ELSEVIER

Development
Editorial overview
Barry Dickson and Christopher A Walsh

Current Opinion in Neurobiology 2004, **14**:1–5

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2004.01.016

Barry Dickson

Institute of Molecular Biotechnology (IMBA), Austrian Academy of Sciences, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria
e-mail: dickson@imba.oeaw.ac.at

Barry Dickson is a senior scientist at IMBA. His group studies the molecular mechanisms of axon guidance and target specificity in *Drosophila*. One focus of this work is the regulatory mechanisms that operate as commissural axons grow towards, across and beyond the CNS midline.

Christopher A Walsh

Bullard Professor of Neurology, Investigator, Howard Hughes Medical Institute, Harvard Medical School, BIDMC, Room 816, HIM, 4 Blackfan Circle, Boston, MA 02115, USA
e-mail: cwalsh@bidmc.harvard.edu

Christopher Walsh is interested in genes that regulate the development of the cerebral cortex. Mutations in these genes in humans cause autism and epilepsy, as well as mental retardation and other learning disorders. These genes are vital to the normal development of the cortex, and it is possible that they might have been altered during evolution to allow the unique aspects of the brain that underlie human cognitive abilities to develop.

Introduction

In their editorial overview in these pages a few years ago, Lumsden and Jan [1], borrowing a phrase from Winston Churchill, concluded that we were now at the ''end of the beginning'' of developmental neurobiology. There can be little doubt that developmental neurobiology is now a mature field. The sense of awe that one inevitably experiences when contemplating the nervous system has now been enriched (but not supplanted) by a modest level of understanding. Some general principles have emerged, and many previously unknown biochemical 'activities' now have a precise molecular definition. But this does not mean that the excitement is over. Quite the contrary. Maturity brings with it a deeper appreciation for the things that one does know, and a more acute awareness of the many more that one does not.

In bringing together this set of reviews, our goal was to gather a broad set of perspectives on different problems and different systems that would reflect this mature state of the field. Several reviews illustrate the detailed knowledge we have now gained in certain areas; others question received wisdom or highlight some gaping holes in our understanding; and yet others describe new approaches or systems that might help to fill these holes. We have grouped them into four areas. First, we look at the origins of cell diversity in the nervous system. We then move on to the mechanisms that establish the initial patterns of neuronal connectivity, and from there to the experience-dependent mechanisms that fine-tune these connections in response to the real world. Finally, we sample just a few of the exciting areas at the interface of developmental neurobiology and medicine.

Cell diversity in the nervous system

One of the many remarkable features of the nervous system is the extraordinary diversity of the cells of which it is comprised, both neurons and glia. How are so many distinct classes of neurons and glia generated, and at well-defined positions and numbers? The first four reviews in this issue highlight recent progress towards understanding some of the developmental strategies that generate many different variations on the same basic theme of the neuronal phenotype. In keeping with the topic of variations on a common theme, we have also selected reviews that share a common theme — sensory cell diversity — yet range across a broad selection of different sensory modalities (mechanosensation, chemosensation and vision) and model systems (worms, flies, fish and mice).

One way to generate cellular diversity is through an asymmetric cell division, by which one cell gives rise to two distinct daughter cells. Conceptually, this may sound quite simple, but the devil is in the details. What are the determinants that are segregated into only one of the two daughter cells, how are they segregated, and how do they specify cell fate? Answers to these questions are now emerging from genetic studies of the fly's external sensory

organs, as well as the division of neuroblasts within the central nervous system. In the first review, Bardin *et al.* provide a broad overview of this fast-moving field.

We then move from the fly to the fish, and from mechanosensation to vision, as Malicki examines patterning and cell fate specification in the zebrafish retina. In the developing vertebrate retina, a pool of multipotent progenitors gives rise to six different types of neuron and one type of glia — all produced in a precise sequence, position, and ratio. Efforts to understand how these cell fates are specified have focused mostly on the mouse and *Xenopus* retina. The zebrafish is a relative newcomer to this field, bringing the power of forward genetics to bear on these questions. As Malicki reports, many mutants with specific defects in retinal patterning have now been isolated, and the cloning and characterisation of these genes is providing new insights into the complex interplay of intrinsic and extrinsic factors that control cell fate decisions in the vertebrate retina.

For the next two reviews, we turn to chemosensory systems, sampling from almost opposite ends of the spectrum. *Caenorhabditis elegans* has just 32 chemosensory neurons, with at least 14 different types. Lanjuin and Sengupta review recent work revealing how diversity arises within this relatively small set of neurons. Transcriptional networks that generate distinct neuronal subclasses are the main focus, but the review also highlights some of the more surprising mechanisms the worm uses to maximise neuronal diversity — to the extent that the left and right neurons of a bilateral pair adopt distinct fates. In one case, asymmetry is generated stochastically, and relies on signals passed between the processes of the two cells. In another case, a cell on the left side of the animal expresses a microRNA that causes it to become different from its homologue on the right side. Are these just esoterics of *C. elegans* development; clever tricks the worm uses to get the most out of its tiny set of chemosensory neurons? Or are similar mechanisms also at work in more complex nervous systems? Time and a lot of hard work will tell, but given the remarkable evolutionary conservation of so many other aspects of neural development, the answer to this question is not really in doubt.

While *C. elegans* gets by with just 32 chemosensory neurons, the mouse has millions just in its main olfactory epithelium. These olfactory sensory neurons (OSNs) represent at least a thousand distinct types, each defined by the expression of particular odorant receptor (OR). The key to understanding cell diversity in this system is therefore to explain how each OSN selects a specific OR. It is now widely accepted that each OSN does indeed express just a single OR (the 'one receptor, one neuron' hypothesis), and that it somehow selects this one OR for expression early in its differentiation. While often regarded as an established fact, direct proof of this hypothesis is hard to come by, and so the evidence to support it remains largely circumstantial. Mombaerts thoughtfully reviews this evidence. The hypothesis stands up well, but Mombaerts raises an interesting alternative that is equally consistent with all the available data. According to his 'oligogenic' hypothesis, an OSN would not necessarily choose a single OR from the outset, but may select none, one, or a few. Mechanisms of positive and negative selection could then set in, as they do, for example, in the immune system. In this way, cells that express either no or multiple ORs might be eliminated, leaving just those that express a single OR (or a 'compatible' combination of ORs, such as one functional OR and one pseudogene).

Colognato and ffrench-Constant complete the set of reviews on cell diversity with a detailed look at the most numerous and (until recently) under-appreciated cells of the nervous system: the glia. Their review ranges across topics such as glial cell fate, proliferation, differentiation, and migration. One of the important emerging themes is the surprising 'developmental plasticity' of the glial lineage, with recent findings suggesting that, in several different regions of the brain, some neurons may actually originate from 'glial' lineages.

Wiring up the nervous system

The past decade or so has seen rapid progress in identifying the molecules and mechanisms by which neurons form specific connections during development. Intense efforts have been directed towards understanding how axons are guided along specific pathways towards their targets. This work has led to the identification and functional analysis of several highly conserved families of axon guidance cues and their receptors. Although this has been enormously satisfying, it is at the same time sobering to contemplate some of the daunting challenges that remain: how much of axon pathfinding can be explained by this now familiar set of molecules? How do axons adjust their sensitivity to guidance cues as they extend along each leg of their journey? And how do these cues work to steer the axon? And in contrast to the great inroads that have been made into understanding axon development, we are still largely ignorant of the mechanisms that regulate dendrite development. We also need to find out how synaptic connections are specified. Despite promiscuous contact between axons and dendrites of many different neurons, synapses form only between specific pairs of neurons at specific sites. How is this controlled? These issues are addressed in the next six reviews.

The series on connectivity kicks off with a review by Yoshikawa and Thomas. The catalogue of axon guidance molecules continues to expand, driven on by both genetic and biochemical studies in a variety of different systems. Recently, some rather surprising new members have been added to this list. These are a set of molecules better

known for their functions as morphogens: the bone morphogenetic proteins (BMPs), Wnts, and Hedgehogs. The first indications that these molecules might also act as axon guidance cues began to trickle in several years ago. But acceptance of this idea has been slow, as for these molecules it is imperative — but exceedingly difficult — to distinguish between their functions as morphogens and guidance cues. Do they guide the axon directly, or just pattern the tissue in which it grows? As Yoshikawa and Thomas discuss, compelling evidence has now been amassed from several different systems that these molecules do indeed have a direct action in guiding axons.

Several guidance molecules, including both 'classic' and 'morphogen' cues, were initially characterised in the context of axon divergence at the midline — either the midline of the *Drosophila* ventral nerve cord, or the floor plate of the vertebrate spinal cord. Another major midline structure in vertebrates is the optic chiasm, where retinal axons must also choose either a contralateral (crossing) or an ipsilateral (non-crossing) projection. In a thoughtful commentary, Williams *et al.* compare these different systems, and point out that, despite their superficial similarities, not all midline structures are the same. Perhaps the clearest illustration of this is the recent finding that the same guidance systems may be at work in the different systems, but do different things. For example, Slit proteins and their Robo receptors are thought to control axon crossing at the fly's ventral nerve cord and the vertebrate floor plate, but in the optic chiasm Slits and Robos instead appear to guide all axons — contralateral and ipsilateral alike — along the optic pathways both before and after the chiasm. What then are the cues that control the choice of a contralateral or ipsilateral pathway at the chiasm? Williams *et al.* go on to review a series of elegant studies implicating the ephrin-Bs in this decision, and tracing the chain of events back to transcriptional programs that regulate the expression of the corresponding EphB receptors in the retina.

Retinal axons are not only guided by ephrin-Bs at the optic chiasm, but later they also use ephrin-Bs to locate their correct topographic targets along the dorsoventral axis of the tectum. This is but one illustration of a general and crucial issue in axon pathfinding: how does an axon respond in the right way to the right cue at the right time? As reviewed by van Horck *et al.*, recent evidence points to an elaborate set of regulatory mechanisms that control the growth cone's sensitivity and response to various cues as it extends along its path. The review maintains the focus on studies of retinal axon guidance, which have provided much of this evidence. One of the most surprising findings, coming from work in the Holt laboratory, was the demonstration that local protein synthesis and degradation in the growth cone are induced by guidance cues and are necessary for steering. Endocytosis has recently been identified as yet another regulatory mechanism. How these processes impinge on growth cone steering is still hotly debated.

The reviews by Yoshikawa and Thomas, Williams *et al.* and van Horck *et al.* nicely show how much has been learned from detailed studies of two different sets of axons: retinal ganglion cell axons in vertebrates, and commissural axons in both vertebrates and invertebrates. In the next review, Ghysen and Dambly-Chaudière introduce an emerging model system that holds the promise of greatly furthering our understanding of many different aspects of neuronal connectivity, in particular the dynamic nature of cell and axon migration and the coordinated activities of multiple cell types. This is the zebrafish lateral line, the sensory system by which the fish detects and responds to changes in the motion of the water. Detailed analysis of lateral line development in wild type fish, as well as the first few mutants, has defined the underlying cellular interactions and begun to reveal some of the molecular mechanisms. This is a timely review, as we can clearly look forward to a wealth of fascinating new insights coming from this system in the near future.

Whereas the development of axons has been so intensively studied in recent years, dendritic development has received relatively little attention. The pioneering genetic studies of *Drosophila* dendrite development by Jan and co-workers over the past few years have now brought dendrites back into the limelight. This work is reviewed by Grueber and Jan. Clever genetic strategies are now rapidly advancing our understanding of the cellular and molecular interactions that control dendritic growth, branching, tiling and remodelling. One important theme emerging from these and other studies is that axons and dendrites navigate independently but coordinately to their respective target areas, apparently using many of the same cues.

Once axons and dendrites have reached their common target regions, synapses form between specific partners at specific contact sites. The mechanisms that control synaptic specificity are still not as well understood as those that regulate axon guidance, but rapid progress is now being made in a number of different systems. This work is reviewed here by Shen. As he points out, some of the principles of axon pathfinding may also apply to target specificity — such as the important role for 'guidepost' cells and the hierarchical nature of targeting decisions (with preferred and alternative sites for synapse formation). At the molecular level too many similarities are emerging, in particular with the prominent role of immunoglobulin superfamily members.

Synaptic plasticity

Although these early steps in establishing neuronal connectivity are 'hard wired', neuronal activity has long been

known to play a crucial role in the subsequent remodelling of synaptic connectivity. In the next set of three reviews, Foeller and Feldman, Kandler, and Kasthuri and Lichtman discuss the roles and mechanisms of this activity-based plasticity.

In the rodent somatosensory system, axons from each whisker form a somatotopic map in the cortex, known as the barrel map. During a critical period of neonatal development, this barrel map is fine-tuned in response to sensory experience. As Foeller and Feldman discuss, developmental plasticity relies on a variety of synaptic mechanisms, including both long-term potentiation (LTP) and depression (LTD), and involves not only excitatory but also inhibitory circuits. This kind of plasticity may be responsible for the remarkable reorganisation of sensory maps in a variety of animal systems.

The role of inhibitory circuits in synaptic reorganisation is further explored by Kandler, who focuses on the auditory system. Recent work has revealed a dramatic remodelling of inhibitory synapses shortly after the onset of hearing. Kandler reviews work showing how this restructuring relies on both spontaneous and sensory-evoked neuronal activity, and revealing both the general principles and the cellular mechanisms that underlie plasticity in this system.

Any mechanistic account of the structural changes in synaptic circuits must be built upon a detailed knowledge of what changes actually take place. Imaging synapses in living animals over extended periods of time poses some formidable technical challenges. Recent advances in the use of genetically encoded fluorescent markers and two-photon microscopy have sparked renewed vigour in this endeavour. Kasthuri and Lichtman provide a brief account of earlier attempts at *in vivo* imaging, before going on to discuss these new technologies, some of the critical insights they have already provided, and the technical challenges that still remain.

Development, disease, and repair

Increasingly, issues in developmental neurobiology and medicine are becoming intertwined. Insights from developmental studies are offering new therapeutic prospects, whereas studies of human diseases are revealing key genes and processes in neural development. The last set of reviews in this issue cuts broadly across this vast landscape of clinically oriented research.

Brain size varies tremendously in mammals, with humans famously having the largest brains of all. There is increasing interest in understanding how brain size is regulated, with key insights coming from studies of human microcephalic disorders. Woods and co-workers have identified the genetic basis for some of these developmental disorders, and he reviews this work here. These genes appear to regulate neurogenesis, cell fate, and cell migration. An intriguing idea is that changes in these genes during evolution may have contributed to the diverse shapes and sizes of the mammalian brain.

Recent progress in understanding the mechanisms that regulate axon growth during development has fuelled renewed hope that, in the not-to-distant future, it may be possible to entice severed axons to regrow and reform functional connections in the adult spinal cord. Schwab offers a realistic assessment of these prospects. His review focuses on Nogo and other inhibitors of neurite growth in the mature CNS, highlighting recent work that identifies the corresponding receptors and downstream signalling pathways. Rapid progress in this area has in part been attributable to the well-known roles of many of these signalling molecules during axon development. Schwab critically reviews a series of promising studies in which interfering with these pathways at various levels with various strategies has allowed some degree of regeneration and functional recovery in rodent spinal cord injury models.

Finally, one of the most remarkable forms of adult plasticity is that of persistent stem cells in the adult brain, which can replace lost neurons or continue to add new neurons throughout life in some regions of the brain. Morshead and van der Kooy provide a critical review of the evidence for the existence of these stem cells, their location, and their properties. The driving force here is the hope that a better definition of what these stem cells are and how they can be purified should lead to better techniques for manipulating their amazing capabilities.

Looking ahead

It is always a hazardous exercise to try to predict what lies ahead, particularly in science. The only reasonable guesses one can make are that current trends will continue, and that new technologies will bring a fresh approach and a deeper understanding to some old issues. In surveying the field over the past few years, and as reflected in the reviews assembled here, one clear trend is the push to an ever more detailed account of the molecular mechanisms underlying key developmental processes. The combined attack of molecular biology and genetics on developmental neurobiology through the 1980s and 1990s yielded a long list of molecules involved in various processes. But how do these molecules work? Geneticists (ourselves included) are wont to claim that a gene 'controls' a particular process, with 'controls' being little more than a euphemism for 'is required in some unknown way for'. Fortunately, this is beginning to change, as researchers seek to determine what that word 'controls' actually means, mechanistically, in these cases. This will be even more difficult than it was to find these genes and molecules in the first place. But two technological developments will help greatly in this endeavour. The first is the ever-increasing precision with which

single genes can be manipulated in the living organism, often even in single cells (Grueber and Jan provide nice examples of this). The second is the power of *in vivo* imaging, as described here by Kasthuri and Lichtman. The frantic gene-hunting exercise over the past decade or so, while still far from over, is now giving way to a period of hypothesis-driven investigation, with answers coming at unprecedented spatial and temporal resolution.

Another clear trend is the shifting emphasis back to the differences rather than the similarities between systems. For example, in axon guidance, not all growth cones respond the same way to certain cues, not even the same growth cone at different times, and seemingly similar guidance processes are not always built upon identical molecular mechanisms. This trend is particularly evident here in the commentaries from Williams *et al*. and van Horck *et al*. The high degree of conservation of developmental molecules and mechanisms across species that became apparent during the 1980s and 1990s was important in both revealing the general principles and bringing together findings and approaches from diverse systems. But worms, flies, fish, frogs, mice and humans undeniably have vastly different nervous systems. These differences do not arise by fundamentally different mechanisms, as once was thought, but rather as the accumulation of countless subtle differences. This should not surprise us. Such subtleties are the stuff of evolution. We should embrace these differences, and seek to explain them.

Finally, developmental neurobiologists have long held out the hope that their work will ultimately lead to effective treatments to deal with disease and injury to the adult nervous system. Recent work on axon regeneration and adult stem cells, as reviewed by Schwab and Morshead and van der Kooy, suggests that such treatments are now a realistic prospect. We should not think, nor create the impression, that these treatments are only just around the corner. Amid much hype, the reviews by Schwab and by Morshead and van der Kooy are refreshingly cautious, pointing out some of the enormous challenges that still remain. Nevertheless, the hope is now real. Perhaps it is yet another sign of its maturity that the field of developmental neurobiology may soon be ready to give something more than mere knowledge back to the public that supports it.

Reference

1. Lumsden A, Jan Y-N: **Editorial overview. The end of the beginning?** *Curr Opin Neurobiol* 1997, **7**:3-6.