**The Giovanni Armenise-Harvard Foundation**
**Thirteenth Annual Symposium**
**June 5-8, 2010**
**Grand Hotel Villa Tuscolana, Frascati, Italy**
**Neuronal Function and Neurologic Disorders**

## About the Symposium

In the end, everyone agreed it had been a great trip, not only to Italy and to the sweet, green hills that surround Rome, but the 13th Annual Symposium of the Giovanni Armenise Harvard Foundation was an exhilarating trip through the most complex object ever studied by science: the brain. From its development to its basic functions and disorders, the nervous system was at center stage during the three-day meeting, this year devoted to Neuronal Function and Neurologic Disorders.

Frascati, where the symposium took place, is one of the main towns in the Castelli ("castles") area close to Rome and is renowned for its beautiful hills and lakes, as well as for its food and wine. Chosen since the 16$^{th}$ century by the Roman aristocracy as an ideal location to build their beautiful villas, Frascati today is also an important center for Italian science. It is home to several research centers such as the National Institute for Nuclear Physics, the European Space Agency and the National Agency for Alternative Energy, and it was a perfect location for the symposium, which took place at the Grand Hotel Villa Tuscolana between June 5 and 8, 2010.

Twenty-three researchers, most leaders of their groups in Boston, Padua, Rome, Milan and Zurich, presented their latest research, in many cases freely discussing preliminary and unpublished data with their colleagues. In introducing the talks, Michael Greenberg, head of the Neurobiology Department at Harvard Medical School, stressed that one of the main goals of the meeting was to foster collaboration between Armenise researchers based in the US and those working in Italy or elsewhere. The exchange and lively debates following each talk were clear proof that this goal had been reached.

The symposium covered most of the hottest topics in neuroscience, one of the most challenging and, at the same time, exciting fields in contemporary biology; a field with much unchartered territory still to explore and the promise of a tremendous impact on our understanding of how we function. As they

rolled out, the presentations described an ideal path from the early moments of brain development to the diseases that typically affect it with age. The first group of talks concentrated on the rules governing synapse formation and refining. Others showed how sensory systems (sight and smell in particular) can be used as a model to study the fundamental rules that allow neurons to exchange signals and translate external stimuli into decisions and behaviors: in other words, how the brain does its fundamental job on a daily basis. The study of both the visual and olfactory systems has a well-established tradition at Harvard, a tradition that has resulted in two Nobel prizes (to Hubel and Wiesel for work on the visual system, to Axel and Buck for the study of smell) whose legacy was evident during the workshop.

The ultimate goal that drives brain science, though, is to prevent and treat disease, be it inherited disorders such as Rett Syndrome, or age-related conditions like Alzheimer's disease, which is rapidly becoming one of the western world's big health issues. A third group of presentations dealt with ongoing work to understand the molecular mechanics behind these conditions, and the quest for new drug targets.

The research presented in Frascati also highlighted the richness, power and complexity of experimental methods being used in neuroscience. Genes can be manipulated to probe their functions; imaging techniques are becoming more and more versatile, ranging from the use of fluorescent protein markers to advanced microscopy; bioinformatics allow researchers to handle massive amount of data; at the same time, well established, old-fashioned techniques such as behavioral studies on animal models remain essential.

A rich poster session complemented the event, giving many younger researchers a chance to present their work and contributions to the field.

**Molecular Aspects of Mental Retardation: Insights from the Fragile X Syndrome**

Claudia Bagni
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The Fragile X Syndrome (FXS) is the most common genetic cause of inherited mental retardation, characterized by visible clinical symptoms in addition to mental retardation, and caused by the absence of a gene identified in 1991 called FMR1. The main feature of the disease is a very severe developmental delay with IQ dropping down to 40. Yet when affected kids are very young, they show almost no sign of the disease. After the age of four, though, the age when synaptogenesis is supposed to be completed, they begin to show both physical and cognitive features of the disease.

Claudia Bagni's group has devoted the last ten years to the study of the Fragile X Syndrome, believing that it can provide useful insight into other forms of mental retardation. "What struck me ten years ago when I started to work on Fragile X" she says "was the spine dysmorphogenesis observed in post-mortem brains from human patients as well as in the mouse model to study the Syndrome. In addition, it was becoming clear that a specific mRNA regulation was occurring at synapses and could be involved in the spine dismorphogenesis. Spines, the short branches of dendrites that allow for synapse formation, appear abnormally long and thin in Fragile X patients, suggesting that incomplete synaptogenesis may be a key factor behind their mental retardation.

Bagni's group has shown that at the base of the spine, a cluster of ribosomes are actively engaged in regulating translation of dendritically localized mRNAs. This, together with other findings from other laboratories at the beginning of the '80s had led to the idea, now widely accepted, that in highly polarized cells like neurons, gene expression is controlled by localizing subsets of neuronal RNA which are then translated only when required (upon synaptic activity for example). Ribosomes found in dendrites and at synapses, translate mRNAs encoding for different proteins-all involved in forming and maintaining an autonomous synapse. But how are some mRNAs able to travel along dendrites to reach the point where they need to be translated to form a working synapse? How is this fine-tuned regulation accomplished?

FMRP (the protein encoded by the FMR1 gene and lacking in Fragile X patients) normally binds RNA molecules, and has different functions in regulating the RNA metabolism in neurons. Bagni's focus is on understanding what exactly goes wrong when FMRP is absent or mutated in the molecular chain of events that should lead to mature and working synapses.

A key role is surely played by the small non-coding BC1 RNA, which acts together with FMRP to repress translation of some FMRP target mRNAs during synaptogenesis. Since those targets encode for key synaptic proteins, Bagni is proposing that this dysregulation, occurring specifically during spine formation, could contribute to the spine dysmorphogenesis observed in Fragile X patients.

Bagni is currently continuing work on a very interesting molecule that plays a crucial part of this mechanism of regulation, called CYFIP1, which seems to be fundamental in spine morphogenesis and RNA translational control and relies on FMRP to do its job. Interestingly, deletions and duplications of this molecule have been reported for other forms of mental retardation, like Autistic Spectrum Disorder, and for schizophrenia, suggesting that spine dismorphogenesis could be a common feature shared by many different brain diseases.

**Modulation of Individual Synapses in the Mammalian Brain**

Bernardo Sabatini
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Though they do not often get to show their talent in the laboratories where they are usually kept by scientists, mice are great explorers. In the wild, they show a remarkable ability to explore and memorize new environments up to 1 square kilometer wide, locating and remembering food sources or possible threats. To say that such abilities depend on what happens in the mouse brain and at the level of synapses in particular, may sound like common sense. Yet, the mechanisms that regulate synapses allowing for essential cognitive functions such as arousal, vigilance and learning, remain mostly unclear.

Bernardo Sabatini's lab at Harvard Medical School is studying the mouse brain, trying to identify the "neural correlates" of vigilance and learning: the molecular pathways that allow the animal to analyze and store spatial information. Sabatini's main goal is to understand synaptic plasticity—the changes in the strength of nerve signal transmission across synapses that are crucial to learning and memory. In particular, Sabatini and his group study the modulation role played by neurotransmitters such as dopamine and acetylcholine in the hippocampus and striatum, brain areas which are critical for arousal, vigilance, locomotion, and reward-based learning. Though the main focus of the group is on basic research, their work has an obvious clinical relevance, as perturbations of these systems contribute to human neuropsychiatric diseases such as Parkinson's disease and drug addiction.

Since their work requires visualizing events at the level of the individual synapse, Sabatini and his group had to develop novel imaging technologies for their work. In particular, a custom-designed 2-photon scanning laser microscopy system that allows them to directly stimulate individual postsynaptic terminals while monitoring evoked electrical and biochemical signals. At Harvard, the group has pioneered the development of optical techniques for observing the interactions of dendritic spines during synaptic events, uncovering many of the mechanisms that enable individual synapses to control the consequences of their stimulation.

In a recent paper in *Nature Neuroscience*, Sabatini and his colleagues used such techniques to study the modulation of glutamatergic synaptic transmission in neurons found in the mouse striatum. The striatum is the first processing stage in the basal ganglia, a collection of nuclei whose function is critical for the generation of coordinated, purposeful movements. Regulation of striatal activity by dopamine is important for a variety of psychomotor functions, including habit learning and serial movement. Striatal D2-type dopamine receptors (D2Rs) regulate striatal synaptic plasticity and have been implicated in the pathophysiology of neuropsychiatric disorders, including Parkinson's disease and schizophrenia. Yet the mechanisms underlying dopaminergic modulation of glutamatergic synapses had not been fully explained. The researchers found that D2R activation reduces corticostriatal glutamate release and attenuates both synaptic- and action potential-evoked calcium influx into dendritic spines by approximately 50%.

At the symposium, Sabatini also showed how these techniques can be used to investigate the synaptic action of acetylcholine, particularly its action on NMDA and AMPA type glutamate receptors, and presented some new and unpublished data on the possible role of this neuromodulator in vigilance and memory formation.

**Mechanisms of Experience-Dependent Retinogeniculate Plasticity: A Role for Stargazin in Synapse Remodeling**

Chinfei Chen
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Understanding what happens during the first crucial phases of brain development and how brain circuits are refined by experience is a fundamental task for neuroscientists. We know genes provide a blueprint for brain maturation, but at some point plasticity must be driven by sensory stimuli from the outside world, in order to fine-tune the system.

Chinfei Chen's laboratory at Boston Children's Hospital is studying this process using as a model the mouse retinogeniculate synapse, the connection between retinal ganglion cells and relay neurons in the thalamus. This experimental model system is very useful for developmental studies because distinct phases of circuit refinement have been well delineated. And that seems to be a good model for studying other areas as well.

The first phase begins right at eye opening although, strangely, it has nothing to do with it: keeping the animal in the dark before eye opening does not seem to alter the synaptic function. This phase is dependent on spontaneous activity and characterized by elimination and strengthening of most of the retinal inputs. A second phase occurs about a week after eye-opening. During this period, plasticity is robust and drives further remodeling of this synapse in response to visual experience. This time, the presence of visual stimuli is crucial: vision is needed to maintain the refined neuronal circuit. Dark-rearing the animal at this stage, around 20 days after birth destabilizes the circuit and results in dramatic changes in innervation.

By recording from fresh-cut brain slices, Chen is trying to elucidate the mechanisms that drive plasticity in these two different phases. In particular, she has been focusing on the "stargazer" mouse, a mouse model engineered to lack stargazin. This protein, identified in the late 1990s, is a transmembrane-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor regulatory protein (TARP), and has been shown to regulate traffic at AMPA receptors and modulate their kinetic properties at other synapses.

Chen's studies reveal that the initial phases of synapse formation, elimination and strengthening are not significantly disrupted in stargazer mice. However, during the later experience-dependent (after 15-16 days) phase of synapse development, the neuronal circuit becomes abnormal in the mutant mice, as afferent innervation of relay neurons increases, and retinal inputs become weaker.

Stargazin expression patterns in the retina and in the visual thalamus demonstrate a post-synaptic role for this protein. Furthermore, stargazin expression is regulated by visual experience, the driving force of the later phase of retinogeniculate synapse remodeling. Altogether these results suggest an important role for stargazin in the experience-dependent phase of retinogeniculate development.

**Pruning CNS Synapses: An Unexpected Role for Glia and the Classical Complement Cascade**

Beth Stevens
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Growing up is about making choices: what to keep, and what to get rid of, what is really important and what is not. The same is true for the brain. During the first phase of the nervous system's development, there is a period of furious growth, when developing neurons form synapses in quite a chaotic manner. At some point though, synapses that do not perform well enough or that at are not really necessary, are suppressed, or pruned, like dead or excess branches in a tree.

Neuroscientists know this is a crucial phase of brain development, yet they do not know exactly how it is regulated. In other words, how choices are made. Understanding how synapses are normally eliminated during development could also help them understand to prevent synapse loss and cell death in many different neurodegenerative diseases.

Beth Stevens' lab is trying to identify the molecules that "tag" unwanted synapses, unleashing the immune system against them so that they are suppressed. Recently they have discovered a key role in this process is played by the classical complement cascade, a part of the innate immune system made of a group of proteins that are activated in sequence when they encounter an antigen. The typical initiator of this cascade, the one that wakes up and triggers the domino when it encounters an antigen is the C1q protein, which, it turns out, can be found at high levels in developing neurons.

Its role is confirmed by the fact that mice lacking this protein have defects in synapse refinement and elimination. In other words, they have too many synapses, resulting in abnormal brain functioning. So, there is strong evidence that, in the developing brain, the cascade molecules "tag" inappropriate synapses, those that haven't passed the test to become part of the mature brain. But who exactly does the dirty job of suppressing them?

Stevens' group is now studying the role microglia could play in the picture. These cells, poorly understood even by glia specialists, are the resident phagocyte cells of the Central Nervous system. In other words, they spend their time eating unwanted cells, including dead neurons after strokes or brain lesions. In addition, they have receptors for complement. Putting all the pieces together, Stevens is working on the hypothesis that microglia do also take care of unwanted synapses in the immature brain. In other words, there seem to be "something" in the developing brain that unleashes microglia against unwanted synapses, tagged by molecules of the complement cascade, while that "something" seems to become silent in the adult brain.

Interestingly, studying an animal model of glaucoma, Stevens and colleagues have found that C1q becomes abnormally upregulated in retinal ganglion cells, suggesting that synapse elimination, mediated by molecules of the complement cascade occurs as an early event in the disease

**Regulation of Neuronal Connectivity by Ubiquitin Signaling**

Azad Bonni
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First identified in 1975, ubiquitin, a small protein found in all eukaryote organisms, rose to fame in the early 1980s after Arron Ciecanhover, Avram Hershko and Irwin Rose (who would later get the 2004 Nobel Prize in Chemistry for their work) discovered its essential function as the cell's "garbage man." Ubiquitin attaches to proteins that must be eliminated from the cell's metabolism, a work done by proteasomes by breaking the bonds that keep proteins together.

Yet ubiquitin performs a number of other essential functions in different cell populations. Azad Bonni and his group are studying the role of the ubiquitin family (ubiquitin and the molecules that bind to it contributing to its functions) in the brain. Using the cerebellar cortex as a model, they have shown that the ubiquitin system regulates neuron morphogenesis and connectivity during brain development.

As early as 2004, the Bonni Lab at Harvard Medical School has identified a key function for the ubiquitin ligase Cdh1-anaphase promoting complex (Cdh1-APC) in controlling axon growth and patterning in the rat brain. In the following years, their studies have revealed that, by regulating gene expression, Cdh1-APC may play a pivotal role in the control of axon growth in the nervous system. Cdh1 knockdown by RNAi specifically promotes the growth of axons, in cerebellar slice and in vivo RNAi experiments in the postnatal cerebellum, Cdh1 was found to control the layer-specific growth of granule neuron axons and parallel fiber patterning.

Bonni believes that understanding the role of Cdh1-APC will enhance our understanding of axonal morphogenesis in the developing brain, and even provide the foundation for the development of drugs that might ultimately be used to stimulate axonal regeneration following injury and disease.

Recently the lab has been focusing on the major mitotic ubiquitin ligase Cdc20-APC. This protein complex is known to drive mitosis in cells: Cdc20 binds to APC to activate mitosis. Yet Bonni's group has discovered that "the dog can learn new tricks": in a paper published last year in *Cell*, they have shown how Cdc20 also has essential functions in neurons after cellular division, driving the elaboration and arborization of dendrites in postmitotic neurons. A further confirmation comes in rat pups missing Cdc20 in the cerebellar cortex, which have impaired formation of dendrites.

Remarkably, Cdc20 in neurons appears to be concentrated at the centrosome, the organelle that regulates cell life cycle, where it operates to promote dendrite morphogenesis, suggesting a novel function for the centrosome in neuronal development.

This new role of Cdc20-APC in the formation and elaboration of dendrites in the brain has important implications for the study of many biological processes related to neuronal connectivity and plasticity.

**Botulinum Neurotoxins Reveal Novel Features of the Pre-Synapse**

Cesare Montecucco
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As poisons go, the toxin produced by the bacterium clostridium botulinum is close to perfection. It is fast and terribly effective, causing a muscular paralysis that, at its worst, can result in death from respiratory failure if the toxin is assumed even in minute quantities. At the same time, when injected in low dosages, its action is very specific, tightly localized, and surprisingly long lasting. The toxin does not spread around, but remains confined around the point of injection, its effects lasting for up to six months. This remarkable feature allows the use of some particular subtype of the Botulinum toxin in cosmetic surgery, to induce a local paralysis that can hide the effects of time and suppress wrinkles.

Cesare Montecucco and his group are working to reveal the strategy used by the Botulin neurotoxin to disrupt nerve function, trying to identify the tricks that make it so effective, specific and long lasting. To complicate things further, there are seven known types of these neurotoxins in nature, and Montecucco is also trying to explain why they have slightly different effects.

Once inside the body, the toxin acts on a very specific site: the neuromuscular junction, the point where a motor neuron connects to the muscular fiber. There, it blocks the transmission of the nerve impulse to the muscle. Its strategy for entering neurons involves four steps: first, it binds to nerve terminals; second, it enters nerve cells through synaptic vesicles (the “bubbles” where neurotransmitters are stored); third, it uses the vesicle acidity to trigger its own translocation into the cell body. Fourth, once inside the cell's body, the toxin cleaves one of the three SNARE proteins, a family of proteins that mediate the release of the neurotransmitters that command muscle movements.

It has been suggested by other researchers that, to do their job, the SNARE proteins need to group together and form a complex of two chained proteins that somehow “zip” the vesicle and the membrane close to each other, preparing the vesicle's fusion. The key of the toxin's action, then, is in the way it prevents SNARE proteins from forming this complex and doing their job. Montecucco's hypothesis is that the SNARE complex is not enough for the protein to function properly. SNAREs actually have to form a supercomplex, made of 8 to 12 SNARE complexes arranged in a rosette around the vesicle.

Different types of Botulin neurotoxin can cleave different parts of the SNARE protein, so preventing the formation of the complex itself or only of the supercomplex. In particular the A type, the one used in clinic, cleaves only a very small terminal part, so preventing the formation of a stable rosette, while the complex itself remains functional. “A bit like in politics” jokes Montecucco, “when one member who does not play the game is enough to prevent the coalition from working.”

**The Transcriptional Landscape of Dopaminergic Neurons**

Stefano Gustincich
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Back in the 1990s, when thousands of scientists set to work on the Human Genome project, many of them expected it would provide the answers to all of their questions, or at least to those more crucial for medical science: what makes some people more or less prone to one disease or another; why do not all medications work the same way in all patients; why do some of us age better than others.

Alas, the opposite was to happen. Its invaluable contributions to medical science notwithstanding, the Human Genome Projects has brought along more questions than answers, revealing layers and layers of complexity beyond the "simple" sequence of genes. In particular, the way genes are transcribed (that is, translated from DNA into RNA which can then serve as a blueprint for proteins) has proved to be incredibly complex and, from a certain point of view, more crucial than genes themselves. Even parts of the genome that were once considered "junk" because they do not code for proteins, are actually transcribed, and play a big part in regulating gene transcription, for example turning genes on and off when necessary. Exit genome, enter transcriptome, now the new code word.

Things get even more complex in the brain, which is made of very heterogeneous neuronal populations. Understanding how genes are transcribed in different kind of neurons may be the key to explain how the brain performs different functions, and how such functions are lost in neurodegenerative diseases.

Such a complex job requires entirely novel technologies. Stefano Gustincich and his group are trying to develop them, starting from a particular set of neurons: dopaminergic neurons, a varied group of cells involved in many different brain functions. In particular, a subclass of dopaminergic neurons called SN neurons are involved in regulating voluntary movements and postural reflexes, while another, VTA cells, play a fundamental role in reward and attention. When they stop working well, they can cause several neurodegenerative and psychiatric disorders, in particular Parkinson's disease (PD).

Gustincich is working to describe what he calls the "transcriptional landscape" of dopaminergic neurons, combining transgenic labeling, Laser Capture Microdissection and gene expression profiling, to understand what parts of the genome are actually transcribed in these cells, and what do they correspond to.

Such a complex job often requires entirely new technologies. Gustincich and his group have used in particular three platforms: the SISSA-RIKEN cDNA microarrays, exon arrays from Affymetrix and nanoCAGE (Cap Analysis Gene Expression) a technique they described in a 2010 article in Nature Methods, that allows to start from short RNA sequences, even degraded ones, that correspond to the initial portion of transcripts and map them to the genome (identifying the areas of the DNA sequence they come from). Working on the mouse genome, using transgenic mice to identify dopaminergic neurons, the researchers have identified more than 17 million RNA "tags" from 2000 dopaminergic neurons. Their ongoing work implies trying to understand the role played by the many different transcription products they have identified, for example understanding how genes form networks where the expression of one gene control the others; the role of antisense RNA, single-stranded RNA sequences, complementary to messenger RNA, whose presence in the transcriptome turns out to be unexpectedly high, or the function of repetitive elements, sequences that are present in thousands copies in the genome and represent retrotransposons.

**Impact of ADAM10 in Synaptic Function and Disease**

Monica DiLuca
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One crucial moment in the chain of events leading to Alzheimer's disease is the cleavage of the Amyloid precursor protein (APP). When this protein, whose functions have not yet been totally understood is chopped to pieces, some of these pieces can end up aggregating and forming the plaques which are the most characteristic feature of the disease.

The cleaving work is done by a family of enzymes called alpha secretases, members of the ADAM ('a disintegrin and metalloprotease domain') family, which are expressed on the surfaces of cells and anchored in the cell membrane. One member of the family in particular, ADAM10 is the most credible candidate for the cleavage of APP. Monica di Luca's group is trying to clarify the physiological role of this enzyme, and to understand what can cause its interaction with APP to become pathologic.

ADAM10 is at home in the synapse, where it is part of the excitatory post synaptic environment. Here it binds to the synapse associated protein 97 (SAP 97). Its synaptic localization and the capability to shed multiple synaptic substrates, including APP and N-Cadherin, suggests that ADAM10 has a central role in synaptic formation and plasticity. Yet the protein is also found in the adult synapse.

Monica di Luca's group is studying the interaction between ADAM10 and SAP97 using hippocampal and cortical cultures, as well as the effects of altering this interaction on APP metabolism.

Di Luca's group has showed that activation of ADAM10 and its localization at synaptic membranes control the structure and function of glutamatergic synapses. They used ADAM10 inhibitory cell-permeable peptides to interfere (both in vitro and in vivo in a mouse model) with ADAM10 synaptic localization and activity, and managed to decrease the ability of ADAM10 to mediate processing of its synaptic substrates, in particular its interaction with SAP 97.

This disrupting of the normal synaptic activity of ADAM10 seems to be the cause for its pathogenic cleavage of APP. Working on a mouse model, the researchers found that interfering with the ADAM10/SAP97 complex for two weeks by means of cell-permeable peptides is sufficient to derail APP metabolism, promoting amyloidogenesis and reproducing the initial phases of sporadic Alzheimer's disease. After two weeks of treatment, Di Luca and colleagues detected progressive Alzheimer's-like neuropathology in the animals, with an increase of Amyloid-β aggregates production and of Tau hyperphosphorylation and a selective alteration of NMDA receptors in mouse brain. Behavioral and electrophysiological deficits were also induced by peptide treatment, thus proving this strategy useful to model early stages of the disease in mice.

The interaction between ADAM10 and SAP97, then, may prove a key to understanding the chain of events that leads to Alzheimer's disease, and may in the future become a target for therapeutic strategies.

**From Filopodia to Synapses: The Cell Biology of Neuronal Wiring**

Elisabetta Menna
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One of the great questions in neuroscience is how neurons communicate with each other and form synapses, both during development and during plasticity in the adult brain. The first step in this process is the formation of filopodia both on axons and on dendrites. Axonal filopodia are characterized by the presence of synaptic vesicles (from which neurotransmitters are released) before synapses are formed. On the other hand, dendritic filopodia are the precursors of spines. Taken together, they are the first hint of what will one day be a complete synapse.

Michela Matteoli and her group, Elisabetta Menna in particular, are working out the molecular machinery that drive the formation of filopodia, focusing in particular on a family of proteins whose function is to bind actin, their main component.

The starring role is played by MAPK, a molecule that regulates the dynamics of synaptic vesicles, and also the formation and elongation of filopodia, and by EPS8, a protein that is phosphorylated by MAPK in the developing brain. Matteoli's group has found that EPS8 works as an actin capping protein in neurons. It normally binds to actin filaments, but its removal, consequent to the phosphorylation by MAPK, facilitates the elongation of the filament thus resulting in filopodia formation.

But how does EPS8 regulate the formation of filopodia? “To answer, we started looking at the distribution of this protein in neural cells” says Matteoli, “and found it is localized in axonal and dendritic filopodia, at their very tip”.

Notably, when they grew cell cultures from EPS8 deficient mice, Menna and Matteoli found that EPS8 deficient neurons had an excess number of filopodia. The counterproof came from putting back EPS8 in the knockout mice. The result, this time, was neurons deprived of filopodia and endowed instead with lamellipodia, flat membrane expansions which form in the presence of high concentrations of actin capping proteins.

The next step was to understand which signal regulates the binding and, more important, the unbinding of EPS8 from actin filaments. Matteoli's suspicions concentrated on BDNF, since exposure to this growth factor increases the formation of filopodia in neurons. Tests in hippocampal cell cultures confirmed that BDNF increases the amount of the soluble portion of EPS8, suggesting that the latter is detaching from actin filaments and “uncapping” them. The filament is then free to elongate and form a filopodium. NBDNF treatment acts through MAPK-dependent phosphorylation of Eps8 residues S624 and T628. Accordingly, an Eps8 mutant, impaired in the MAPK target sites (S624A/T628A), is not detached by actin filaments upon BDNF treatment and inhibits BDNF-induced filopodia. For the first time the complete signaling cascade that links extracellular factors (i.e. BDNF) with the structural modifications of the neuron, through the inactivation of a key regulator of actin dynamics, is therefore described.

At this point the big question is: does an excessive production of filopodia result in a more interconnected brain, with more synapses? And, is it really such a bad thing? Both cell cultures and knockout mice confirmed that removal of EPS8 results in an apparently normal brain but with more synapses and spines. “But more is not always better”, says Matteoli. Behavioral studies show indeed that EPS8 knockout mice are not able to learn like other mice. They take more time to learn to recognize novel objects, and are impaired in exploring environments, possibly as a consequence of the excess of synapses and spines. Given the evidence that excessive synapse formation is one typical feature of many forms of mental retardation, Matteoli's group keeps working on EPS8, whose disruption might prove one of the key factors leading to such diseases.

**New Developments in Prion Biology**

Adriano Aguzzi
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About 15 years have passed since an unexpected epidemic of the so-called “mad cow” disease sent panic throughout the UK and Europe. But prions, the infectious agent responsible for that disease (more properly called transmissible spongiform encephalopathies) still puzzle biologists.

“When we think about infectious diseases, we usually think about bacteria and viruses,” says Adriano Aguzzi. Living organisms, well, more or less in the case of viruses, propagate by replicating nucleic acids. To understand how prions can fit in the picture, Aguzzi suggests a thought experiment. Think of a planet whose inhabitants are bottles, filled with a supersaturated solution. At some point a crystal forms, which propagates inside the bottle, and might even drop and propagate from one bottle to another. This is more or less what prions do causing a morphological alteration in the brain that propagates without any need to affect DNA or RNA. The prion, an abnormally folded and aggregated protein, propagates itself by imposing its conformation onto cellular prion protein ($PrP^C$) of the host.

Prions have been at the center of Aguzzi's work for years, and are gaining more and more attention in life sciences because it now appears that many diseases, including Alzheimer’s disease, may be explained as prion-like diseases: the result of morphological alterations in essential proteins. “But I prefer to call them prionoids”, says the Swiss researcher, “the word prion should be reserved for something that spread with measurable rate and causes human to human transmission.”

Now things are getting even more interesting because biologists are discovering molecules that behave like prions, but do not bring any damage. On the contrary, they can confer evolutionary advantages. Molecules stored in the secretory granules of mammals, for example, can behave like prions, forming aggregates that contribute to purify secretions, and these discoveries are causing a true paradigm shift in biology.

For Aguzzi, though, the key question of his 15 year obsession is: how do prions manage to get to the brain? And what triggers their toxicity? We know that $PrP^C$ is necessary for prion replication and for neurodegeneration, yet the causes of neuronal injury and death are still poorly understood. Prion toxicity may arise from the interference with the normal function of $PrP^C$, and therefore understanding the physiological role of $PrP^C$ may be a first step to clarify the mechanism underlying prion diseases. Studying Prp-deficient mice, Aguzzi's team found they lack myelin, the coating of nerve fibers. So it seems Prp is needed to produce it.

Trying to go further and to replicate what happens in vivo, Aguzzi and colleagues prepared cerebellum slices, exposed them to prions for a couple of hours, washed them and then kept them under observation for a long time, observing an exponential increase in prion replication over time. Some of the slices were kept for six months. “Now we have a model that allows us to study actual degeneration” says Aguzzi, “in particular we will be able to test compounds that do not block prion replication but uncouple it from downstream events, and so can help us understand the whole chain of events.”

One of the group's latest interests is the role of microglia in prion diseases. “Some people think microglia are the bad guys and release all kind of neurotoxins; others think they are simply trying to do the best possible job to remove aggregates.” Aguzzi made a transgenic mouse that expresses suicide genes in the microglia, obtaining slices with almost no microglia. Then they treated them with prions, and the result was an enormous accumulation of prions, suggesting that microglia are the good guys after all, trying their best to stop prion aggregates from invading the brain.

**Dysregulation of Cellular $Ca^{2+}$ Handling in Cell Models of Familial Alzheimer Disease**

Tullio Pozzan
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Calcium is a fundamental keyword in the cell's vocabulary. Many fundamental processes, in particular in the brain, use it as a signal, and disruptions in calcium circulation can make essential living functions go awry and cause diseases.

Tullio Pozzan is studying the role calcium signalling might play in Alzheimer disease (AD), in particular in a small percentage of Alzheimer cases that are inherited as a familiar, dominant trait.

The cause for these forms of Familiar Alzheimer Disease (FAD) lies in mutations of three genes, presenilin (PS)-1 and 2 and the Amyloid-Precursor Protein (APP). Pathological mutations in the PS1 and PS2 genes lead to the overproduction of the less soluble A-beta peptides, the more toxic APP cleavage product thought to play a major role in the pathogenesis of both AD and FAD. Many research groups over recent years have revealed strong – and controversial – effects of PS mutations on calcium signalling. According to some, this group's PS mutations lead to an increased calcium content in cellular stores and consequently to an exaggerated release upon stimulation, that might be involved in brain degeneration. Other groups have reported opposite results, and Pozzan is trying to make things clearer, by specifically and directly measuring calcium concentration in the organelles of model cells bearing ps2 and ps 1 mutants.

To begin with, his group has demonstrated that the expression of PS2 - and, to a lower extent PS1, causes a reduction of calcium within the ER and the Golgi apparatus. At this point Pozzan also thought it was worth having a look at his "beloved organelle", as he calls it: the mitochondria, the cell's resident power plant, which Pozzan has spent a good part of his career studying. Mitochondria are capable of efficiently take up Ca2+ released by other organelles, the endoplasmic reticulum in particular, and modification in Ca2+ handling by mitochondria is known to be a key step in triggering the death of cells, neurons in particular. It had also been reported that PS 1 and PS 2 are enriched in the regions of contact between mitochondria and the endoplasmic reticulum. Knowing how this organelle handles calcium, Pozzan expected that reduction of calcium in the endoplasmic reticulum would result in a much greater reduction inside mitochondria, as it is usually the case.

He was initially puzzled to observe that this was not happening. Then he and his group went on to discover that FAD linked PS2 mutants favor the physical interactions between ER and mitochondria, an effect that is less pronounced in wild type PS2 and is not shared by FAD linked PS1; as a consequence, the expression of FAD PS2, despite causing a partial depletion of calcium in the endoplasmic reticulum, favors calcium transfer between the reticulum and mitochondria, somehow rebalancing calcium in the latter and possibly even causing a deleterious overload of calcium in the mitochondria. Calcium may then play an important part in Alzheimer's Disease, although one that will need further studies.

**System Analysis of Endocytosis by Functional Genomics and Quantitative Multi-Parametric Image Analysis**

Marino Zerial
*Max Planck Institute of Molecular Cell Biology and Genetics MPI-CBG, Dresden,Germany*

Like most modern cities, cells have big traffic problems. Nutrients must come in, signaling molecules must communicate their instruction to the nucleus before being destroyed; incoming pathogens must be destroyed. Most of this work is managed by endosomes, organelles that form inside cells, encapsulate compounds and bring them towards the center or at the periphery where they are needed. Such organelles are fundamental to regulate cell life and to differentiate cell types so that they can perform their functions. Endosomes are typically produced in the cell periphery, where they undergo fusion; they then take their cargo, concentrate it, move to the center of the cell, are converted, degrade their cargo. This motility inside the cell is very important, and is often lost in diseases. But what is the molecular machinery behind it?

Marino Zerial is studying which genes regulate the number and shape of organelles, and how. He defines his group's work as a kind of reverse engineering, like the one you would have to use if you were to understand how a TV set works simply by opening it and looking at its components. First, of course, you need to describe all the components, in this case all the possible types of endosomes. Then you have to look for the emerging properties of the system, things that no single component can do on its own, but that result from the interaction of different parts.

Zerial's group is focusing on Rab GTPases, a class of proteins that act as membrane organizers, literally shaping the identity of organelles. For example, the transition of cargo from an early endosome (one that gathers cargo at the periphery of the cell) to a late one that prepares for degradation towards the center, corresponds to a remodelling of the membrane, which first responds to one type of Rab protein, Rab 5, and then abruptly becomes susceptible to another, Rab 7. The change of Rab changes the trafficking properties, for example preparing degradation.

In order to achieve more predictive power on the cell's behavior, Zerial embarked on a large genomic screening, where perturbations of single genes encoding Rab proteins where correlated with observed changes in the organelles populations inside the cell. For each mutation, several different properties of endosomes were measured, including number, size, shape, quantity of cargo, localization inside the cell. The goal is to understand properties at the system level, how altering one parameter affects the others.

Some of the emerging correlations are obvious: for example, number and size of endosomes are inversely correlated which is easy to understand, given that membrane is a finite resource, so cells have to choose between fewer, larger organelles or more, smaller ones. More interestingly, the distance from the nucleus is negatively correlated with the number of endosome, but positively correlated with size. In other words, you can have either many small organelles in the periphery or a few large ones in the center. And this is an important, independent confirmation of the idea that the cell's trafficking system works by moving endosomes towards the center as they become larger and loaded with cargo.

Zerial's analysis, involving perturbations in about 160.000 genes, has only begun, and will allow ever more precise predictions on how living cells regulate their complex inner life.

**Drug Delivery through TRP Channels**

Bruce Bean
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Fighting pain is one of the oldest and most fundamental problems of pharmacology. Yet existing pain treatments were developed by trial and error, before an understanding of the circuits by which pain signals reach the brain. “Until recently, textbooks taught medical students that pain is processed by the same circuits used by normal sensory stimuli, just firing faster” Bruce Bean explains. “But we now know that pain is actually transmitted by specialized neurons devoted only to pain signals.” Bean is now working to develop more effective pain treatments that exploit this specificity, acting on the pain-generating neurons without interfering with other essential functions, like movement or perception.

Bean's work started from one of the best known chemicals that block pain: lidocaine, which works by blocking the flow of sodium from the cell, so interrupting nerve signaling. Lidocaine, though, is not very selective. In addition to fighting pain, it blocks function of all neurons, not just those responsible for pain. This causes motor paralyses, numbness and other unwanted side effects. The idea that Bean and his collaborator Clifford Woolf came up with was to get lidocaine (or, better, one of its derivatives) to act only on pain-specialized neurons.

Lidocaine exists in two chemical forms that interconvert with each other: a hydrophobic form (an protonated free base), and a charged (protonated) form. In order to understand which one is more effective in blocking sodium channels, pharmaceutical companies in the 1960s experimented with a lidocaine derivative, QX-314, which is permanently charged. It turned out it does nothing if applied outside the cell, but worked very well once inside, effectively blocking sodium channels. In other words, it needs some help to enter the cell before doing its job and blocking sodium channels from inside. In contrast, the uncharged free base form of lidocaine is active even when applied externally, because it can diffuse rapidly through the cell membrane.

One characteristic feature of pain neurons are ion channels called TRPV1. They are responsible for the feeling of painful heat, and are also stimulated by compounds like capsaicin, found in hot peppers, paprika, and other “hot” foods. TRPV1 channels are unusual in having a very large pore. Bean and his colleagues exploited this large pore as a way of selectively introducing QX-314 into pain-sensing neurons. By applying QX-314 and capsaicin in mice, they could obtain a long lasting inhibition of sodium channels in pain-sensing neurons: capsaicin opens TRPV1 channels, letting QX314 in, and the latter in turn blocks sodium channels from inside. Since QX-314 alone cannot enter cells, there is no effect on neurons that do not express TRPV1 channels. Behavioral studies in mice confirmed that the reduction in pain perception (the animal can resist higher temperatures or stronger pressure applied to its paw) does not affect attention and movement. Also, because QX-314 is trapped inside cells, its action lasts far longer than that lidocaine, whose action is short because it can diffuse out as easily as it diffuses in.

It looks like a perfect match, except for the fact that capsaicin is itself painful, an obvious problem for clinical use in humans. Something else must be used instead to open TRPV1 channels. Remarkably, it comes out that good old lidocaine can do the trick because it is itself a TRPV1 channel opener at the doses given for local anesthesia. Bean and Woolf are now experimenting with a co-application of lidocaine and QX314. The lidocaine produces a short-acting local anesthesia that blocks all types of neurons, while the QX-314 enters only into TRPV1-expressing pain-sensing neurons and produces a long-lasting, selective block of pain sensation. The result could be a new generation of local anesthetics, whose effect would not be accompanied by the usual numbness and local paralysis.

**Impaired Innate Fear Responses in TRPC5 Null Mice**

David E. Clapham
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*Department of Neurobiology, Harvard Medical School, Boston, MA*

Can there be something like a switch to turn off fear? If so, many of us would surely be happy to find it. Well, in mice at least, David Clapham and his colleagues at Boston's Children's Hospital seem to have found it, and have used it to obtain the rodent equivalent of a bungee jumper.

The switch they found is TRCP5, one of the TRP channels, a vast class of channels that bring sodium and calcium inside neuronal cells. In vitro studies indicate that this particular channel regulates the morphology of growth cones, the extensions of developing axons seeking their targets, as well as neurite extension rates. But their function in synapses is not well understood.

The TRPC5 channel is expressed almost everywhere in the brain: in the auditory cortex, in the somatosensory cortex and in the hippocampus, but most prominently in the amygdala. This brain region, part of the limbic system, is located deep within the medial temporal lobes of the brain of complex vertebrates, and has a primary role in processing and memorizing emotional reaction The lateral amygdalae receive input from the sensory systems and send impulses to the rest of the basolateral complexes and to the centromedial nuclei, involved in emotional arousal in rats and cats. The amygdala is, in essence, the gateway of fear. When a mammal encounters a fearful stimuli such as the smell of a predator, it enters the brain and produces a reaction by transiting through the amygdala.

To explore the role of TRPC5 in vivo, Clapham and colleagues generated transgenic mice in which the TRPC5 gene was knocked out. The quite astonishing result, described in a paper published last year in *Cell* by Clapham and Antonio Riccio, was a mouse that looked normal, had normal reflexes, but was...fearless, as it became apparent in detailed behavioral studies. Both innate and learned fear responses were lower than those of wild type animals. TRPC5-knockout mice went to the center of the cage more often than the others. They spent more time in the center of the cage where normal mice fear to tread. They were also more “sociable”: when a new mouse was put in the cage, the TRPC5 knockout mouse spent more time around it and had more nose-to-nose contacts than the wild type. As to baseline synaptic transmission, membrane excitability, and spike timing-dependent long-term potentiation at cortical and thalamic inputs to the amygdala (all measures of a regular electric activity in the brain) the knockout mice were largely normal.

The $TRPC5^{-/-}$ knockout is, in Clapham's words, an “anxiolytic phenotype” which means make TRCP5 may become a very good candidate as a target for new anxiolytic drugs.

**Glycomics of the Mitogenic Niches in the Brain**

Rosalind Segal
*Harvard Medical School, Boston, MA*

The development of the nervous system, from a few undifferentiated stem cells to a wide variety of different neuron populations capable of diverse functions, is one of biology's true wonders. Rosalind Segal's laboratory at Harvard is looking into it, studying how the nervous system develops from neural stem cells to neurons that function within a neural circuit. In particular, they focus on the extracellular cues, such as growth factors and morphogens that direct this complex process.

Neural stem cells are self-renewing precursors capable of giving rise to additional stem cells and to differentiated neurons and glial cells. A feature of the specialized niches where stem cells are found in the developing and mature brain is that they contain both critical protein growth factors and specialized proteoglycans. Sonic Hedgehog (Shh) is one such growth factor.

Despite taking its name from a videogame character, Sonic Hedgehog has a terribly serious role in mammals. This protein, the most studied in a family that also includes Desert Hedgehog and Indian Hedgehog, has a key role in regulating vertebrate organogenesis, such as the growth of digits on limbs and morphological organization of the brain. During development, it diffuses to form a concentration gradient and has different effects on the cells of the developing embryo depending on its concentration. In essence, it guides the formation of body parts by labelling the areas where cells of one type or another have to go and group. It then remains important in adult organisms, controlling division of stem cells.

In the developing brain, Sonic Hedgehog is essential for normal proliferation of neuron precursors. As is often the case in medical science, this is made evident when things go wrong: over activity of Shh causes cancer, while its underactivity is even more devastating, causing holoprosencephaly and cyclopia.

Segal and her team are studying the mechanisms by which Shh regulates neural stem/precursor proliferation in the cerebellar cortex, and other mitogenic niches. Using genetic approaches to disrupt the binding of Shh to proteoglycans, they have found that Shh interactions with specialized proteoglycans are needed for a proliferative response to Shh. As mutations that activate the Shh signaling pathway cause brain tumors and other cancers, Shh-proteoglycan interactions are likely to be important in oncogenesis. The group is currently investigating the ways in which inhibitors of Shh signaling might be used in treating brain tumors that arise from neural stem/precursors.

“We are beginning to identify a glycocode, understanding what makes glycands specific for binding to Shh and promoting proliferation in different cell types,” she explains. This glycocode will be of extreme importance for a better understanding of development and of cancer.

“We are beginning to identify a glycocode, understanding what makes glycands specific for binding to Shh and promoting proliferation in different cell types,” she explains. This glycocode will be of extreme importance for a better understanding of both normal development and of cancer.

**Trans-synaptic Coordination of Synaptic Development by the Conserved microRNA miR-8**

David Van Vactor
*Department of Cell Biology and Department of Genetics, Harvard Medical School, Boston, MA*

David Van Vactor likes to compare the work he and many other biologists are doing to the work of cartographers. “Over the centuries we have learnt to map the visible world with incredible precision, going from early maps to navigation technologies. Now we are finally mapping the invisible cellular networks of living organisms. In the case of neuroscience, functional genomic tools allow us to chart the surface of the brain and the nervous system down to the single cell.”

Using drosophila as a model, Van Vactor's group (also including Tudor Fulga, Carlos Loya and Cecilia Lu) is trying to map the cellular network responsible for muscular control, and to understand the regulation of synapse morphogenesis at neuromuscular junctions. In particular, the group has investigated the role of microRNA in trans-synaptic coordination between motor neurons and muscle target cells during various stages of development.

MicroRnas are short RNA sequences corresponding to what was once considered junk Dna (parts of the genome that do not code for proteins). On the contrary, they have a crucial role in regulating gene expression and gene silencing, and have become one of the hottest topics in life sciences.

Studying microRna with the “usual” techniques (mutating the corresponding gene and observing the effects on the phenotype) has often proven difficult, because related microRNAs often came to the rescue and performed the duties of the disabled family member, thus undermining the experiment.

Van Vactor's group has solved the problem by developing a novel tool, a microRNA sponge (miR-SP) which was described in an article in *Nature Methods* at the end of 2009 and developed with his collaborators Tudor Fulga and Carlo Loya. The sponge is a DNA sequence that matches key portions of particular microRNAs. Each sponge binds to, or soaks up, a specific microRNA or family of microRNAs, allowing scientists to isolate and understand its functions.

Using the miR-SP system, Van Vactor and his colleagues identified an essential role of the microRNA miR-8 in neuromuscular junction formation. Its role goes from the initial refinement of the point of contact between nerve and muscular target into a working synapses, to the later expansion of the synapse in response to the ongoing growth of target cells.

The team also made a surprising discovery—though miR-8 is present on both sides of the synapse, and its main function is to regulate presynaptic morphogenesis, it mostly plays its regulatory role post synaptically, regulating target genes on the receiving end. However, as the development of the neuromuscular Junction proceeds to maturity, MiR-8 controls presynaptic morphogenesis through inhibition of the conserved actin-regulatory protein Enabled. The role of Enabled reveals a transsynaptic mechanism by which muscle controls presynaptic development. The precise cellular mechanism however is now under intensive study in Van Vactor's lab and is still a mystery. In this later phase, miR-8 function becomes restricted in the presynaptic part, suggesting the existence of a mechanism that protects post-synaptic key target genes in mature neurons.

Profiling miR-8 mutants has already allowed the group to classify several genes downstream of miR-8 that contribute in different ways to the development of the neuro-muscular junction, but their work, like that of any cartographer, is still ongoing.

**Sensory Processing: Insights from a Simple Brain**

Rachel I. Wilson
*Harvard Medical School, Boston, MA*

Once a poor relative of research on the visual system, the study of the olfactory system has now come of age as witnessed by the Nobel Prize awarded to Richard Axel and Linda Buck in 2004.

Biology has always progressed by studying simple model systems to discover mechanisms that work in larger systems too, and the antennal lobe that serves as olfactory organ in the drosophila now looks like an excellent model to study how stimuli are processed in the brain. Rachel Wilson's lab uses in vivo whole-cell recording from the drosophila antennal lobe to study one classic problem in brain science: how real estate is allocated in the brain, i.e. how brain regions and circuits are mapped and put in correspondence with sensory organs depending on their activity, and how the brain can use plasticity to respond to changes.

The compartmental organization of the antennal lobe makes it relatively easy to map connections between neurons, and each compartment (or “glomerulus”) corresponds intuitively to a discrete processing channel in the network. Moreover, this circuit contains relatively small numbers of neurons, and genetic tools allow us to label identified cells for recording.

The antennal lobe receives direct input from olfactory receptor neurons (ORNs). Every ORN expresses a single odor receptor gene, and all the ORNs that express the same gene project their axons to the same compartment in the antennal lobe, or glomerulum. There they make synapses with projection neurons (PNs), and each PN extends a dendrite into a single PN. Individual odors typically bind to multiple receptors, so odors are encoded by a combination of glomeruli. Glomeruli are also interconnected by a network of local neurons (LNs).

Wilson’s recent experiments focus on the role of these LNs in mediating cross-talk between olfactory glomeruli. Her lab’s results show that these LNs have diverse properties. Some are inhibitory, and their function is to suppress neurotransmitter release from ORN axon terminals. This turns down the “gain” on olfactory signals. Wilson and her colleagues speculate that this could be useful in preventing olfactory signals from saturating when odors are strong.

Other LNs are excitatory. These excitatory LNs can spread depolarization from a glomerulus that was directly excited by its ORNs to other glomeruli that were not directly excited by their ORNs. The function of this excitatory cross-talk is uncertain, but Wilson suggests that it might be useful in promoting sensitivity when odors are weak.

Ongoing work in the Wilson lab is seeking to understand the mechanisms and functions of these inhibitory and excitatory networks of interneurons.

**Seeing Circuits for Smell**

Bob Datta
*Department of Neurobiology, Harvard Medical School, Boston, MA*

Bob Datta's research is focused on one of the most fundamental problems in neuroscience: How is information coming from the environment processed in the brain and used to produce a behavioral response? All animals have evolved mechanisms to detect critical information in the environment, transmit this information to the brain, and process this information into an internal representation of the external world. We know this happens all the time of course, but describing the chain of molecular events that link perception to behavior is another matter.

Datta and his colleagues are concentrating on the olfactory system which can be described as a sensory map coupled to higher brain centers that both generate innate behaviors in response to fundamentally important odors, and link newly discovered odors with adaptive behaviors. While much is known about the general mechanisms used to detect odorants in the periphery, little is known about how olfactory sensory and projection neurons construct an internal sensory map that can trigger specific behaviors.

In a paper published in *Nature* 2008, Datta and his group applied their research method (which combines optical, behavioral, genetic, and molecular approaches) to the study of courtship in *Drosophila*, and explained how a single pheromone acting through the same set of sensory neurons can cause different behaviors in male and female flies.

In *Drosophila*, courtship involves complex and yet stereotyped dimorphic behaviors that are triggered by a male-specific pheromone, cis-vaccenyl acetate (cVA). This pheromone is detected by the odorant receptor Or67d, expressed by a subclass of olfactory neurons, which converge on the DA1 glomerulus on the antennal lobe. Whereas in males, activation of Or67d1 neurons by cVA prevents a male from courting  of other males; in females their activation promotes receptivity to males.

Datta and his colleagues suspected that functional dimorphisms in this neural circuit might be responsible for the dimorphic behavior. By using functional imaging of the DA1 glomerulus, based on two-photon laser scanning microscopy to activate the photoactivatable green fluorescent protein, they showed that the projections from the DA1 glomerulus to the protocerebrum are actually different in male and female flies. Projection neurons in males have more axonal branches that extend to the ventromedial region of the protecerebrum, compared to females.

The neural logic used by fruit flies to associate odors and innate behaviors seems to be shared, in part at least, by the mouse brain. Datta and his colleagues used newly developed neural labeling and imaging techniques to study the structure of brain circuits responsible for fear responses in the mouse. In rodents, once again, olfactory sensory neurons converge on specialized glomeruli which in turn are connected to the cortex, where their signals can trigger stereotyped behavior, like those shown in presence of a predator. By probing one of these circuits, Datta showed that the pattern of axonal ramification from glomeruli involved in detecting fear-related odors is highly specific, and that this specificity seems to be genetically controlled. Once again, then, the pattern of arborization from each single glomerulus seems to be the key to its ability to trigger innate responses.

**Ligands for Olfactory Trace Amine-Associated Receptors**

Stephen D. Liberles
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Science can be a really tough job at times. Ask people in Stephen Liberles' lab, who even had to tour the zoos of the Boston area, facing lions and tigers nose to nose to collect their urine. The reason they needed those samples was that they were trying to identify, literally, the essence of fear.

Most of the brain's work in animals is to transform sensory stimuli in instinctive behavior. This may sound like common sense, yet tracing the molecular mechanisms that link perception with action in the brain is very difficult. The Liberles group is trying to do it, using the olfactory system in the mouse as a model.

Sensory neurons in the mouse nose detect odors and pheromones using over 1,600 different G Protein Coupled Receptors (GPCRs). Liberles recently identified a new family of mammalian olfactory receptors, trace amine-associated receptors (TAARs), which appear to influence various innate behaviors.

TAARS are found in many vertebrates, from the Zebrafish (which have 113 of them) to mice and rats (15 and 17 respectively) to humans (only 6). Their genetic sequence closely resembles that of receptors for serotonin, histamine and dopamine. The projection patterns of TAAR-expressing sensory neurons project to the olfactory bulb, and the neural circuits they activate deeper in the brain, are unknown. Also ligands for the majority of TAARs are not known, and finding them is an important step towards determining whether these receptors have a specialized physiological role.

Liberles and colleagues first identified molecules that could bind to 13 TAARS expressed in the rodent olfactory system, and found that several of them were bioamines found in urine. They then concentrated on specific members of the family, searching for the molecules that trigger them.

In particular they studied one specific TAAR which was activated by bobcat urine. After purifying this agonist in bobcat urine, they found it to be a biogenic amine.

To assess whether this molecule is specific to bobcat or works like a more general signal for carnivores, David Ferrero in Liberles' lab collected samples from 38 mammalian species (this is the part of the experiment that required touring zoos and cages), purified them and looked for concentrations of that particular chemical. He found that, though it occurs in many different species, it is found in higher concentrations in the urine of large carnivores. Behavioral studies on rats exposed to this chemical in cages confirmed that it can selectively trigger typical aversive responses, such as avoiding the corner of the cage treated with some drops of it

**Odorant Receptor and Circuit Information in the Olfactory System.**

Claudia Lodovichi
*Venetian Institute of Molecular Medicine (VIMM), CNR, Neuroscience Institute, Padua, Italy*

Claudia Lodovichi belongs to a growing number of neuroscientists who consider the olfactory system (OS) an interesting model to study the mechanics and the logic of neuronal wiring in the mammalian brain. The relatively simple anatomy and topography of the OS allow researchers to study how signals travel from the periphery of the OS to its centre, as basic perceptions turn into odor recognition and behavioral responses.

At the Venetian Institute of Molecular Medicine in Padua, Lodovichi and her group concentrate on the olfactory system of rats and mice. In the olfactory epithelium, the most peripheral structure of the olfactory system, olfactory sensory neurons (OSN) expressing different odorant receptors (OR), are randomly intermixed. In other words, the olfactory epithelium has no topographical organization; in this it stands in sharp contrast to the peripheral structures of other sensory systems, e.g., vision and hearing. Spatial order is, however, achieved in the olfactory bulb (OB), where OSN expressing the same OR converge with exquisite precision to form glomeruli in specific position in each OB.

A unique feature in the topographic organization of the OS in mammals is the dual role of the OR. In one role, in the nasal epithelium, it detects odors; in the other it guides axonal convergence of olfactory sensory neurons to form glomeruli in the olfactory bulb.

This duality has been demonstrated by genetic experiments which showed that altering the sequence of an odor receptor gene leads to a change in the sensory map; and has been corroborated by the expression of the OR not only at the cilia-dendrite level, but also at the axon termini-growth cone of OSN.

It has long been well established that the signalling pathway coupled to the OR expressed at the cilia involves cyclic adenosine monophosphate (cAMP), calcium ions ($Ca^{2+}$) and cyclic guanosine monophosphate (cGMP). In contrast, nothing has hitherto been known about the functional characteristics of the OR expressed at the axon termini-growth cone. Lodovichi and her group decided to investigate whether the signalling molecules coupled to the OR at the axon termini are, or are not, the same as the ones at the cilia.

By studying the spatial distribution and the temporal dynamics of cAMP, $Ca^{2+}$ and cGMP in living OSN in vitro and in vivo, Lodovichi and her group demonstrated, for the first time, that the OR at the axon termini-growth cone is actually capable of binding odors, and that this binding is coupled to local increases of cAMP, cGMP and $Ca^{2+}$.

The nature of the activation mechanism of the OR at the axon termini remains an open question, and Lodovichi's group is using a variety of approaches in an attempt to answer it.

**Visual Processing Based on a Retinal Spike Latency Code**

Markus Meister

*Center for Brain Science and Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA*

Our vision is actually the result of a continuous editing process: in humans and most other animals, the moments when the eyes are relatively still and vision is focused are interrupted by rapid gaze shifts called “saccades”. During these few milliseconds, vision becomes blurred, the visual image sweeps rapidly over the retina.

Then vision becomes focused again and the brain must make sense again of the scene that appears on the retina. During saccades, most retinal ganglion cells are strongly suppressed. After the image comes to rest, many ganglion cells fire a burst of spikes, and this burst of spikes during the fixation period is all the retinal information available for processing the new scene. Markus Meister and his group are interested in understanding how information encoded in these bursts is processed by the brain.

The conventional wisdom about brain computation is that information is mostly encoded in the average firing rate of a neuron. Yet this cannot be true in the case of rapid visual processing. It takes us only 100 ms to recognize high-level content of a new image. Along the pathways used for visual perception, each neuron has only 10 ms in which to communicate to the next synaptic stage. That leaves time for only 1 or 2 action potentials at most.

For many ganglion cells the timing of the very first spike in this volley reveals much of the image information, and generally more than contained in the subsequent firing rate This suggests that downstream brain circuits could indeed begin image computations very rapidly, using just one action potential per retinal ganglion cell at the onset of fixation.

To add to this complexity the retina has about sixty different cell types, which can be distinguished anatomically or biochemically or genetically. In the output layer alone, at the level of retinal ganglion cells, there are about 15-20 different types. Each of them maps the visual field completely, but each responds to a different visual feature.

To understand how processing is performed at the onset of a fixation, Meister's group presented the retina with a grating of white and black bars, appearing and disappearing so to reproduce the rhythm of saccades, reappearing every time with a different spatial offset.

By recording from ganglion cells, researchers could see that every appearance of the grating produced a burst of spikes, but with some differences: the most evident difference was related to timing of spikes. Some stimuli caused cells to fire early, and some to fire later. How much information is conveyed by the simple timing of the first action potential, and how much by the number of spikes before the next saccade?

It turns out that, in the case of detecting oriented lines at least, firing rate is much less informative than timing. The brain can extract quite a lot of information simply from the timing of the first action potential. Timing of the spike seems to be driven by luminosity of the stimulus: a predominantly bright image will trigger a pathway that is slower and reaches an activation threshold a bit later, in comparison to an image that is predominantly dark.

That leaves us with the question of how the cortex processes this information: can the cortex compute directly with spike latencies or does this code need to be translated into the standard language of firing rate. Meister suggests a simple and yet powerful model for computation where a single cortical neuron receives spikes from many different ganglion cells (via the thalamus), and the postsynaptic potentials

simply add up until a threshold is reached. This model of processing is called a tempotron, because it works on the temporal superposition of action potentials from presynaptic neurons. It was found that a tempotron that is presented with spike trains recorded from the retina can effectively distinguish between many different visual stimuli. This raises the possibility that cortical circuits might accomplish the rapid feats of visual perception by computing in a mode that requires just a single action potential per neuron.

**Self-Inhibiting Neurons of the Neocortex**

Alberto Bacci
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In music, silence is as important as notes. The same is true in the brain where inhibition is as important as excitation in regulating communication between neurons. Inhibition brings balance in neuronal networks by preventing excitation that would otherwise spread uncontrollably through synapses between neurons and by keeping it confined where it is needed and significant.

Most of the inhibition work is done by interneurons (neurons whose axons are limited to a single brain area) which mostly use GABA as a neurotransmitter. During the latest years, Alberto Bacci's work has been focusing on a particular class of inhibitory interneurons, called fast spiking basket cells (for their characteristic high-frequency firing) , found in the cerebral cortex (including the neocortex and hippocampus), the site where sensory information is integrated into complex cognitive functions. The activity of inhibitory interneurons is crucial, as they represent the basic elements that provide cortical feedforward and feedback inhibition, so preventing, among other things, the development of epilepsy. Moreover, like orchestra conductors, these inhibitory interneurons can generate, pace and modulate the oscillatory activity of large neuronal populations.

In a landmark paper published in 2003 in the *Journal of Neuroscience*, Bacci and his group demonstrated that these neurons can generate self-inhibition using GABAergic autaptic contacts: a sort of "auto-synapses" where the neuron's axon crosses the same neuron's dendrites, creating a very efficient feedback system. In other words, when triggering an action potential these neurons do not only send inhibitory signals to other neurons, but also to themselves, a mechanism which seems fundamental for their action. At the workshop, Bacci presented some new data which can further elucidate the role of autaptic inhibition in neuronal networks.

In particular, this potent form of self-inhibition seems to be instrumental in synchronizing networks formed by GABAergic interneurons by dictating the pauses and therefore temporarily organizing the time in which inhibitory cells are allowed to fire. Once these interneurons fire synchronously, their global organized activity will be more efficient in conducting the concerted firing of principal excitatory neurons, thereby promoting global network synchrony.

More recently, the work of the group has shown that when fast spiking neurons discharge at high frequency, as it happens in some specific brain states, fast spiking cells produce a prolonged and disorganized inhibition onto themselves (via autaptic contacts) and other targets within cortical circuits, including principal neurons. This phenomenon is mediated by asynchronous release of GABA at each synaptic and autaptic contact and lasts for several seconds after interneurons ceased to fire. Bacci's group investigated the function of this prolonged and delayed inhibition and found that it changes the capability of the postsynaptic neuron of integrating incoming inputs into precise and reliable outputs. It seems, in other words, that when fast-spiking interneurons fire at high frequencies they promote network desynchronization, whereas when they spike at lower frequencies they synchronize the network.

More recently, the work of the group has also focused on inhibitory neurotransmission in the hippocampus, and on how it can explain the mechanism of action of some widely used antidepressants.