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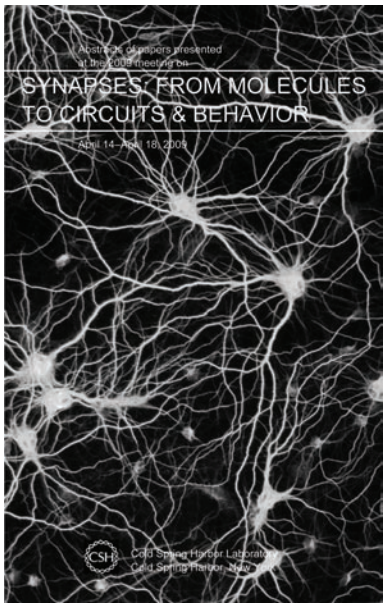
meeting report

Constructing a road map from synapses to behaviour

Meeting on Synapses: From Molecules to Circuits & Behavior

Hee Jung Chung[1]*& *Hey-Kyoung Lee*[2]*+

[1]University of California San Francisco, San Francisco, California, USA, and [2]University of Maryland, College Park, Maryland, USA



The Cold Spring Harbor Laboratory meeting on Synapses: From Molecules to Circuits & Behavior took place between 14 and 18 April 2009, in Cold Spring Harbor, NY, USA, and was organized by H. Cline, R. Huganir & T. Sudhof.

Keywords: synaptic plasticity; synapse formation; neural circuits; functional mapping; experience-dependent plasticity

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See Glossary for abbreviations used in this article.

Introduction

Synapses are the fundamental nodes of neuronal circuits in the CNS that underlie cognition and behaviour. Since their first visualization by S. Ramón y Cajal, synapses have been the subject of intense investigation at multiple levels, and through diverse experimental approaches and preparations. The Cold Spring Harbor Laboratory meeting on Synapses: From Molecules to Circuits & Behavior brought together a diverse group of neuroscientists who provided synopses of the latest advances in the field. Several emerging trends were highlighted at this meeting, including the combination of cutting-edge, comprehensive anatomical and functional *in vivo* imaging with sophisticated genetic manipulations in model organisms to visualize the neuronal circuits involved in sensing and behaviour (Fig 1). The use of large-scale genomic and proteomic approaches to understand synapse development and function was also emphasized, as was the need to generate large-scale public databases to facilitate future research on brain function (Fig 1C). The field of synaptic plasticity focused on the identification of new molecules and mechanisms, whereas sensory experience-dependent change at synapses and circuits has emerged as an important theme in neural plasticity (Fig 1A, B). Here, we highlight some of these themes and discuss future directions.

How large or small can we go in mapping the brain?

The function of the brain is, in part, restricted and defined by its structure, especially by the connectivity among neurons. The continuing efforts to map the functional and anatomical circuitry of the brain were highlighted in several talks, including one by keynote speaker R.C. Reid (Boston, MA, USA). Two-photon microscopy has revolutionized the study of brain function because it provides functional imaging with spatial and temporal information at single-cell resolution, even in the brains of live animals (Fig 1B). *In vivo* two-photon Ca^{2+} imaging revealed that neurons in the visual cortex can be grouped into functional organizations by their response properties (Ohki *et al*, 2005), which suggests that these groups of neurons could be connected to produce a functional unit. To determine whether this is the case, Reid's group is developing a method to reconstruct the visual cortex at a large scale with EM resolution. The challenges to this new approach are the analysis of extraordinarily large three-dimensional data sets and individual variability across experimental subjects. The latter could affect the general applicability of information about brain circuitry, but is less problematic for mapping the *Drosophila* CNS. A.-S. Chiang (Hsinchu, Taiwan) introduced an initiative to generate a searchable database on the connectivity map of the *Drosophila* brain (Lin *et al*, 2007). To overcome individual variability, Chiang's group developed a software algorithm that linearly morphs each

[1]Department of Physiology, University of California San Francisco, San Francisco, California 94158, USA
[2]Department of Biology, College of Chemical and Life Sciences, University of Maryland, College Park, Maryland 20742, USA
*These authors contributed equally to this work
+Corresponding author. Tel: +1 301 405 9784; Fax: +1 301 314 9358;
E-mail: hlee21@umd.edu

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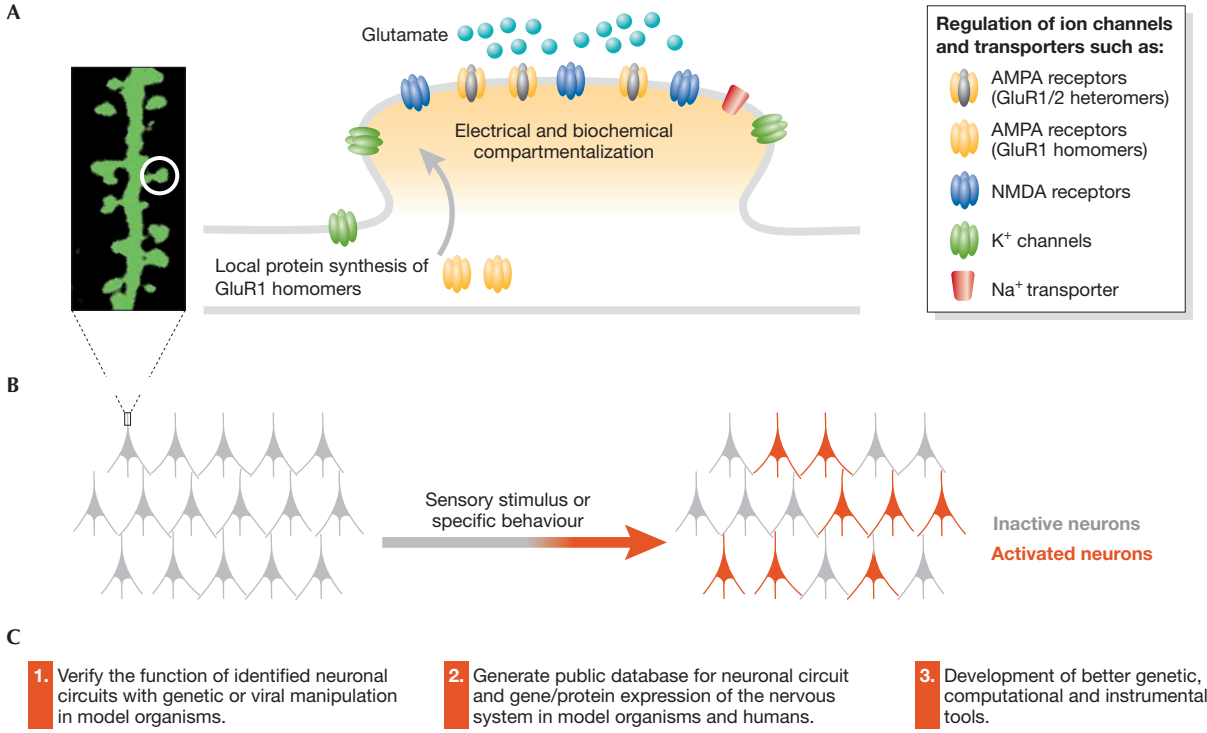

Fig 1 | An integrated view of synaptic research at various levels of complexity. (**A**) Plasticity of synapses and dendritic spines. On the left, the dendritic spines of a pyramidal neuron are visualized using GFP labelling. Dendritic spines are postsynaptic specializations of excitatory synapses, and act as electrical and biochemical compartments. The activity-dependent regulation of glutamate receptors and other ion channels is involved in excitatory synaptic plasticity. (**B**) Functional and anatomical mapping of neuronal circuits. Techniques such as *in vivo* two-photon Ca^{2+} imaging can reveal a functional network of neurons that are activated by sensory experiences or specific behaviours (shown in red). (**C**) A full understanding of the neural basis of cognition and behaviour requires the tools to manipulate functional circuits specifically. These efforts will benefit from the development not only of publicly accessible databases of brain maps, but also of better imaging, genetic and computational tools in model organisms. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GFP, green flourescent protein; GluR, glutamate receptor; NMDA, N-methyl-D-aspartic acid.

visualized neuron to the 'average' *Drosophila* brain and obtained high-resolution images of transgenic *Drosophila* brains expressing fluorescent proteins such as EGFP in specific types of neuron. The identification of neuronal types using this method allowed the generation of the fly connectome database, which can be searched by neurotransmitter, pre- and postsynaptic target structures, and brain area. An open-access database named Flycircuit will soon be available to aid the large community of neuroscientists who use *Drosophila* as a model system.

In line with the spirit of generating a public database beneficial to researchers worldwide, H. Zeng (Seattle, WA, USA) presented the efforts of the Allen Institute for Brain Science. In addition to the Allen Brain Atlas (www.brain-map.org), which is widely used for searching gene expression profiles in the brain, Zeng provided an update on several continuing projects at the institute, which include the generation of transgenic mice that have specific cell types labelled—with EGFP, for example—or that can express or inhibit a particular gene in a cell type-specific manner through the use of Cre-inducible promoters. These developments will aid the functional study of distinct neuronal types within the brain, especially in areas such as the cerebral cortex where diverse populations of neurons are often difficult to distinguish anatomically.

mRNA and miRNA in synapse formation

The regulation of mRNAs could be a crucial molecular event for synapse formation during learning and memory in *Aplysia*. S. Puthanveettil from E. Kandel's group (New York, NY, USA) used proteomics and microarray technology to analyse the proteins and RNAs that are present in the cargos of kinesin heavy chain (KHC), a motor protein involved in fast axonal transport and crucial for the induction of 5HT-dependent long-term facilitation of the gill withdrawal reflex (Puthanveettil *et al*, 2008). In addition to proteins, the KHC cargos contain several hundred mRNAs involved in synapse formation, maintenance and function. A subset of these mRNAs is upregulated by 5HT, suggesting that activity differentially regulates the synaptic transcriptome.

In addition to mRNAs, miRNAs have emerged as regulators of synapse formation. miRNAs are approximately 22-nucleotide-long non-coding RNAs that silence translation through either the induction of mRNA decay or the inhibition of translational initiation (Corbin *et al*, 2009). In a forward genetic screen, C. Lu from D. Van Vactor's group (Boston, MA, USA) discovered that miR-8, a highly conserved miRNA, is essential for synaptic growth and morphogenesis in the NMJ of *Drosophila* larvae. Signalling and trans-synaptic cell adhesion proteins of presynaptic and postsynaptic

Glossary

5-HT	5-hydroxytryptamine, commonly known as serotonin
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
B2-nAChR	β2-nicotinic acetylcholine receptor
CA1	cornu ammonis 1 (group of hippocampal pyramidal cells)
CaMKII	calcium/calmodulin-dependent protein kinase II
CNS	central nervous system
CREB	cAMP response element binding
EGFP	enhanced green fluorescent protein
EM	electron microscopy
FRET	fluorescence resonance energy transfer
GFP	green fluorescent protein
GluR	glutamate receptor
LTP	long-term potentiation
mEPSC	miniature excitatory postsynaptic currents
miRNA	microRNA
mRNA	messenger RNA
NMDAR	N-methyl-D-aspartic acid receptor
NMJ	neuromuscular junction
PKA	cAMP-dependent protein kinase
RMG	a pair of neurons that innervate muscles in the head through the NMJ in the nerve ring of *C. elegans*
SNP	single nucleotide polymorphism

compartments are among the predicted miR-8 targets. Tissue-specific silencing of miR-8 suggested that temporal and spatial regulation of proteins by miRNAs might have a role in synapse formation and stabilization.

What are dendritic spines good for?

Several speakers discussed how dendritic spines could function to mediate synaptic plasticity and generate a reliable neuronal output. R. Yasuda (Durham, NC, USA) characterized Ca^{2+}-dependent signalling in a single dendritic spine by using two-photon FRET imaging of Ras and CaMKII activity after the induction of LTP through single-spine glutamate uncaging. Unlike activated Ras, which rapidly diffuses out of the activated spine and spreads to neighbouring spines (Harvey *et al*, 2008), activated CaMKII is largely confined to the activated spine owing to a surprisingly fast inactivation kinetic of approximately 10 seconds (Lee *et al*, 2009). These results suggest that the spatiotemporal confinement of activated CaMKII probably underlies the synapse specificity of LTP (Fig 1A).

Spines act not only as biochemical compartments but also as electrical ones (Araya *et al*, 2006). R. Araya from R. Yuste's group (New York, NY, USA) suggested that one of the consequences of electrical compartmentalization is the ease of recruiting voltage-gated conductances for generating dendritic spikes. N. Spruston (Evanston, IL, USA) followed up on his previous finding that the proportion of perforated synapses and density of AMPARs in CA1 apical dendrites increased as a function of distance from soma (Nicholson *et al*, 2006), and showed that synaptic strength and density decrease as a function of distance from a dendritic branch point. These new results argue against a global normalization of synaptic strength and support a two-stage model of synaptic integration in which the generation of spikes in dendritic branches is followed by the integration of branch responses in the soma and the axon.

Power to postsynaptic ion channels and transporters

The regulation of postsynaptic neurotransmitter receptors is widely known to modulate synaptic function; however, this meeting emphasized the role of transporters and other ion channels in this process. H. Man (Boston, MA, USA) highlighted the importance of the Na^+/K^+-ATPase (NKA) in synaptic function (Fig 1A). NKA is a transporter that regulates intracellular Na^+ and K^+ gradients. Man's group found that NKA is enriched in synapses and associated with AMPARs. Interestingly, NKA inhibition reduces synaptic transmission by inducing AMPAR endocytosis and degradation (Zhang *et al*, 2009), suggesting that the Na^+ gradient in spines regulates synaptic activity. The K^+ channels that are present at excitatory synapses—including G-protein-activated inwardly rectifying (GIRK) channels and A-type channels (Kv4.2)—also have important roles in excitatory synaptic plasticity (Fig 1A). H. Chung, from the group of L. Jan (San Francisco, CA, USA), reported that NMDAR activation increases the surface expression of GIRKs at dendrites and spines by promoting channel recycling through the dephosphorylation of GIRK2 at Ser 9 (Chung *et al*, 2009a). Furthermore, GIRK2 knockout mice specifically lack depotentiation but maintain normal LTP (Chung *et al*, 2009b). By contrast, D. Hoffman (Bethesda, MD, USA) showed that the transient A-type K^+ current (I_A) regulates LTP. The A-type K^+ channel subunit Kv4.2—which is located in dendritic spines—is internalized after synaptic stimulation and during LTP (Kim *et al*, 2007). Furthermore, the regulation of Kv4.2 expression alters the subunit composition of synaptic NMDARs (Jung *et al*, 2008), which could account for their effect on LTP.

Maintaining the status quo of synapses and circuits

The need to maintain function despite the continuing changes in neural activity that arise from sensory input or internal cues is a formidable challenge faced by the nervous system. A. Barth (Pittsburgh, PA, USA) suggested that the function of NMDARs could switch from being crucial for the induction of synaptic plasticity to maintaining stability in neural circuits as a new mechanism for maintaining homeostasis in the barrel cortex layer 2/3 neurons (Clem *et al*, 2008).

Several talks highlighted the fact that the regulation of AMPARs—especially the Ca^{2+}-permeable GluR1 homomers—has a role in homeostatic synaptic plasticity (Fig 1A). H.-K. Lee (College Park, MD, USA) showed that homeostasis in the visual cortex is mediated by the regulation of synaptic AMPAR subunit composition. Visual deprivation globally increases the amplitude of AMPAR-mediated mEPSC in the 2/3 layer of the visual cortex, which occurs throughout development into adulthood (Goel & Lee, 2007). In juveniles, the scaling up of synapses is associated with the appearance of Ca^{2+}-permeable AMPARs, and crucially depends on phosphorylation of the GluR1 subunit on a PKA site. Results from dissociated primary cultures suggest that local synthesis of GluR1 underlies the scaling up of synapses through inactivation (Sutton *et al*, 2006). L. Chen (Berkeley, CA, USA) showed that signalling through the retinoic acid receptor-α (RARα) has a role in the local dendritic synthesis of GluR1 during inactivity (Aoto *et al*, 2008) by relieving the translational repression of GluR1 mRNA (Poon & Chen, 2008). On the presynaptic side, M.A. Sutton (Ann Arbor, MI, USA) reported that inactivity could recruit retrograde signalling of brain-derived neurotrophic factor to increase neurotransmitter release.

Mapping of neural circuits for sensory experience

The sensory neocortex is often organized as functional columns. To investigate how the functional circuits develop, S.H. Shi (New York, NY, USA) labelled ontogenetic clones of excitatory neurons using

retroviral vectors and performed multi-electrode whole-cell recordings. His group found that synapses develop preferentially among sister excitatory neurons (Yu *et al*, 2009), suggesting that the radial clones of excitatory neurons could act as a substrate for the formation of functional units of neural circuits in the sensory neocortex.

The development of precise receptive fields is crucial for sensory processing. For example, in the visual system, binocular alignment of the receptive fields of the two eyes is essential. To investigate the mechanism for establishing binocular receptive fields, F. Engert (Boston, MA, USA) used two-photon Ca^{2+} imaging in the optic tectum of zebrafish larvae to characterize direction selectivity in this brain region. The unilateral ablation of the optic tectum allows the remaining tectum to receive binocular inputs (Ramdya & Engert, 2008). The Engert group found evidence that local inhibitory interneurons are involved in providing directional selectivity in the rewired tectum. In mice, the distinct patterns of retinal spontaneous activity seem to dictate the refinement of the retinotopic map and eye segregation of retinocollicular synapses. By using β2-nAChR knockout mice—that lack early retinal waves—and rescue experiments to restore β2 in the retina, M. Crair (New Haven, CT, USA) found that local retinal waves are important for retinotopic map refinement, whereas global waves are required for eye-specific segregation (Shah & Crair, 2008).

Sophisticated tools are available for identifying and manipulating the function of specific types of *Drosophila* neurons in information processing. G. Miesenböck (Oxford, UK) put forward the hypothesis that weak signals in some neural circuits can be enhanced by nonspecific—even noisy—background activity. Miesenböck proposed that the excitatory local neurons in the antennal lobe, which are activated by a broad array of odours, could provide such a noise to facilitate the detection of faint odours (Shang *et al*, 2007). Consistent with this idea, the specific silencing of excitatory local neurons by expressing the temperature-sensitive dominant-negative form of dynamin decreased the odour-evoked activation of postsynaptic neurons.

Mapping neural circuits for behaviour

C. Bargman (New York, NY, USA), the second keynote speaker of the meeting, highlighted the role of gap junction-mediated transmission in neural circuit function during innate social behaviours of *Caenorhabditis elegans* (Macosko *et al*, 2009). Her group identified the RMG inter/motor neuron as the hub of a regulated circuit that controls social aggregation and related behaviours. Gap junctions connect the RMG to several classes of sensory neurons, which provide the sensory input—obtained from cues such as O_2, pheromone or nociception—needed to trigger aggregation. Such anatomical organization can be visualized as a hub-and-spoke circuit in a wiring diagram. By using SNP analysis, cell-type specific rescue and laser ablation, the Bargman group identified the neuropeptide receptor gene *npr-1*, which is expressed in the RMG neuron, as essential for social aggregation behaviour.

The importance of obtaining detailed functional maps of neuronal circuits as a first step to understanding behaviour was also reinforced in other studies. S. Waddell (Worcester, MA, USA) identified a subset of dopaminergic neurons that innervate the mushroom bodies of *Drosophila* as part of a neural circuit that controls motivated behaviour. The loss of function of the neuropeptide F receptor in these dopaminergic neurons specifically impairs the behavioural expression of appetitive odour memory—a type of long-lasting memory in the fly (Krashes & Waddell, 2008). Where and how is memory formed and stored? By using microarray and genetic analyses, A. Kauffman from C. Murphy's group (Princeton, NJ, USA) identified CREB targets that are changed by associative long-term memory formation in *C. elegans*. By examining a GFP reporter of CREB activity, they further identified a neuronal circuit for CREB-dependent long-term memory formation.

What is next?

This meeting brought together researchers studying synapse function at the level of a single protein with those who work at the systems level. These two approaches to research must clearly converge if we are to obtain a full understanding of how synapses function and form the neuronal circuits that underlie behaviour. The study of molecular and cellular mechanisms of synapse development, function and regulation will continue in future years and lead to further development of smart molecules and reporters, as well as cutting-edge imaging techniques. In addition, the development of transgenic animals with fluorescent cell-type specific labelling will facilitate our ability to visualize and manipulate specific neurons in a circuit, in order to delineate their function. The functional and anatomical mapping of neural circuits that are important for specific behaviours is already at the level of single neuronal type in simple model organisms such as *Drosophila* and *C. elegans* (Fig 1B). Such precise manipulations will improve only with efforts to build public databases that feature neuronal circuitry and with further development of sophisticated genetic tools (Fig 1C). However, the same task is still a challenge in mammals because their neural circuits are yet to be defined and neuronal type-specific control of activity is still relatively limited. However, several presentations from this meeting suggest that major efforts in this direction have already begun. We acknowledge that future progress will require us to push the limits of existing approaches, as well as to develop new tools and approaches (Fig 1C). Such efforts, if successful, would satisfy our desire to understand the neural basis of cognition and behaviour, as well as to facilitate better detection, prevention and treatment of neurological diseases and mental disorders. We look forward to approaches that characterize not only the main information highways, but also the details in the road map from synapses to neuronal circuits and behaviours.

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Hee Jung Chung

Hey-Kyoung Lee