EMBO reports

meeting report

Neuroscience from different angles

Student symposium: From genes to thoughts

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The first EMBL student symposium on Neurobiology was held at the European Molecular Biology Laboratory in Heidelberg, Germany on October 20 and 21, 2000.

Introduction

On October 20 and 21, 2000, over 200 students came to the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany for a unique symposium. As the title 'From genes to thoughts' suggests, the symposium covered most aspects of modern neurobiology, from detailed structural studies to the most advanced models of *in silico* built neural networks. The idea came from a group of EMBL PhD students who invited 14 young researchers and leading scientists to give introductions into their fields of research, spiced with recent results. The meeting initiated a series of European student conferences that are fully organized by students and are held in an informal setting, with the aim of bringing interested students together with selected speakers from different disciplines of modern biology.

Molecular mechanisms of neural functions

Jean-Pierre Changeux (Institut Pasteur, Paris, France) opened the meeting with a general introduction to the hierarchical organization of the brain. During phylogeny and ontogeny, successively higher levels of neural complexity are established, making it impossible to mechanistically and directly define 'thoughts' from a mere analysis of genes. Changeux presented a model for how reward systems might stabilize circuits of neurons. He postulated that allosteric receptors could mediate the strengthening of synapses, discussing as an example the regulation of the nicotinic acetylcholine receptor (nAChr). Showing some of his recent work, Changeux described mice lacking the β-2 (nicotine binding) subunit of nAChr. On a behavioral level, a self-administration test with nicotine revealed that the β-2 knock-out mice 'smoked' less than did the wild-type controls. On a physiological level, administration of nicotine in the brain of knock-out animals did not elicit the release of dopamine, a neurotransmitter already known to be involved in many reward systems. On a single cell level, nicotine failed to activate nAChr and did not elicit any physiological response.

In an effort to understand neurotransmission at an atomic level, Josep Rizo (Dallas, TX) used nuclear magnetic resonance (NMR) spectroscopy to obtain structural insights into neurotransmitter release. This process involves the so-called SNARE complex, which is formed by the vesicle protein synaptobrevin, the plasma membrane protein syntaxin and the membrane-associated protein SNAP 25, and is essential for the fusion of synaptic vesicles. Rizo's studies show that syntaxin can undergo a large conformational switch from an open to a closed state. In the open conformation, syntaxin participates in the formation of the SNARE complex, whereas when locked in the closed state by the binding of MUNC-18 (a protein involved in many intracellular fusion events), the SNARE complex can not be formed. Syntaxin can also interact with synaptotagmin, but only when

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the latter has calcium bound. These calcium ions neutralize repulsive charges that otherwise repel the two proteins.

The role of calcium in regulating gene expression after stimulation of a neuron was discussed by Hilmar Bading (Cambridge, UK). He described how different signaling cascades lead to the expression of different genes through spatially distinct elevations of calcium levels. While a rise in nuclear calcium leads to activation of transcription mediated by the cyclic-AMP-response element (CRE), increased cytoplasmic calcium stimulates the serum response element (SRE). Bading and colleagues found that cytoplasmic and nuclear calcium both trigger the phosphorylation of the CRE binding protein CREB at its activator site serine 133. Whereas this is not sufficient to activate transcription yet, it enables the transcriptional coactivator CREB binding protein (CBP) to bind to CREB, forming a complex primed for activation by the nuclear (but not cytoplasmic) calcium-sensitive CaM kinase IV. Interestingly, the route of calcium entry also plays a role in determining the response. When calcium enters through L-type calcium channels, CBP is activated and recruited to CREB, and transcription is strongly induced. Calcium flux through NMDA receptors induced by exposure to glutamate also activates CBP. However, CBP is only recruited to CREB transiently, if at all, under these conditions. Consequently, the transcriptional response is weak. Finally, a third pool of calcium can also modulate gene expression: submembraneous calcium transients lead to the phosphorylation of p42/44 MAP kinase and to CAM kinase IV-independent phosphorylation of CREB. Bading's studies beautifully illustrate the combinatorial organization that relates intracellular calcium signaling to the activation of different programs of gene expression.

Development of nervous systems

An issue of debate in evolutionary biology is whether the central nervous systems (CNS) of vertebrates and invertebrates have a common precursor or have evolved independently (Figure 1). Detlev Arendt (EMBL, Heidelberg, Germany) compared the patterning molecules that are involved in the development of the CNS in distantly related species. He discussed the concept of a dorsoventral axis inversion, which postulates that 'ventral' for insects amounts to 'dorsal' for vertebrates, indicating that the CNS of insects and vertebrates did not evolve independently. Supporting this concept is the fact that the two systems use homologous genes to achieve opposing tasks, e.g. *Drosophila* DPP has dorsalizing activity in the fruit fly, while the mouse homolog, BMP-4, has ventralizing activity. Another set of genes that allows the study of complex mechanisms in simpler model organisms includes Pax6, which is important for eye development in mouse. In Polychaeta, the Pax6 homolog is expressed in their most primitive eye. Consisting of only two cells, one photoreceptor and one pigment cell, the eye of the polychaete Platynereis could be close to an ancient common precursor for both insect and vertebrate eyes. These results argue that distantly related model systems can yield insights into fundamental neurobiological problems.

Joe Culotti (Toronto, Canada) investigates the pathways taken by migrating neurons in the nematode *Caenorhabditis elegans*. Early screens resulted in a multitude of mutants that displayed uncoordinated movements. Many of the genes mediating these

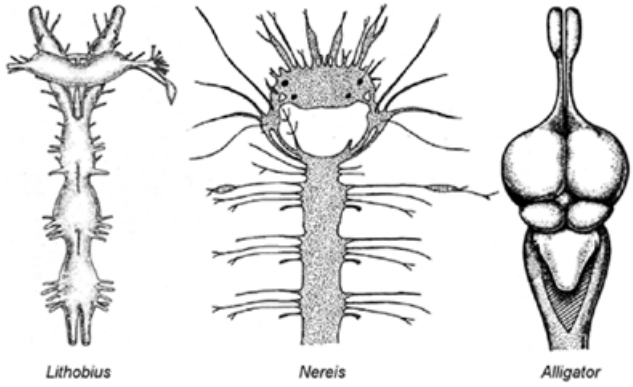

Fig. 1. Drawings of adult central nervous systems (CNS) in distantly related Bilateria. The dorsal and ventral CNS found in Bilateria could have evolved independently or by axis inversion from a common ancestor. Arendt and co-workers have found support for the concept of axis inversion on the molecular level (see text). Figure modified from different sources including Arendt and Nübler-Jung (1999) and reproduced with permission from The Company of Biologists Ltd.

effects are now cloned and some have homologs in vertebrates. For example, UNC-6 (netrin in vertebrates), along with the UNC-6 receptors UNC-5 and UNC-40, is responsible for guidance of migrating growth cones along the dorsoventral axis. However, it is clear that these are not the only molecules involved in the process of guiding neurons along, down, up and across the worm. Culotti and colleagues isolated a novel TGF-β homolog (UNC-129) that is expressed in a gradient along the dorsoventral axis, under the control of a forkhead transcription factor (UNC-130). Both molecules are required for circumferential migrations of neurons. Interestingly, UNC-130 also plays a role during embryonic development, independently of UNC-129.

Switching gears, Jonas Frisén from the Karolinska Institute in Stockholm, Sweden reported evidence against the traditional view that, once the brain has established full function, no additional neurons can form. He described the discovery of neural stem cells, which continue to regenerate as many as 100 000 neurons per day in the olfactory bulb of the mouse and develop into neurons, astrocytes and oligodendrocytes. Bona fide neural stem cells can be cultured *in vitro*, where they form clonal aggregates (neurospheres) of 30–40 cells. *In vivo*, these cells all line up along the ventricular epithelium. These rarely dividing ependymal stem cells give rise to fast dividing progenitor cells localized in the subventricular zone, and these produce neuroblasts that migrate as far as the olfactory bulb. Interestingly, the *de novo* generation of neurons seems to be a regulated process that can also occur later in life, for example, during brain injury. Frisén demonstrated this by injuring the dorsal funiculus of the spinal cord of mice, which induced a dramatic increase in the proliferation of ependymal stem cells. These experiments demonstrate that even an adult brain has the potential to generate neurons *de novo*, and therefore can repair injuries. Future research will focus on strategies for stimulating this self-repair capacity in diseases that are linked to brain lesions (e.g. Alzheimer's or Huntington's disease), as was addressed later in the meeting by

Maeve Caldwell (Cambridge, UK). Even more strikingly, adult neural stem cells are pluripotent; Frisén introduced stem cells from adult mice into both chicken and mouse embryos and showed that they localize to various tissues where they acquire the respective fate of that particular tissue. So far, however, none of the many laboratories working on neural stem cells has reported these cells to contribute to the germ line. However, this might well turn out to be due to technical obstacles.

Learning, memory and neural networks

Three different kinds of memory are known: short-, medium- and long-term. Randolf Menzel (Freie Universität Berlin, Germany) uses honey bees to examine the neural and cellular mechanisms for, and the contents of, these memory states. Menzel immobilized bees and looked at their olfactory systems after reward learning of odors. He found that multiple learning trials led to stable long-term memory, and a single learning trial to short- and medium-term memory, with an early consolidation phase in the minute range. A single neuron was found to represent the reward pathway in the bee brain. Stimulation of this neuron, paired with odor, could replace the sugar reward in a learning exercise. Using calcium-imaging techniques, Menzel showed that every odor stimulates a distinct pattern of activity in the glomeruli of the antennal lobes. In these lobes, the activities of both protein kinase A (PKA) and protein kinase C rise during learning. The role of PKA activity in the antennal lobe for the transition from medium- to long-term memory was determined by measurements during photolysis of caged cAMP. A single learning trial now leads to the establishment of long-term memory. When PKA was down-regulated by injection of an anti-sense RNA specific for the catalytic subunit, the formation of long-, but not short-term, memory was inhibited. Menzel concluded that PKA plays an important role in the transition from short- to long-term memory.

Like honey bees, zebra finches are not just cute but provide an efficient model system to study mechanisms of learning. Natalia Denisenko-Nehrbass (CNRS, Paris, France) presented an exciting study on the molecular mechanisms governing song learning. Unlike canary birds, which modify their songs throughout their lives, zebra finches learn their song once and repeat it ever after. Denisenko-Nehrbass and colleagues isolated the high vocal center (HVA), a region of the brain known to be important for song development, and identified a class 1 aldehyde dehydrogenase (zRalDH) involved in the learning of songs. In song nuclei, zRalDH synthesizes retinoic acid, a molecule that has been implicated in many developmental processes of the CNS. Inhibition of this enzyme interfered severely with song development, supporting its importance in establishing memory.

With a fast leap into the potential of computational biology, Giorgio Ascoli (Fairfax, VA) turned the attention of the audience towards the construction of virtual brains *in silico*. Ascoli's group has designed new software that generates neural structures in virtual reality (www.krasnow.gmu.edu/L-Neuron/). These models (which strikingly resemble the real neurons first drawn by Cajal) can be virtually wired together to simulate entire parts of the brain, such as the hippocampus. As the anatomical accuracy of neural network models is crucial for the computational analysis of structure–function relationships, Ascoli hopes for the creation of an anatomical brain library (www.nimh.nih.gov/neuroinformatics/index.cfm), which he considers to be as important as deciphering the human genome.

Probing neural dynamics in cortical networks, Ad Aertsen (Freiburg, Germany) focused on how the brain manages to relate perception to behavior in a meaningful manner. His group studies the problem by analysing single- and multi-electrode recording data from the mammalian neo-cortex. Aertsen defines a 'functional group' of up to 50 simultaneously active neurons as cells that display a temporal relationship and share a common fate. Such a group is not a stable anatomical assembly of neurons, but changes for each task in a highly dynamic fashion. Depending on what an animal is doing, certain combinations of neurons will be orchestrated to display a specific 'melody of activity'. When a trained monkey expects an external signal, synchronous spiking can be observed in multi-electrode recordings. But only when the signal is delivered, is a change of neural activity registered in the synchronized cells. The precision with which these signals are synchronized poses a theoretical problem: these neurons must fire 10 times more precisely than expected from the known biophysical properties of their membranes (capacitative membrane constant). Using computational models, Aertsen and his colleagues found that, indeed, 1 ms synchronous spikes can survive in a network as long as at least 50–100 synchronized cells are available to maintain the signal.

Diseases of the nervous system

The last session of the symposium was dedicated to the molecular mechanisms underlying diseases of the nervous system and their potential therapies. Ursula Bellugi (La Jolla, CA) focused on Williams syndrome (WS), a genetic disease that affects 1 in ~25 000 individuals and results in cognitive and physical abnormalities. WS patients achieve the same overall IQ score as patients with Down syndrome (DS). However, the language abilities of WS patients are sometimes even more elaborate than those of unaffected children of the same age. On the other hand, people with WS have stronger spatial defects than do DS patients (Figure 2). Interestingly, in line with their remarkable creativity, children with WS seem to be much more outgoing and friendly to strangers than are unaffected kids. Molecular genetics has revealed that a single-copy deletion, spanning ~17 genes, pseudogenes and transcription factors on chromosome 7, is responsible for the disease. Bellugi and her team are starting to link some of these genes to special aspects of the disease by identifying rare patients who only show some features of the disease and have smaller deletions. Indeed, neither mental retardation nor disease-associated facial features are seen in people with only the central five genes missing. At the end of her fascinating seminar, Bellugi described how WS patients see us, the supposedly 'normal' people: 'cold, but with a lot of love and understanding, most of them could develop the ability to express emotions, to feel music, laugh and enjoy life'.

From this promising outlook for overcoming our own shortcomings, Danilo Tagle (Bethesda, MD) turned to a description of Huntington's disease (HD). HD (affecting 1 in ~10 000 individuals) is characterized by selective neural loss (especially in the striatum and the cerebral cortex), leading to involuntary movements (chorea) and intellectual impairment. The HD gene,

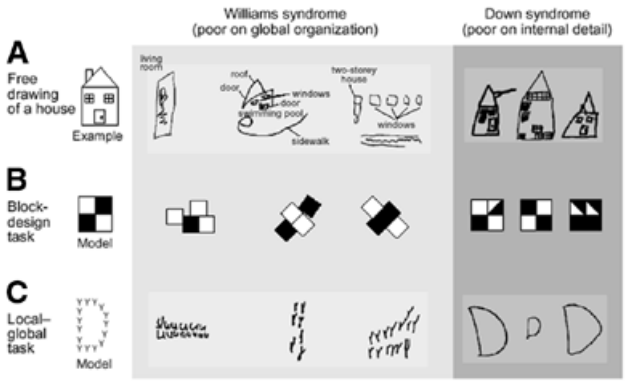

Fig. 2. Patients with Williams syndrome (WS) and Down syndrome (DS) show different spatial deficits. (**A**) The houses drawn by adolescent and adults with WS contain many features of houses but are not organized coherently. On the contrary, the drawings of houses by age-matched and full scale IQ-matched DS adults are less detailed but have the correct appearance. (**B**) Block-design task; both individuals with WS and DS fail, but in different ways: WS patients show disjointed and fragmented designs, while DS patients maintain the overall configuration but make mistakes in internal details. (**C**) Delis hierarchical processing task: subjects are asked to copy a large global figure made of smaller local forms (a 'D' made of 'Y's). Once more, the failure in both groups is different: people with WS have a tendency to reproduce the local elements spread all over the page while persons with DS tend to produce only the global forms. Figure reprinted from Bellugi *et al.* (1999), with permission from Elsevier Science and Ursula Bellugi (The Salk Institute for Biological Studies, La Jolla, CA).

huntingtin, lacks homology to any other gene, is widely expressed, and localizes in the cytosol where it interacts with several proteins. In HD patients, huntingtin displays an expansion of a CAG trinucleotide repeat coding for a stretch of glutamines in the first of its 67 exons. This repeat length is highly polymorphic in the healthy population, ranging from 5–35 repeats; in HD patients, the extended range lies between 26 and 121 repeats. The longer the repeat, the earlier the disease manifests itself. However, no function has been assigned to the protein yet. Mouse knock-out experiments showed that it is required for embryonal development. Therefore, Tagle concentrated his efforts on making three knock-in mice with repeats of 16 (wild type), 48 and 89 CAGs. Elongation of the CAGs indeed caused a progressive neurological phenotype that included motor and behavioral abnormalities and a restricted pattern of neural cell death. However, it remains to be fully proven that only the CAGs and not any other part of the protein are fully responsible for the disease.

Picking up on the Huntington problem, Maeve Caldwell ventured towards possible therapies for neurodegenerative diseases. Transplantation of neural tissue (from aborted human embryos) has been successfully applied in the treatment of Parkinson's disease to replenish dopaminergic neurons in the striatum of patients. However, ethical and practical problems led Caldwell to study neural stem cells as a potential alternative. She developed a cocktail of growth factors that yields, for each stem cell culture, 65% of neurons, a substantial improvement over previous attempts and a promising step towards future therapies for neurodegenerative diseases.

Degeneration of neurons can be painful in many ways. In the final lecture, Andy Randall (SmithKline Beecham, Harlow, UK) vividly showed how a modern industrial approach to remedy this problem combines large-scale screening with targeted knock-out technology and a good team of smiling electrophysiologists. Capsaicin, from red hot chili peppers, elicits a sensation of burning pain. It is used in some anti-arthritis creams to desensitize pain receptors and thereby relieve the patients. Randall described how the capsaicin (a vanilloid) receptor (VR1) and a range of related ion channels were cloned. He found that, upon capsaicin binding, the temperature- and pH-sensitivity of the channel increase. Randall and colleagues then screened a library of over 400 000 different chemicals and obtained a specific inhibitor of the channel. This compound is now being investigated for its potential use in pain treatment. To investigate the *in vivo* function of VR1, the team created a knock-out mouse. There was no significant difference between mice lacking the VR1 receptor and the wild type when tested for heat response, indicating that other receptors also play a role in detecting heat. However, the knock-out animals show a complete loss of inflammation-induced thermal hyperalgesia, which raises the hope that a specific VR1 antagonist will prove useful in treating inflammatory pain.

Conclusions

This tour through neurobiology featured some interesting novelties. For the first time, an international symposium at EMBL was entirely organized by students. Not only top quality speakers, but also a large group of interested students from as far as Japan, were attracted by this new educational concept of exposing students to experts from different disciplines within an exciting area of modern biology. The symposium was greatly appreciated, not only by the students. The speakers were enthusiastic about the meeting, and perceived it as an opportunity both to meet colleagues and to exchange ideas on a much broader selection of topics in neurobiology than is normally allowed by more specialized, ad hoc meetings. Furthermore, the speakers managed to combine broad introductions with the presentation of novel, unpublished data during both their talks and the following discussions. Although designated a student conference, the symposium also attracted a number of more senior scientists. They clearly enjoyed what originally must have motivated them to become scientists—the fascination for biology—something that, much too often, seems to be lost in the day-to-day business of the life sciences.

In his final address, Fotis Kafatos, Director General of EMBL, congratulated the organizers, pointed to the uniqueness of this meeting and promised to support future meetings of this type in coming years. Indeed the second European PhD student symposium will be held at EMBL on November 9 and 10, 2001 around the theme of evolution (www.EMBL-Heidelberg.DE/Conferences/evosymp/).

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The authors and organizers of the symposium (from left to right), who all contributed equally are: Michi Hannus*, Olaf Selchow, Freddy Frischknecht*, Valentina Greco, Stefania Castagnetti* (standing), Thomas Schell*, Giuseppe Testa*, Tor-Eric Rusten and Berthold Rutz*; not in the photograph: Katja Zanier.*

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