using SILAC (Stable Isotope Labeling with Amino acids) and mass-spectrometry enables measurement of protein half-life, and newly synthesized proteins can be measured with BONCAT (biorthogonal noncanonical amino acid tagging). Cumulated data show that neuronal and synaptic proteins have unusually long half-lifetimes, typically on the order of days to weeks, and even longer. Long-lived proteins include histones, mitochondrial, nuclear pore, myelin-associated, and extracellular matrix proteins; short-lived proteins tend to include proteins related to nucleic acid processing, membrane-associated proteins, secreted proteins, signaling proteins, and chaperones. Synaptic proteins might be selectively stabilized at synapses, yet, half-lives of specific proteins belonging to the same complexes can deviate significantly. Lysosomal-mediate protein degradation is a major cellular pathway regulating protein turnover Boecker and Holtzbaaur review how autophagy and endo-lysosomal degradation, both converging at the point of cargo delivery to lysosomes, regulate vesicular protein turnover, and highlight specific protein targets of bulk autophagy and selective autophagy in axons, dendrites, and pre-synaptic and post-synaptic compartments. Vanhauwaert et al. offer additional insights on protein turnover control in quality control of mitochondria.

Although our understanding of the biochemical under-pinnings of synapse function has made significant strides, detailed mechanistic understanding of the biochemical machinery remains at the frontier of molecular neuroscience. As chemical synaptic transmission is usually triggered by an abrupt change in local membrane potential, the details of how different ion channels sculpt the action potential as it travels down the axon is an exciting new area reviewed by Alpazar et al. Unc13, a protein discovered in the original and most powerful genetic screen in *C. elegans*, is crucial for all known forms of chemical synaptic

transmission. Dittman reviews the many mysteries that have emerged in understanding exactly how this protein controls the exocytic protein machinery. Of the many types of neurotransmitter used in the brain, dopamine has a privileged role, particularly in the striatum. The machineries responsible for controlling dopamine secretion is reviewed by Liu and Kaeser. The conversion of the secreted chemical signal back into an electrical one is carried out in dendrites. The initial detection of a given neurotransmitter on the postsynaptic dendrite is usually handled by ionotropic receptors, assembled into complexes that determine the precise localization and turnover of these ion channels. Tomita reviews what is currently understood regarding GABA receptor assemblies and how this impacts function. For excitatory transmission the depolarization of the dendritic membrane often triggers Ca^{2+} entry through voltage-gated Ca^{2+} channels. Sather and Dittmer discuss how these channels are also controlled by the endoplasmic reticulum in dendrites through novel regulator processes. Understanding mal-adaptive changes in synapse function in response to chronic exposure to drugs of abuse has been an important goal that lies at the intersection of synapse biology and addiction research. Stellwagen et al. review the new appreciation for astrocytes and microglia in contributing to adaptive and mal-adaptive responses. Recently the concept of 'membrane-less' organelles has emerged in many areas of cell biology, where protein assemblies can be driven by numerous weak multi-valent interactions through intrinsically disordered protein domains. Feng et al. review evidence for the presence of these novel protein-phases in both the pre-synaptic and post-synaptic compartments. A key but relatively recently appreciated aspect of synapse function is the constant need for fuel to keep the machinery running. Rossi and Pekkurnaz review how mitochondria at synapses are able to change their behavior in response to changes in energetic requirements.

Expansive data from single-cell sequencing and neuronal type and compartmental proteomic studies indicates that neuronal identity is strongly linked to its pattern of connectivity. The roles of local protein synthesis have been continuously examined as technology improves in detection sensitivity and specificity. The use of RiboTag mouse, combined with ribosome profiling, is powerful in identifying *in vivo* local transcriptome. mRNAs encoding a number of transmembrane proteins, including ion channels, are found within the presynaptic transcriptome and protein translation machinery is present in mature axons. Biever et al. review recent evidence supporting presynaptic translation and intra-axonal synthesis of transmembrane proteins. The complexity of localized translational control lies in the dynamic interaction between regulatory elements embedded in the transcript and a rich toolbox of transacting factors, including translational machinery components, RNA binding proteins and miRNAs. Heterogeneity of ribosomes may underlie compartmental-specific local translation. Improvements in imaging technology has enabled tracking of mRNPs, which are made of higher-order assemblies of RNAs and proteins with unprecedented temporal resolution Das et al. review both visualization and bioinformatic methodologies for analyzing neuronal mRNPs. For example, an algorithm based on Bayesian model selection and Hidden single-molecule detection Markov Modeling helps to characterize the transient transition states of individual mRNA transporting granules, and captures the real-time dynamics of protein synthesis in living neurons. Current studies demonstrate that composition of mRNPs is dynamic across space and time. The generation of new reagents, such as transgenic mice expressing PP7 stem-loops knocked-in to the 3'UTR of the activity-

regulated gene *Arc*, offers a promising tool to address *in vivo* how neuronal activity regulates local proteomes. The main action in the generation of neuronal proteome diversity remains in the nucleus. Soto and Lipscombe review recently publications on the cellular mechanisms that regulate alternative splicing, highlighting the specific action of select RNA splicing factors in regulating neuronal membrane protein isoforms. They also discuss how the mechanistic dissection of the SMN (Survival of Motor Neuron) protein in alternative splicing leads to the development of therapeutic strategies in treating human patients.

Throughout the lifetime, neurons need to protect themselves from acute damage and chronic stress or disease, and fight against cellular destruction. Ding and Hammarlund highlight the recent findings on injury induced-axon degeneration, focusing on the signaling network involving the axon survival factors NMNAT2. Emerging studies from *C. elegans* also uncover a unique form of axon degeneration, due to absence of mitochondria. Vanhauwaert et al. also cover how quality control of mitochondria is linked to chronic neurodegeneration in mammals. All together, these studies point to critical understanding of energy regulation and cytoskeleton integrity to be key steps in protecting damaged axons and stressed neurons from self-destruction. The theme of dysregulation of neuronal proteins in human diseases is further stressed in the review by Caldeira et al. Additionally, emerging evidence for non-coding RNAs points to their powers in tuning neuronal network, and mis-regulation of network activity contributes to neuropsychiatric disorders, as reviewed in Zampa et al. The brain is intimately involved in correct regulation of all of the body's vital organs through an constant exchange of information informing on critical physiological parameters like blood pressure and fluid balances. In some cases dedicated neuronal circuits play a key role in determining vital parameters such as thirst and satiety as discussed by Ichiki et al. One critical interface between the brain and the rest of the body is the blood-brain barrier formed by specialized endothelial cells whose cell biology controls the detailed exchange of macromolecules into and out of the cerebrospinal fluids. Ayloo and Gu discuss recent progress in showing the critical importance of transcytosis across this epithelium and how it is regulated.

The set of reviews encompassed in this issue exemplifies how molecular neuroscience spans a wide set of problems from atomic level resolution of protein function to whole animal physiology.

References

- Editorial overview: Molecular neuroscience Ryan Timothy A, Jin Yishi Pages iii–vi
- Phase separation as a mechanism for assembling dynamic postsynaptic density signalling complexes
Feng Zhe, Chen Xudong, Zeng Menglong, Zhang Mingjie Pages 1–8
- Neuronal and synaptic protein lifetimes Cohen Laurie D, Ziv Noam E Pages 9–16
- Unc13: a multifunctional synaptic marvel Dittman Jeremy S Pages 17–25
- Alternative splicing of neuronal genes: new mechanisms and new therapies Lipscombe Diane, Soto
Eduardo Javier Lopez Pages 26–31
- Transcytosis at the blood–brain barrier Ayloo Swathi, Gu Chenghua Pages 32–38
- Microtubule control of functional architecture in neurons Kelliher Michael T, Saunders Harriet AJ,
Wildonger Jill Pages 39–45
- Mechanisms and regulation of dopamine release Liu Changliang, Kaeser Pascal S Pages 46–53

- Non-coding RNAs: the gatekeepers of neural network activity Zampa Federico, Hartzell Andrea L, Zolboot Norjin, Lippi Giordano Pages 54–61
- New insights on synaptic dysfunction in neuropsychiatric disorders Caldeira Gladys Lima, Peça João, Carvalho Ana Luísa Pages 62–70
- Pumping the brakes: suppression of synapse development by MDGA–neuroligin interactions Connor Steven A, Elegheert Jonathan, Xie Yicheng, Craig Ann Marie Pages 71–80
- Molecular constituents and localization of the ionotropic GABA receptor complex *in vivo* Tomita Susumu Pages 81–86
- Surveillance and transportation of mitochondria in neurons Vanhauwaert Roeland, Bharat Vinita, Wang Xinnan Pages 87–93
- Vesicular degradation pathways in neurons: at the crossroads of autophagy and endo-lysosomal degradation Boecker C Alexander, Holzbaur Erika LF Pages 94–101
- Septin functions during neuro-development, a yeast perspective Falk Julien, Boubakar Leila, Castellani Valérie Pages 102–109
- The travels of mRNAs in neurons: do they know where they are going? Das Sulagna, Singer Robert H, Yoon Young J Pages 110–116
- Subcellular control of membrane excitability in the axon Alpizar Scott A, Cho In Ha, Hoppa Michael B Pages 117–125
- Compartmentalized distributions of neuronal and glial cell-surface proteins pattern the synaptic network Apóstolo Nuno, de Wit Joris Pages 126–133
- Neural populations for maintaining body fluid balance Ichiki Takako, Augustine Vineet, Oka Yuki Pages 134–140
- Local translation in neuronal processes Biever Anne, Donlin-Asp Paul G, Schuman Erin M Pages 141–148
- Powerhouse of the mind: mitochondrial plasticity at the synapse Rossi Meghan J, Pekkurnaz Gulcin Pages 149–155
- Synaptogenic pathways Kurshan Peri T, Shen Kang Pages 156–162
- Control of neural development and function by glial neuroligins Sakers Kristina, Eroglu Cagla Pages 163–170
- Mechanisms of injury-induced axon degeneration Ding Chen, Hammarlund Marc Pages 171–178
- Glial regulation of synaptic function in models of addiction Stellwagen David, Kemp Gina M, Valade Simone, Chambon Julien Pages 179–185
- Regulation of voltage-gated calcium channels by the ER calcium sensor STIM1 Sather William A, Dittmer Philip J Pages 186–191