Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Neurobiology

New technologies

Editorial overview

Erin Schuman and Xiaowei Zhuang

Current Opinion in Neurobiology 2010, **20**:608–609

Available online 9th September 2010

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2010.08.019

Erin Schuman

Max Planck Institute for Brain Research, Synaptic Plasticity, Max von Laue Strasse 3, 60438 Frankfurt, Germany
e-mail: schumane@brain.mpg.de

Erin Schuman is a professor and director of Synaptic Plasticity at the Max Planck Institute for Brain Research in Frankfurt. Her group is interested in the molecular and cellular mechanisms that underlie information storage in neuronal networks. Her research uses electrophysiology, imaging, molecular biology, and biochemistry to understand the cell biological processes that control synaptic function and plasticity. The lab also examines information storage in the hippocampal network in rodents and humans.

Xiaowei Zhuang

Department of Chemistry and Chemical Biology, Department of Physics, Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138, United States

Xiaowei Zhuang is a professor of chemistry and chemical biology and professor of physics at Harvard University and a Howard Hughes Medical Institute investigator. Her research lab develops advanced optical imaging techniques, in particular single-molecule and super-resolution imaging methods, to study various problems in the areas of biophysics, cell biology, and neurobiology.

Many fundamental discoveries in neurobiology have been fueled by new technologies that allow or improve the manipulation, visualization or analysis of synapses, neurons and circuits. This issue of CONB presents some of the recent exciting technological developments on these fronts, including advances in optogenetics, microfluidics, bio-orthogonal labeling, high-resolution imaging, image segmentation, and electrophysiology.

In neurons, as in other cells, the specificity of signaling relies on both the temporal kinetics of intracellular cascades as well as their localization within restricted spatial domains. Optogenetic tools allow controlled manipulation of neural signaling by light with high spatiotemporal resolution. Two reviews in this issue address recent advances made in two complementary approaches of optogenetics. Bamburg *et al.* focus on light-activated channel rhodopsins, such as the depolarizing channel rhodopsins 1 and 2, and the hyperpolarizing Cl-pump halorhodopsin. Biophysical properties of these light-activating channels are described, along with recent advances made in targeting and addressing these molecules. The authors also present exciting applications of these tools in circuit analysis and in the treatment of neurodegenerative diseases. Rana and Dolmetsch describe the recent development and design of artificial photoreceptor proteins that possess a light-sensing domain from a natural photoreceptor and an effector domain from a target protein of interest. These synthetic photoactivatable proteins make use of the light-oxygen-voltage or phytochrome domains. Upon exposure to light, these domains undergo reversible conformational changes that modulate the activity of an effector domain through steric occlusion or allosteric changes, resulting in a modulation of signaling. These modular and functionally diverse signaling platforms provide a new set of tools for genetically targeted optical control of cellular signaling.

The use of fluorescent proteins has transformed the study of cellular dynamics in all types of cells, including neurons. The size of fluorescent protein tags as well as the overexpression of fluorescent fusion proteins can sometimes perturb the folding, trafficking, and function of the protein of interest. In this issue, Dieterich describes methods that can bypass these concerns by using small, membrane permeable, chemical probes that can be incorporated into endogenous proteins. These probes provide a molecular handle that can be specifically 'grabbed' (using click chemistry) by a second chemical group, such as a fluorescent molecule or an affinity tag. This allows one to visualize tagged protein *in situ* or to purify the protein(s) via affinity purification. The author describes in detail one particularly exciting application of this technology, the specific labeling of all newly synthesized proteins in neurons, via the use of non-canonical amino acids, which possess an azide or alkyne group. Subsequent tagging with fluorescent or affinity

probes, enables one to visualize or purify the population of newly synthesized proteins. The most recent innovation of this technology is the ability to restrict the incorporation of the amino acid to particular cell types, which enables researchers to visualize and evaluate new protein synthesis in their favorite neural circuit or cell type.

Over the past 15 years or so, it has become clear that the responsiveness of neurons to neurotransmitter is largely governed by the availability of neurotransmitter receptors at the synapse. Until quite recently, the dominant view was that most receptors at synapses are relatively stable-synaptic strength is then modified by the addition or subtraction of relatively small population of receptors to this stable pool. Recent studies using single-particle tracking (SPT) have challenged the notion of stable long-term residency of receptors. Gerow and Triller provide a compelling discussion and review of SPT experiments that have elucidated the dynamic behavior of neuronal membrane proteins, in particular neurotransmitter receptors. These studies show that receptors can exchange rapidly between synaptic and extrasynaptic compartments. Receptors are stabilized via the interaction with binding partners, like adhesion molecules and scaffold proteins. These observations indicate that the stability of synapses is maintained and modified by the concerted action of many synaptic molecules that possess different diffusion properties and different affinities for interacting partners, including scaffolding proteins.

Much of our knowledge of the cell biology of synaptic transmission has been driven by the use of dissociated neuronal preparations in which the architecture of neural circuits is effectively reduced to two dimensions to enhance our ability to visualize and manipulate synapses. The process of dissociating neurons and replating them in a dish, however, disrupts the native connectivity and can lead to aberrant networks that do not resemble those formed *in vivo*. Taylor and Jeon describe a new generation of microfluidic devices that can be used to direct the placement of neurons, the growth of dendrites and axons, and the establishment of synaptic connections between two defined neuronal populations. The microfluidic devices have been designed to interface with high resolution and time-lapse microscopy. In addition, different compartments (cell bodies, dendrites, and axons) can be fluidically isolated, allowing experimenters to manipulate, independently, subcellular compartments with antagonists, neurotransmitters, siRNA constructs, etc.

Visualization of brain structures has relied heavily on various optical imaging methods, such as confocal and multiphoton microscopy. However, the spatial resolution of light microscopy is limited by diffraction to several hundred nanometers. This resolution makes it difficult to trace neural circuits and resolve many important subneuronal structures, such as synapses. Dani and Huang describe in their article several recently developed super-resolution fluorescence microscopy methods that break the diffraction limit. The principles of these methods are summarized, as well as practical aspects of their implication. Comparison of these optical methods with electron microscopy is made so as to help researchers properly choose suitable imaging approaches for their own systems. They further describe several exciting directions for these emerging methods in neuroscience, ranging from resolving brain subcellular structures to tracing neural circuits.

Brain circuit analysis with high-resolution optical or electron microscopy is generating an enormous amount of data. If one were to reconstruct the entire wiring diagram of a mouse brain with 10 nm image resolution, 10^{18} voxel of image data will be generated. In fact, a complete circuit tracing with high fidelity will likely require higher image resolution, which means an even larger data volume. Without automated computer algorithms, segmentation of such a large volume of imagery data would be inconceivable. Jain *et al.* review some of the general principles of image segmentation as well as recent development in automated segmentation using machine-learning approaches. In particular, they focus on improvement of metrics for evaluating segmentation performance based on topological features and methods for searching optimized algorithms based on these metrics. Chklovskii *et al.* review recent progress made in image processing and describe a semi-automated pipeline developed at Janelia Farm for reconstruction circuit diagrams from serial-section transmission electron microscopy images. This pipeline has been applied to reconstruct the connectivity diagrams of the lamina and medulla neuropiles of the Drosophila optical lobe.

Understanding how the brain represents and decodes sensation, perception, and action requires access to the electrical activity of neuronal populations. Historically, this has been accomplished by recording extracellularly from individual neurons in anesthetized animals. Recently, several groups have pioneered the use of multielectrode arrays to record the activity of many individual neurons in freely moving animals, mostly rodents. Shenoy and co-workers describe a new generation of electrophysiological systems that are battery-operated and lightweight; this system can acquire spikes, local field potentials, and electromyography signals, and can also stimulate based on real-time processing of recorded signals. This new technology will enable investigators to record neural activity for days during natural behaviors such as locomotion, navigation, and social interaction in freely behaving primates.

Together, these nine articles described a variety of exciting new tools for structural and functional characterizations of the brain. We hope that you will enjoy reading these articles and are inspired to implement some of these technologies in your experiments.