

Turning neurons into a nervous system

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The RIKEN Center for Developmental Biology recently held its 2008 Symposium 'Turning Neurons into a Nervous System' in Kobe, Japan. The program, organized by Masatoshi Takeichi, Joshua Sanes, Hideki Enomoto and Raj Ladher, provided a rich sampling from current work in developmental neurobiology. Researchers from Japan, Europe and the USA gathered at this meeting to share insights into neural development and to admire the opening of the cherry blossom season.

Introduction

True to its name, the symposium began with a talk by William Klein (M.D. Anderson Cancer Center, TX, USA) on retinal ganglion cell specification and ended with Barry Dickson (Research Institute of Molecular Pathology, Vienna, Austria) describing the genetic control of mating behavior. From *Drosophila* to humans, the major model systems were well represented at this meeting, including the mouse – the mammalian model of choice. Participants in the meeting were fortunate to hear a special lecture from one of the founders of modern mouse genetics: Nobel Laureate, Mario Capecchi (HHMI, University of Utah, UT, USA).

As at any developmental neurobiology meeting, an overarching theme was the balance of hardwired and activity-dependent mechanisms at each stage of development. Both mechanisms were proposed to shape the distinctive dendritic and axonal arbors that characterize particular types of neuron. Several talks emphasized the hardwired nature of early development in sensory systems. There was equal emphasis, however, on the mechanisms of postnatal plasticity, which might, under the right circumstances, reshape even the adult brain. The disruption of activity-dependent mechanisms that mold the postnatal brain was, moreover, the center of a bold new hypothesis regarding the genetics of autism. Each of these sub-themes is discussed in more detail below.

From new cells to new models

A new cell type

For well over a hundred years, neuroanatomists have catalogued the morphological features of different neuron types and speculated on their functional correlates. Surprisingly, the diversity of neuron types is still not fully appreciated. Joshua Sanes (Harvard University, MA, USA) described a previously unknown class of retinal ganglion cells (RGCs), revealed by the transgenic labeling of cells that express the junctional adhesion molecule B (Kim et al., 2008). These RGCs are scattered across the mouse retina with their dendrites aligned from dorsal to ventral (Fig. 1A). The cells respond to stimuli that move across the retina from dorsal to ventral, a striking example of the functional significance of arbor shape. Because the lens of the eye inverts the image that falls on the retina, these RGCs detect upward

motion in the outside world. Why a specific cell population should be set aside for this function remains a mystery, and suggests that other, equally specialized, retinal cells will eventually be found.

Novel axon guidance cues

A half dozen established families of guidance molecules dominate the field of axon guidance. Vascular-derived endothelins (Edns) are newcomers, and were discussed by Takako Makita from David Ginty's laboratory (HHMI, Johns Hopkins University School of Medicine, MD, USA) (Makita et al., 2008). As presented by Makita, this lab's findings show that newly born sympathetic neurons in the mouse embryo follow vascular trajectories for guidance, adding to a growing appreciation of the role that surrounding non-neural tissues have in regulating neural development.

Autism and activity-dependent genes

Autism is characterized by impaired social interactions and language use, indicative of a profound disruption to brain function that might be particularly devastating to early childhood development. The pattern of inheritance of autism indicates that it is due to mutations at several, potentially interacting, loci. Christopher Walsh (HHMI, Harvard Medical School, MA, USA) used single nucleotide polymorphism (SNP) genotyping to search for autism-related mutations in families with high rates of autism. The list of candidate genes overlapped with a set of genes defined as 'activity dependent' in an independent study by Michael Greenberg (Harvard University, MA, USA). These collaborative findings suggest the provocative hypothesis that, in autism, activity-dependent processes that are crucial to normal postnatal development are impaired (Morrow et al., 2008).

Neuronal polarity and dendrite patterning

Mineko Kengaku (Riken Brain Science Institute, Wako, Japan) proposed a role for activity- and innervation-dependant mechanisms in shaping the dendritic arbor of a cerebellar Purkinje cell. The mature arbor lies flat in a single sagittal plane, but an immature Purkinje cell extends dendrites in multiplanar arrays (Fig. 1B). The elimination of excess dendrites accompanies the elimination of multiple climbing fibers that contact the immature Purkinje cell. Only one climbing fiber will innervate the mature neuron. If climbing fibers are inefficiently removed in mouse mutants, or if they are synchronously activated, the multiplanar tree is not remodeled.

Dendritic trees form under two broad constraints, with details specific to neuronal cell type. Branches of the same neuron divide and separate in a cell type-specific manner, and the entire arbor may either avoid the arbor of a neighboring neuron or overlap with it. This suggests a system of identity recognition by which neurons distinguish self from non-self. The most detailed model of identity recognition by neurons has been worked out in *Drosophila*, following the identification of orthologs of the mammalian gene *Dscam* (Down's syndrome cell-adhesion molecule). Alternative splicing of *Dscam* in *Drosophila*, but not in vertebrates, generates several thousand different receptor isoforms that display homophilic binding.

Dietmar Schmucker (Harvard Medical School, MA, USA) discussed 'self-avoidance' between dendrites of the same neuron, mediated by receptor isoforms of *Dscam* (Hughes et al., 2007). Dendrite arborization (da) neurons in *Drosophila* larvae extend two-

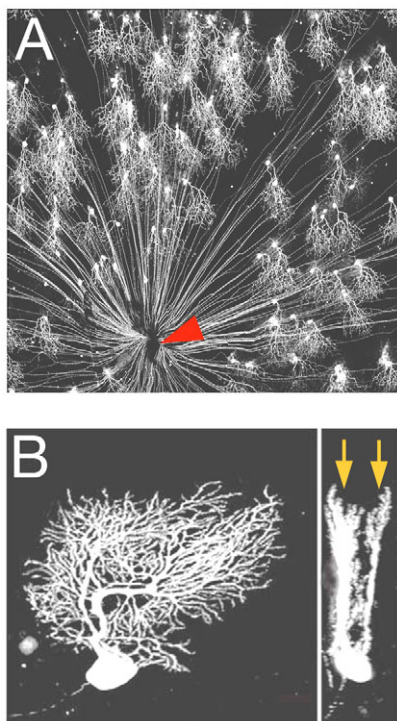


Fig. 1. Cell morphology: development and function. (A) Retinal ganglion cells of an embryonic day/postnatal day mouse that express a fluorescent form of junctional adhesion molecule B. These cells are scattered over the retina with their dendrites aligned from dorsal to ventral (dorsal is uppermost). Axons stream down to the optic nerve (red arrowhead). Image courtesy of In-Jung Kim and Joshua Sanes (Harvard University, MA, USA). (B) An immature Purkinje cell and its dendritic arbor (left panel). Viewed orthogonal to the major axis of the arbor (right panel), dendrites are seen in two planar arrays (yellow arrows). Elimination of excess dendrites by an innervation-dependent mechanism leads to an arbor in a single plane. Image courtesy of Mineko Kengaku (Riken Brain Science Institute, Wako, Japan).

dimensional dendritic trees. Loss of *Dscam* causes the dendrites of an individual *da* neuron to clump together; introducing a single *Dscam* isoform into a neuron restores dendrite separation. In a subset of *da* neurons, the dendritic arbors of neighboring cells avoid one another. This dendritic ‘tiling’ is unaffected by the loss of *Dscam*. However, segregated arbors can be forced to tolerate one another, and to overlap, by introducing different *Dscam* isoforms in each cell. Equally, two overlapping arbors can be forced to separate by introducing a single isoform of *Dscam* in both cells.

Lawrence Zipursky (HHMI, University of California at Los Angeles, CA, USA) noted that *Dscam2*, rather than *Dscam*, generates the receptors responsible for ‘tiling’. He further emphasized that *Dscam* diversity is required for normal circuitry to form in *Drosophila*. When the large array of *Dscam* isoforms is reduced to just one, circuitry is severely disrupted throughout the nervous system (Hattori et al., 2007). Zipursky also presented data from studies of *Dscam* at the atomic level, identifying the structural elements that allow isoform-specific homophilic binding (Meijers et al., 2007; Wojtowicz et al., 2007).

Adrian Moore (Riken Brain Science Institute) used the model system of *Drosophila da* neurons to identify transcription factors that control arbor complexity. Class IV *da* neurons have the most complex dendrite arbors and express both *Cut* and *Knot*. Both *Cut*

and *Knot* transcription factors promote dendrite complexity. *Knot* induces microtubule-filled extensions to form in *da* neurons, mediated by *Spastin*, a protein that is involved in severing microtubules. *Cut* additionally induces dendritic filipodia, an activity that is suppressed by *Knot* in class IV cells, which do not generate dendritic filipodia as a result (Jinushi-Nakao et al., 2007).

Historically, the topic of neuronal polarity has focused on how one of several cell neurites is specified to become the axon. Koizo Kaibuchi (Nagoya University Graduate School of Medicine, Nagoya, Japan) discussed the development of the directional trafficking of proteins along the growing axon. Collapsin response mediator protein 2 (CRMP2) links vesicles that contain the neurotrophin receptor *TrkB* to the microtubule motor kinesin 1, allowing the kinesin 1-mediated axonal transport of *TrkB*. Activation of *TrkB* in turn promotes axon growth. Glycogen synthase kinase 3 β (GSK3 β) blocks the formation of this complex by phosphorylating CRMP2, giving GSK3 β a role in regulating neural polarity, and implying that it must be inactivated in a growing axon. Indeed, GSK3 β appears to be selectively inactivated at the distal end of growing axons (Jinushi-Nakao et al., 2007).

Cerebral cortex development

Development of the cerebral cortex requires several cell types to acquire polarity, among them the radial glial (RG) cell, which generates neurons and extends a process from the ventricle to the pial surface along which newborn neurons migrate. William Snider (University of North Carolina, NC, USA) provided evidence that GSK3 α /GSK3 β and Adenomatous polyposis coli (APC), a substrate of GSK3 β , are regulators of RG cell proliferation and polarity. The double deletion of *Gsk3b* and *Gsk3ain* the cortex of mouse embryos caused hyperproliferation and disrupted RG cell morphology. The conditional deletion of *Apc* caused an even more dramatic disturbance. Videomicroscopy showed RG processes that appeared to be lost: their leading edges moving randomly, rather than heading for the pial surface.

A major step in corticogenesis is the development of a two-way pathway between cortex and thalamus that crosses the striatum and the globus pallidus. Previous studies have shown that thalamocortical axons receive important permissive and instructive guidance cues along this route (Garel and Rubenstein, 2004; Lopez-Bendito et al., 2006). Masatoshi Takeichi (RIKEN Center for Developmental Biology, Kobe, Japan) reported that the pathway between thalamus and cortex fails to develop in mice deficient in OL-protocadherin (also known as protocadherin 10, *Pcdh10*). OL-protocadherin is expressed in the striatum, and several findings point to the striatum as being the site at which axons are blocked in the mutant. The striatonigral pathway is absent and striatopallidal axons are tangled. In vitro, however, axons grow out from dissociated striatal neurons, suggesting that growing striatal axons – and by extension, other axons crossing the striatum – face an inhospitable environment (Uemura et al., 2007). Takeichi provided evidence that OL-protocadherin acts on axonal outgrowth by associating with the Nap1-WAVE protein complex, which is known to regulate cell motility.

Aaron DiAntonio (Washington University School of Medicine, WA, USA) reported a similar loss of connections between thalamus and cortex following deletion of the mouse ortholog of *highwire*, *Phr1* (Bloom et al., 2007) (*Mychp2* – Mouse Genome Informatics). A comparison between mice in which *Phr1* was conditionally knocked out in the entire telencephalon, or just in the cortex, suggested that the key deficit is in the subcortical telencephalon. Although an *Islet1*-expressing permissive corridor for thalamic

axons (Lopez-Bendito et al., 2006) is present in the constitutive *Phr1* mutant, striatopallidal axons again form a tangle. Together, these findings suggest that several permissive conditions are important for the development of the pathway between thalamus and cortex, including the normal growth of axons from local neurons along the pathway.

From retinal ganglion cells to a visual system

William Klein (University of Texas, TX, USA) described a transcription factor network that is required for competence to acquire an RGC fate, for RGC fate specification and for the differentiation of RGCs (Mao et al., 2008; Mu et al., 2005). A mouse homolog of the *Drosophila* proneural gene, *atonal*, *Math5* (*Atoh7* – Mouse Genome Informatics) is required for competence and specification. [Note that the zebrafish *lakritz* mutant discussed below (Fig. 2) lacks *Atoh7* and has no RGCs.] Subsequently, the interplay between *Math5* and *Brn3/Pou4f2*, together with *Isl1*, suppresses *Math5* so that RGC differentiation can proceed. *Pou4f2* further regulates the expression of *comespermin*, a transcription factor that mediates the later differentiation of RGC cells, including myelination. In presenting his gene regulatory network for RGC cells, Klein proposed the intriguing hypothesis that relatively simple transcriptional networks generate specific neuron types, involving dozens (but not hundreds) of transcription factors.

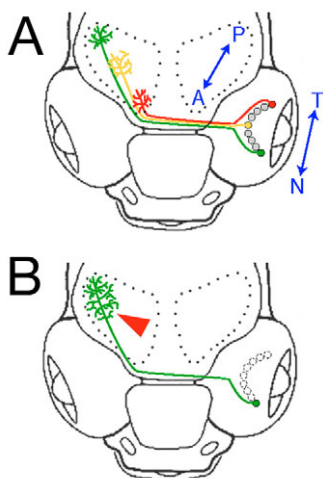


Fig. 2. The role of axon-axon interactions in the formation of the retinotectal map. (A) Dorsal view of the head of a wild-type zebrafish larva, showing the projection of retinal ganglion cells (colored circles) from the eye to the optic tectum (broken outlines).

The terminal arbors of ganglion-cell axons are arranged in a topographic fashion, preserving their neighborhood relationships in the retina. Axons from the temporal retina (red, T) are connected to the anterior (A) region of the tectum; nasal axons (green, N) project to the posterior (P) tectum; and axons from intermediate positions in the retina (yellow) terminate in the center of the tectum. (B) Dorsal view of a *lakritz* (*atoh7*) mutant zebrafish in which a single ganglion cell has developed from a transplanted clone of wild-type cells. The single ganglion cell (green; an example of a nasal cell is shown here) sends a solitary axon into the tectum, where it projects to its topographically appropriate zone, but forms a larger arbor than is normal (red arrowhead). This experiment rules out a requirement for axon competition in retinotectal mapping along the anterior-posterior axis. However, axon-axon interactions strongly influence the stability of branches on the proximal/anterior side of the arbor. Images courtesy of Nathan Gosse and Herwig Baier (University of California at San Francisco, CA, USA).

The output from RGCs to the brain is modulated by interneurons that make synaptic contact with RGC dendritic arbors in the inner plexiform layer (IPL) of the retina. Joshua Sanes presented evidence that four highly related molecules, *Dscam1* and 2, and *sidekicks* (*Sdk*) 1 and 2, are required to match up the correct synaptic partners in the correct sublaminae of the IPL (Yamagata and Sanes, 2008). The four molecules mediate homophilic adhesion (not repulsion this time) and are distributed in appropriate, coordinated patterns in the retina. Thus, *Dscams* and similar receptors can provide at least a simple recognition code in the vertebrate nervous system.

The projection from the retina to the optic tectum (or superior colliculus) is a classic model system for studying topographical map formation in the brain. Neighboring RGCs project to neighboring tectal neurons to generate a continuous map of the visual field in the tectum. Axons from cells along the temporal to nasal axis of the retina, for example, project to neurons along the anterior to posterior axis of the tectum (Fig. 2A). Temporal RGC axons are directed to the anterior tectum by repellent ephrin A/EphA signals in the posterior tectum, but it is unclear how nasal RGC axons are guided to the posterior tectum. One possibility is that unknown molecular guidance mechanisms direct nasal axons; another is that competition among axons for anterior tectal space results in nasal retinal axons being pushed to the posterior. Herwig Baier (University of California at San Francisco, CA, USA) described elegant experiments that appear to settle the question, at least in zebrafish. Small clones of wild-type cells were introduced into zebrafish *lakritz* mutants, which lack RGCs. Some resulting chimaeras had eyes with a single wild-type RGC. Regardless of the position of the RGC in the retina, it projected to the correct target position in the tectum. In particular, axons from nasal RGCs grew straight to the posterior tectum (Fig. 2B). These findings indicate that axon-axon competition is not required for retinotectal mapping (Gosse et al., 2008), although competition may be needed to regulate arbor size. Interestingly, this study generally supports a previous computational model of retinotectal development (Yates et al., 2004).

Plasticity and experience-dependent rewiring

Shortly after birth, many species pass through ‘critical periods’ of neural plasticity during which their brains are particularly malleable to experience. Sensory systems are refined, and a variety of species-specific behaviors are learned. Ocular dominance plasticity is a favored experimental model for studying this phenomenon. Monocular visual deprivation (MD) in young mammals weakens the synaptic circuitry that underlies the visual cortical responses to the deprived eye, and strengthens the synaptic circuits that mediate responses to the non-deprived eye.

Only in recent years has plasticity in visual cortical responses to the two eyes been studied in mice, leading to a range of experimental approaches that are impracticable in cats or monkeys. Takao Hensch (Harvard University, MA, USA) presented new findings implicating the transcription factor *Otx2* in visual cortex plasticity. Cortical infusion of *Otx2* accelerates the overall response to MD in the primary visual cortex (V1). This effect is mediated through GABAergic interneurons that contain parvalbumin (PV). *Otx2* regulates the maturation of these PV interneurons, which in turn trigger general V1 plasticity. A conditional deletion of *Otx2* in the visual pathway prevents plasticity, and this is reversible by benzodiazepine, a GABA-receptor agonist. Of note is that *Otx2* appears to regulate the activity of interneurons but is not expressed in them. A daring hypothesis, well supported by the data presented, is that *Otx2* is made by cells elsewhere in the visual pathway, and is then transported to and released in the visual cortex.

Tobias Bonhoeffer (Max Planck Institute for Neurobiology, Martinsried, Germany) presented evidence that the adult mouse remains sensitive to MD, showing results from technically impressive optical imaging experiments in which dendritic spines in the mouse V1 were imaged before, during and after MD. After 1 week of deprivation, which is longer than is required to induce plasticity in juvenile animals, cells in the binocular region of V1 increased their response to the stimulation of the non-deprived eye. Additionally, dendrites imaged in the binocular region increased their number of spines by 5-25%. After deprivation, visual responses and spine densities returned to normal. However, plasticity can be elicited again with a new, shorter period of monocular sight deprivation, indicative of the first deprivation having a priming effect. This time too, plasticity lasts for longer, indicating that repeated experience enhances cortical plasticity (Hofer et al., 2006).

Conclusion

At this meeting, a remarkable number of excellent talks and posters of unpublished data were presented, many of which could not be discussed in this review. Among the themes not covered here is synaptogenesis, a central interest of many of the researchers present. Notably, this field still lacks candidate molecules that actually induce synapses to form in the CNS of vertebrates. Previous candidates, such as neurexins and neuroligins induce synapses in vitro, but in vivo have other important roles, for example in promoting synaptic function. Key talks on self- and other recognition by neurons in *Drosophila* raise the issue of whether genes exist in vertebrates that can generate multiple cell labels, as the *Dscam* genes do in *Drosophila*. Finally, the day of identifying individual disease susceptibility genes from different families may be over. Broad screens of the genome of large, interrelated families appears much more likely to solve the problem of the genetic causes of complex mental health disorders.

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