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meeting point

Science in Suzhou: establishment and function of neural circuits

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The Cold Spring Harbour Asia conference on 'Assembly, Plasticity, Dysfunction and Repair of Neural Circuits' took place in Suzhou, China, in October 2011. Developmental, cell, molecular and systems neuroscientists convened to discuss the establishment, function and plasticity of neural circuits in diverse organisms.

Marco Polo travelled to Suzhou in 1276 and recorded his impressions. One translation of his travels notes that the city contained "many persons learned in natural science, good physicians, and able philosophers" [1]. With the inception of Cold Spring Harbour Asia in 2010, science is again at the forefront in Suzhou. The October 2011 meeting organized by Barry Dickson (Institute of Molecular Pathology, Austria), Zhigang He (Children's Hospital Boston, USA), Hitoshi Okamoto (RIKEN, Japan) and Yimin Zou (UC San Diego, USA) covered exciting topics in neuroscience, from establishing proper connectivity to plasticity in behaviour.

Four main topics of the meeting were axon growth, guidance and regeneration; synapse formation; function of neural circuits; and plasticity in neural networks. Advances in these areas are highlighted below.

Axons: growth, guidance, regeneration

The meeting was rich in new discoveries on axon growth, both during development and repair. The very beginning of neuronal wiring occurs when a neuron initiates a neurite from its cell body. Frank Bradke (German Center for Neurodegenerative Diseases, Bonn, Germany) used live imaging of cultured neurons to show that neurites initiate at sites of high actin turnover, and that initiation involves microtubule (MT) bundling. How is this process regulated? A likely candidate was the actin-binding protein cofilin, which is active at the right place and time—but cofilin knockouts have normal neurite initiation. This quandary was solved by showing that cofilin functions redundantly with the related protein ADF: neurons that lack both cofilin and ADF cannot initiate neurites. Imaging and EM tomography of these double knockout cells showed that as well as actin disorganization, there was a decrease in MT bundling. These experiments define a pathway for neurite initiation in which cofilin and/or ADF regulate actin turnover, resulting in local MT bundling and the initiation of a neurite.

MT remodelling also occurs after axonal injury, and the role of axonal MTs in determining the extent of axon regeneration is a subject of intensive study. Andrew Chisholm (UC San Diego, USA) demonstrated that MT structure mediates the effect of *efa-6* on axon regeneration in *C. elegans* [2]. The Chisholm and Jin labs performed a large survey of axon regeneration in many genetic mutants and found that *efa-6* is a powerful inhibitor of axon regeneration. Using live imaging of MT dynamics after laser axotomy, the group found that stable MTs increase after injury, and that this increase is enhanced in animals lacking *efa-6*. Consistent with the idea that these changes in MT stability underlie the effect of *efa-6* on axon regeneration, it was shown that overexpression of *efa-6* inhibits regeneration, and the inhibition can be overcome by treatment with the MT stabilizer Taxol. Surprisingly, although *efa-6* encodes a GEF for the small GTPase ARF-6, the GEF domain is dispensable for the ability of *efa-6* to suppress regeneration. Thus, these experiments identify a new mechanism for regulating axonal MTs, with profound consequences for regeneration after injury.

Guidance of an extending neurite is mediated by the growth cone, which is able to respond to subtle concentration gradients of guidance factors. Yimin Zou (UC San Diego, USA) provided new insights into the mechanisms that enable growth cones to follow Wnt5a gradients, which guide commissural axon migrations along the anterior–posterior axis in the spinal cord. He showed that growth cones interpret these Wnt signals using the planar cell polarity (PCP) pathway, the same pathway that is used by epithelial cells to polarize themselves along the tissue plane in response to Wnt signals. However, since growth cones are motile structures, they use these conserved components in a dynamic fashion. Vangl2, which promotes Wnt5a signalling by inhibiting Frizzled3 phosphorylation, is localized to filopodial tips and to nascent filopodia. Decreased Frizzled3 phosphorylation at these sites results in its endocytosis and activation [3]. At the same time, Wnt signals also rely on aPKC, which is a key component of the apical–basal polarity signalling pathway (perpendicular to the planar axis) together with Par3 and Par6, and which regulates Frizzled3 in parallel with Vangl2. Thus, growth cones integrate multiple dynamic signalling mechanisms to convert Wnt5 guidance information into directed migration.

Although many guidance pathways have been identified, our understanding of this neuronal toolbox is still incomplete. Weizhe Hong, a student from Liqun Luo's lab (Stanford University, USA), described his identification of a new homophilic attraction cue: the Teneurins. In the *Drosophila* antennal lobe, connections between particular odorant receptor neurons (ORNs) and projection neurons (PNs) form only at specific glomeruli.

By separately labelling specific ORNs and PNs, it was possible to screen for genes that, when overexpressed, interfered with this connection specificity. The screen identified the Teneurins, proteins that function as homophilic attraction cues. *In vitro*, Teneurins form homophilic complexes. *In vivo*, Teneurins are required in both ORNs and PNs. These data suggest that PNs are targeted by the correct partner ORNs by matching levels of Teneurin isoform expression.

Down syndrome cell adhesion molecule (Dscam) is expressed in thousands of isoforms. In dendrites, individual isoforms mediate self-avoidance between processes from the same cell by transforming homotypic adhesion into repulsion. Consequently, a single Dscam isoform can mediate dendritic self-avoidance. Dietmar Schmucker (VIB, Flanders, Belgium) demonstrated that this simple model does not explain the function of Dscam in axons. His group examined the axonal branching pattern of the posterior scutellar (pSC) neurons in *Drosophila*. Using a combination of BAC transgenics and single-neuron Flp recombination, it was possible to generate individual pSC neurons in which potential Dscam isoform diversity was reduced from 19,008 possibilities to 396. Surprisingly, this reduction eliminated the ability of Dscam to mediate normal axon branching. Thus, Dscam uses a different mechanism to regulate axon branching, opening a new chapter for this fascinating gene.

A poorly understood axon guidance event is the targeting of sensory axons to the skin. Alvaro Sagasti (UC Los Angeles, USA) addressed this question in the Rohon–Beard neurons in zebrafish. He showed that two protein tyrosine phosphatase receptor F isoforms, PTPRFa and PTPRFb, members of the leukocyte common antigen-related (LAR) family of receptor tyrosine phosphatases, are required for this targeting event. Time-lapse imaging revealed that disrupting PTPRFa and PTPRFb eliminates the ability of Rohon–Beard axons to target the skin, even though the axons grow at the normal rate. LAR proteins bind to heparan sulphate proteoglycans (HSPGs), and HSPGs are also required for skin targeting of Rohon–Beard axons. Therefore, one or more HSPG proteins act as a ligand for guiding PTPRFa- and PTPRFb-expressing sensory neurons to the skin.

Receptor tyrosine kinases typically act as on–off switches, and are activated by ligand-induced dimerization. However, Eph receptors, which are the largest known receptor tyrosine kinase (RTK) subfamily, mediate repulsive axon guidance in response to subtle gradients of ephrin. How can an on–off switch mediate the response to a graded signal? Ruediger Klein (MPI of Neurobiology, Martinsried, Germany) described an elegant set of experiments to address how receptor clustering modulates activation during Eph/ephrin signalling. Single or multiple FKBP modules were attached to Eph, allowing the acute and specific induction of either dimers or higher-order clusters. The function of these molecules was tested in cell contraction and growth cone collapse assays. It was found that although dimers have some activity, the main activation threshold occurs at a clustering threshold above dimers. *In vivo*, it was proposed that this threshold for Eph receptor activation could be transformed into a graded response to ephrin cues by the presence of a mixture of different oligomeric states.

Synapse formation

Glia have an important function in regulating synapse formation. Gabriel Corfas (Children's Hospital, Boston, USA) elucidated signalling mechanisms that enable glial cells to promote the formation of synapses in the inner ear. In the vestibular sensory epithelia, a type of glial cell called 'supporting cells' ensheath the contacts between hair cells and the terminals of primary afferent neurons. Corfas and his colleagues found that these glia mediate the formation of hair-cell-sensory neuron synapses, and that this function depends on erbB receptor signalling and brain-derived neurotrophic factor (BDNF; [4]). Vestibular sensory neurons express neuregulin, which acts on supporting cells through its cognate receptor erbB. Disrupting erbB function in the supporting cells impedes synapse formation, a phenotype that correlates with decreased BDNF expression by these glia. Furthermore, knockdown of BDNF expression in supporting cells phenocopies the loss of erbB signalling, while forced expression of BDNF by supporting cells rescues the defects caused by loss of erbB. These data suggest that reciprocal signalling between primary neurons and glia mediated by NRG1/erbB and BDNF/TrkB is essential for normal synapse formation in the inner ear.

Serotonergic neurons do not make synapses onto specific postsynaptic targets, but rather form elaborate branched arbors that contain vesicle release sites and that are ramified over distinct target fields. The mechanisms that regulate the development and targeting of these arbors, and of the serotonin release sites contained within them, are not known. Daniel Colon-Ramos (Yale U, USA) used a genetic screen in *C. elegans* to identify genes that are required for serotonergic arbor formation and found that *unc-40*, which encodes the netrin receptor DCC, is required for this process. As well as identifying a new role for netrin signalling, these results establish a powerful system for the future study of serotonergic neuron development.

Neural circuit function

The meeting highlighted progress in determining brain transformations of sensory stimuli that enable detection of complex features in the environment. Sex-specific sensory cues trigger mate recognition and courtship behaviour in *Drosophila* males. The neural circuitry for courtship is established by the transcription factor Fruitless (Fru^M). Kristin Scott (UC Berkeley, USA) provided evidence that Fru^M-positive leg neurons sense non-volatile pheromones involved in courtship behaviour. Barry Dickson (Institute of Molecular Pathology, Austria) also characterized these neurons and traced the Fru^M circuitry from contact chemoreceptors on male forelegs to a site of sensory integration in the brain. These studies show that Fru^M sensory neurons detect several cues involved in sex recognition. Dissecting the Fru^M pathway is providing insight into how complex behaviours are generated in response to multiple sensory triggers.

Higher order neurons detect local features of the environment that arise from integration of sensory activity over space and time. Tom Clandinin (Stanford U, USA) discussed the function of *Drosophila* second-order visual neurons L1 and L2 in motion detection [5]. Although behavioural studies demonstrate that L1 is essential for responses to motion of light edges and L2 to dark edge motion, calcium imaging studies show that L1 and L2 respond similarly to light increases and decreases. However, pathways downstream from L1 selectively compute responses to sequential dark–bright stimuli, changes in intensity that are associated only with moving bright edges, while pathways downstream of L2 compute bright–dark sequences, changes associated with moving dark edges. Thus, edge selectivity emerges through differential weighting of specific inputs.

The brain synthesizes sensory inputs to encode complex features of the world such as space and time. Neurons in the medial entorhinal cortex (ENT) and the

hippocampus respond as the rat moves about its environment, encoding a map of space. An ENT cell responds at interspersed spatial locations that tile a two-dimensional array, creating a grid-like pattern. A place cell in the hippocampus responds when the animal is in one specific location. Stefan Leutgeb (UC San Diego, USA) discussed studies that tested whether hippocampal place fields are derived from grid cell fields [6]. When subcortical synaptic inputs to the ENT were blocked by drug infusion, grid cell firing patterns were disrupted without affecting place cell firing. These studies argue that there are multiple, parallel representations of space in the brain.

Plasticity in neural networks

Synaptic scaling adjusts neural responsiveness to maintain a dynamic range, promoting activity under conditions where basal activity is low. Lu Chen (Stanford U, USA) reported recent progress in understanding how the diffusible molecule retinoic acid (RA) increases neural excitability. She showed that RA synthesis is increased under various conditions when postsynaptic calcium is low, promoting an increase in AMPA receptors [7]. RA acts through the nuclear receptor RARα—dendritically localized in mature neurons—and requires the ligand-binding and RNA-binding domains but not the DNA-binding domain of RARα, to promote rapid translation of Glur1. This provides a general mechanism in which reduced calcium is counteracted by a rapid, post-transcriptional increase in postsynaptic glutamate receptors.

Cori Bargmann (Rockefeller U, USA) discussed how the *C. elegans* nervous system of only 302 neurons allows for plasticity in behaviour among individuals and across evolutionary time. Changing the sensory receptor repertoire is one key mechanism that alters the animal's interactions with the environment and behaviour. For example, different *C. elegans* strains as well as different nematode species have adapted to growth at high density by independent deletions of similar pheromone receptor genes [8]. Changes in neuropeptide receptors are another common mechanism that alters neural responses and behaviour. *C. elegans* strains differ in their aggregation response in the presence of food largely due to a single amino acid difference in neuropeptide receptor 1 [9]. Strain differences in the tendency to leave food are partly explained by expression level differences in another neuropeptide receptor, tyramine receptor 3 [10]. These studies identify molecular families that are common substrates for adaptation and evolution of behaviour.

Neurons that switch the neurotransmitters they release alter neural networks dynamically with profound effects on behaviour. Nick Spitzer (UC San Diego, USA) discussed recent work examining neurotransmitter switching in response to visual stimuli. In the ventral suprachiasmatic nucleus of *Xenopus laevis* larvae, neuropeptide Y neurons acquire dopamine expression in response to sustained light [11]. The newly dopaminergic neurons drive camouflage behaviour in response to different levels of illumination. To test whether light conditions alter neurotransmitter specification and behaviour in other animals, adult rats were subjected to either long-day or short-day photoperiods. The number of dopaminergic neurons in the paraventricular nucleus decreased in the long-day cycle and increased in the short-day cycle at the expense of somatostatin neurons and was associated with changes in stress responses. These studies demonstrate that neuronal identity is not fixed; instead, activity-dependent neurotransmitter re-specification affords flexibility in modulation.

Motor behaviours are flexible and adapt to environmental conditions. Florian Engert (Harvard U, USA) discussed recent studies examining motor adaptation in response to sensory feedback. These studies simultaneously monitored brain activity and fictive swimming in larval zebrafish exposed to a virtual environment. The relationship between sensory feedback and motor output was altered by manipulating the velocity of the virtual environment as compared to the swim velocity of the fish. The animal increased its motor activity when the velocity of the virtual environment was low and decreased its motor activity when the velocity of the virtual activity was high. Interestingly, neurons in the hindbrain and the cerebellum responded to mismatches between sensory feedback and motor output and might act to detect errors between expected and perceived visual cues. The ability to monitor activity throughout the larval nervous system with cellular resolution will allow fine-scale mapping of sensory–motor integration and the neural basis of motor adaptation.

Hailan Hu (Institute of Neuroscience, CAS, China) discussed the neural underpinnings of a complex social behaviour, social dominance [12]. Mice, as well as many animals that live in groups, establish a social hierarchy. Simple behavioural paradigms that measure hierarchy are willingness to back out of a tube when confronted with another mouse, barbering the whiskers of other mice, urine marking and ultrasonic vocalizations. These measures indicated that social rank is stable and transitive. Monitoring the activity of pyramidal cells in the dorsal medial prefrontal cortex (mPFC) showed that higher social rank correlated with higher synaptic activity. Manipulating activity in the dorsal mPFC altered social rank, with manipulations that increased activity causing an upshift in rank and manipulations that decreased activity causing a downshift in rank. These studies suggest that social status is plastic and can be tuned by activity in the PFC.

For centuries, visitors to Suzhou have enjoyed its classical gardens, which are a UNESCO World Heritage Site and exemplify Chinese landscape architecture. Suzhou is also known for the hairy crabs that inhabit the nearby Yangcheng Lake and that are particularly tasty. Now, with the outstanding science presented at this and other conferences at the new Suzhou Cold Spring Harbour Asia meeting site, Suzhou should also be considered a centre of scientific discourse. We look forward to future meetings (and more crabs).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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