LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

HARVARD UNIVERSITY

LMU–Harvard Young Scientists' Forum

From Molecules to Organisms,
Munich, June 21 – 24, 2009

The LMU-Harvard Young Scientists' Forum (YSF) seeks to unite Ph.D. students and postdoctoral fellows from the Harvard University and the Ludwig-Maximilians-Universität (LMU) with core faculty from the two universities to create a framework for an interdisciplinary exchange of ideas. This year's conference will be held at the Center for Advanced Studies of the LMU Munich.

■ **LMU-Harvard Young Scientists´ Forum at the Center for Advanced Studies (CAS^{LMU}), LMU:**
From Molecules to Organisms, Munich, June 21-24, 2009
■ **Under the auspices of Prof. Dr. Reinhard Putz, Vice-President for International Affairs, LMU**
■ **Academic Board: Prof. Dr. Dirk Trauner (Center for Integrated Protein Science Munich, CIPSM), Prof. Dr. Benedikt Grothe (Munich Center for Neurosciences, MCN)**
■ **Program Management: Anja Rössner (LMU International Affairs)**
■ **Participating academic units: Center for Integrated Protein Science Munich (CIPSM), Munich Center for Neurosciences (MCN), Graduate School for Systemic Neurosciences (GSN)**
■ **Academic Program: Prof. Dr. Benedikt Grothe / Prof. Dr. Oliver Behrend (MCN, GSN), Prof. Dr. Dirk Trauner / Dr. Oliver Baron (CIPSM), Kiri Couchman / Dr. Alex Loebel (Ph.D. / Post-Doc Representative)**
■ **Institutional Responsibility: LMU International Affairs, Center for Advanced Studies (CAS^{LMU})**

Conference agenda

June 20, 2009

Saturday	
	Arrival in Munich, pick-up (if pre-arranged) and transfer to: Hotel Cosmopolitan, Hohenzollernstr. 5, 80801 München, Phone: +49 (0) 89 383810, www.cosmopolitanhotel.de
3.00 – 4.30 pm	**Optional program:** **Guided city tour** **(meeting point: hotel lobby)**
6.45 pm	pick-up at the hotel; walk to Restaurant Seehaus
7.00 pm	**Welcome Dinner (informal) at** Restaurant Seehaus, Kleinhesselohe 3, 80802 München, Phone: +49 (0) 89 3816130

June 21, 2009

Sunday	
	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 218072080
8.30 am	Pick up at the hotel; walk to CAS
9.00 – 9.10 am	**Opening Remarks** **Reinhard Putz**, Vice President, LMU
9.10 – 9.15 am	**"Welcome to CAS"** **Silke Meiners**, Center for Advanced Studies (CAS[LMU])
9.15 – 10.15 am	**Introduction and Faculty Lecture:** "Controlling neuronal activity with molecular switches" **Dirk Trauner**, Dept. of Chemistry and Biochemistry, LMU
10.15 – 10.30 am	Coffee Break
10.30 – 12.30 pm	**Student Session 1** "Methods" (Chair: Benedikt Grothe) "Development of caged opioid neuropeptides" **Matthew Banghart**, Dept. Neurobiology, Harvard

	"Chemical communications", **Ryan Chiechi**, Dept. of Chemistry and Chemical Biology, Harvard
	"Magnetic levitation as a platform for density-based analysis and separations" **Katherine Mirica**, Dept. of Chemistry and Chemical Biology, Harvard
	"A strategy to investigate the regulation of the immune response against cancer through multiphoton intravital microscopy" **Francesco Marangoni**, Division of Rheumatology, Allergy and Immunology, Harvard
12.30 – 2.00 pm	Lunch (catered)
2.00 – 4.00 pm	**Student Session 2** "Neural Coding" (Chair: Dirk Trauner)
	"Odor coding based on spike timing with respect to sniff-triggered oscillations during active sampling" **Kevin Cury**, Dept. of Molecular and Cellular Biology, Harvard
	"Single cell morphology of retinal ganglion cell axon terminals in the mouse brain" **Kate Hong**, Dept. of Molecular and Cellular Biology, Harvard
	"The insect visual system as a model system for peripheral image processing" **Jan Grewe**, Dept. Biology II, LMU
	"A combined electrophysiological and neurogenetic approach to- wards the cellular dissection of motion computation in Drosophila" **Maximilian Joesch**, Dept. of Systems and Computational Neurobiology, MPI of Neurobiology
4.00 – 4.15 pm	Coffee Break
4.15 – 5.15 pm	**Faculty Lecture:** "Eyes smarter than scientists believed: neural computations in the retina" **Markus Meister**, Dept. of Molecular and Cellular Biology, Harvard
5.30 – 7.00 pm	**Poster Session and Get Together**
	Posters: Christopher Fang-Yen, Yoh Isogai, Christian Miller, Nicolas Stephan, Andreas Mayer, Luis Soares, Pratibha Tripathi, Ronald Orth, Alwin Reiter
7.30 pm	**Conference Dinner at** Restaurant Alpenraum, Karlstraße 10, 80333 München, Phone: +49 (0) 89 200030730

June 22, 2009

Monday	
	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 218072080
9.00 – 10.00 am	**Faculty Lecture:** "Pre- and Post-transcriptional mechanisms for suppressing promiscuous transcription" **Stephen Buratowski**, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
10.00 – 10.15 am	Coffee Break
10.15 – 12.15 pm	**Student Session 3** "Molecular Biology" (Chair: Steve Buratowski, tbc)
	"TFIIF binds the RNA polymerase II funnel and stimulates mRNA cleavage" **Anass Jawhari**, Dept. of Chemistry and Biochemistry, LMU
	"A zebrafish model of Alzheimer's disease", **Dominik Paquet**, Adolf-Butenandt-Institut, LMU
	"Directing postnatal astroglia towards distinct neuronal subtypes" **Benedikt Berninger**, Dept. of Physiological Genomics, LMU
	"Neurotrophic function of transcription factor Pax6 in the mature dopaminergic olfactory bulb interneurons", **Jovica Ninkovic**, Institute of Stem Cell Research, Helmholtz Zentrum, München
12.15 – 2.00 pm	Lunch (catered)
2.30 – 6.00 pm	**Organized tour:** **HighTechCampus[LMU] (Großhadern, Martinsried)**
6.15 – 7.00 pm	**MCN lecture:** "Cellular and molecular mechanisms that promote and inhibit spinal cord repair after injury", **John Nicholls**, International School for Advanced Studies, SISSA, Italy
	Evening at free disposal (optional program organised by the local PhD student / postdoc representatives)

June 23, 2009

Tuesday

	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 / 218072080
9.00 – 10.00 am	**Faculty Lecture:** "Chemistry and biology of insect-bacteria mutualisms" **Jon Clardy**, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
10.00 – 10.15 am	Coffee Break
10.15 – 12.15 pm	**Student Session 4** "Chemical Biology" (Chair: Dirk Trauner)
	"On the biogenesis of cyclopiazonic acid" **Xinyu Liu**, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard
	"Regulation of the alkyl-dihydrothiazole-carboxylates (ATCs) by iron and the pyochelin gene cluster in Pseudomonas aerugionsa" **Nawaporn Vinayavekhin**, Dept. of Chemistry and Chemical Biology, Harvard
	"ß-Lactones – from activity based probes to a novel strategy to combat bacterial intfections" **Thomas Böttcher**, Dept. of Chemistry and Chemical Biology, LMU
	"Chemical warfare in the hive: symbiotic bacteria protect honeybees from devastating pathogen", **Renee Kontnik**, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard
12.15 – 2.00 pm	Lunch
2.00 – 4.00 pm	**Student Session 5** "Cellular Interaction" (Chair: Alexander Borst)
	"Modulation of gap junction signaling by intrinsic membrane currents" **Julie Haas**, Dept. of Neurology, Harvard
	"The distribution of neurotransmitter receptors in neurons of the medial superior olive" **Kiri Couchman**, Dept. of Biology II, LMU
	"Role of GAD67 in the function and plasticity of GABAergic synapses" **Geoffrey Lau**, Dept. of Molecular and Cellular Biology, Harvard
	"Multiquantal release underlies the distribution of synaptic efficacies in the neocortex" **Alexander Loebel**, Dept. of Biology II, LMU
4.00 – 4.15 pm	Coffee Break
4.15 – 5.15 pm	**Faculty Lecture:** "Neural processing of optic flow in flies" **Alexander Borst**, Dept. of Systems & Computational Neurobiology, MPI of Neurobiology
5.15 – 6.00 pm	**Follow-Up Meeting**
	Evening at free disposal (optional program organised by the local PhD student / postdoc representatives)

June 24, 2009

Wednesday

8.30 am – 8.00 pm (approx.)	Excursion to Neuschwanstein Castle, Linderhof Castle and Ettal Abbey (pick-up at the hotel)

June 25, 2009

Thursday

8.00 – 10.00 am	Breakfast at the hotel. End of conference; independent departure of participants

Participants*

*Participating Ph.D. students and postdoctoral fellows have been nominated by selected faculty members of LMU and Harvard University (please note the heads of the nominees´ "home laboratories" at the end of each entry).

Harvard University

- **Matthew Banghart**, Postdoctoral Fellow,
  Department of Neurobiology, Harvard Medical School,
  Howard Hughes Medical Institute, Laboratory of Bernardo Sabatini
- **Kenneth Blum**, Executive Director,
  Harvard Center for Brain Science
- **Stephen Buratowski**, Professor,
  Department of Biological Chemistry and Molecular Pharmacology,
  Harvard Medical School
- **Ryan Chiechi**, previously: Postdoctoral Fellow for George M. Whitesides;
  currently: Assistant Professor in the Stratingh Institute for
  Chemistry at the University of Groningen
- **Jon Clardy**, Professor,
  Department of Biological Chemistry and Molecular Pharmacology,
  Harvard Medical School, Broad Institute
- **Kevin Cury**, Graduate Student,
  Department of Molecular and Cellular Biology, Harvard Center for Brain Science,
  Laboratory of Naoshige Uchida
- **Florian Engert**, Professor,
  Department of Molecular and Cellular Biology, Harvard
- **Chris Fang-Yen**, Postdoctoral Fellow,
  Department of Molecular and Cellular Biology, Harvard Center for Brain Science,
  Laboratory of Aravinthan Samuel
- **Julie Haas**, Postdoctoral Fellow,
  Department of Neurology, Harvard Center for Brain Science,
  Laboratory of Carole Landisman
- **Kate Hong**, Graduate student,
  Harvard Medical School, Harvard Center for Brain Science, Laboratory of Joshua Sanes
- **Yoh Isogai**, Postdoctoral Fellow,
  Department of Molecular and Cellular Biology, Harvard Center for Brain Science,
  Laboratory of Catherine Dulac
- **Renee Kontnik**, Graduate student,
  Department of Biological Chemistry and Molecular Pharmacology,
  Harvard Medical School, Laboratory of Jon Clardy
- **Geoffrey Lau**, Postdoctoral Fellow,
  Department of Molecular and Cellular Biology, Harvard Center for Brain Science,
  Laboratory of Venkatesh Murthy
- **Xinyu Liu**, Postdoctoral Fellow,
  Department of Biological Chemistry and Molecular Pharmacology,
  Harvard Medical School, Laboratory of Christopher T. Walsh

- **Francesco Marangoni**, Postdoctoral Fellow, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital and Harvard Medical School, Laboratory of Thorsten Mempel
- **Markus Meister**, Professor, Dept. of Molecular and Cellular Biology, Harvard Center for Brain Science
- **Katherine Mirica**, Graduate Research Assistant, Department of Chemistry and Chemical Biology, Harvard, Laboratory of George Whiteside
- **Luis Soares**, Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Laboratory of Steve Buratowski
- **Nawaporn Vinayavekhin**, Graduate Student, Department of Chemistry and Chemical Biology, Harvard, Laboratory of Alan Saghatelian

Ludwig-Maximilians-Universität (LMU) Max Planck Institute of Neurobiology

- **Oliver Baron**, Managing Director of the Center for Integrated Protein Science Munich (CIPSM)
- **Oliver Behrend**, Managing Director of the Munich Center for Neurosciences - Brain & Mind (MCN)
- **Benedikt Berninger**, Postdoctoral Fellow, Institute of Physiology, Department of Physiological Genomics, LMU, Laboratory of Magdalena Götz
- **Alexander Borst**, Professor, Max Planck Institute of Neurobiology, Department Systems & Computational Neurobiology
- **Thomas Böttcher**, PhD student, Department of Chemistry and Biochemistry, LMU, Laboratory of Stephan Sieber
- **Thomas Carell**, Professor, Vice President of LMU, Department of Chemistry and Biochemistry
- **Kiri Couchman**, PhD student, Department of Biology II, LMU, Laboratory of Benedikt Grothe
- **Patrick Cramer**, Professor, Department of Chemistry and Biochemistry, LMU
- **Stefan Endres**, Professor, Division of Clinical Pharmacology, LMU
- **Stephan Fuchs**, Director, International Office, LMU

- **Jan Grewe**, Postdoctoral fellow, Department Biologie II, LMU, Laboratory of Andreas Herz
- **Benedikt Grothe**, Professor, Department Biology II, LMU
- **Anass Jawhari**, Postdoctoral fellow, Department of Chemistry and Biochemistry, LMU, Laboratory of Patrick Cramer
- **Maximilian Joesch**, Graduate student, Department Systems & Computational Neurobiology, Max Planck Institute of Neurobiology, Laboratory of Alexander Borst
- **Esther Krumbholz**, International Visitors´ Programs, LMU
- **Alex Loebel**, Postdoctoral fellow, Department Biology II, LMU, Laboratory of Andreas Herz
- **Andreas Mayer**, PhD student, Department of Chemistry and Biochemistry, LMU, Laboratory of Patrick Cramer
- **Silke Meiners**, Scientific Coordinator (Natural and Life Sciences), Center for Advanced Studies (CAS[MU])
- **Christian Miller**, PhD student, Department of Chemistry and Biochemistry, LMU, Laboratory of Patrick Cramer
- **Jovica Ninkovic**, Postdoctoral Fellow, Helmholtz Zentrum München, Institute of Stem Cell Research, Laboratory of Magdalena Götz
- **Ronald Orth**, PhD student, Department of Chemistry and Biochemistry, LMU, Laboratory of Stephan Sieber
- **Dominik Paquet**, PhD student, Adolf-Butenandt-Institut, LMU, Laboratory of Christian Haass
- **Reinhardt Putz**, Professor, Vice President of LMU
- **Alwin Reiter**, Graduate student, Department of Chemistry and Biochemistry, LMU, Laboratory of Dirk Trauner
- **Anja Rössner**, International Cooperation Officer, LMU
- **Stephan Sieber**, Professor, Department of Chemistry and Biochemistry, LMU
- **Philipp Stawski**, PhD student, Department of Chemistry and Biochemistry, LMU, Laboratory of Dirk Trauner
- **Alexandra Stein**, Program Coordinator, Graduate School of Systemic Neurosciences
- **Nicolas Stephan**, PhD student, Division of Clinical Pharmacology, LMU, Laboratory of Stefan Endres
- **Dirk Trauner**, Professor, Department of Chemistry and Biochemistry, LMU
- **Pratibha Tripathi**, PhD student, Helmholtz Zentrum München, Institute of Stem Cell Research, Laboratory of Magdalena Götz

Abstracts of lectures and posters

Development of caged opioid neuropeptides

Matthew Banghart
Harvard, Department of Neurobiology

Neuropeptides have profound and complex neuromodulatory effects on neuronal function in the mammalian nervous system. At the cellular level, the basic physiological responses to most neuropeptides have been described, yet the spatial and temporal precision of these signals remains unknown. To enable delivery of neuropeptides on the scale of single synapses with millisecond kinetics, we are developing caged neuropeptides that can be released by exposure to UV light. Our initial efforts have focused on Leu-Enkephalin, a member of the opioid family of neuropeptides, which is selective for the Delta and Mu opioid receptor subtypes. Building on known pharmacology, several candidate caged Leu-Enkephalin analogues containing nitrobenzyl-derived chromophores were designed and obtained. Dose-response relationships were determined using a modified secreted alkaline phosphatase assay that enables Gi-coupled receptors to signal through the Gs pathway. These screens identified caged analogues that exhibit >100 fold reductions in potency with respect to Leu-Enkephalin at both Mu and Delta receptors with no activity at Kappa receptors. Furthermore, no sign of antagonist activity at any receptor was detected. For each analogue, photolysis was studied using UV/VIS spectroscopy, HPLC and mass spectrometry. Preliminary electrophysiological evaluation was carried out in transverse hippocampal slices from P15-18 mice and focused on inhibitory neurons, the predominant cell expressing Mu and Delta opioid receptors in the CA1 region.

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Directing postnatal astroglia towards distinct neuronal subtypes

Benedikt Berninger, Christophe Heinrich, Robert Blum, Rodrigo Sánchez & Magdalena Götz
LMU, Department of Physiological Genomics

We have previously shown that astroglia from the postnatal cerebral cortex can be reprogrammed by neurogenic fate determinants to generate neurons capable of action potential firing (Berninger et al., J. Neurosci 2007). An essential prerequisite for a potential use of astroglia-derived neurons for functional replacement of degenerated neurons or circuit modification would be their capacity (i) to form functional synapses and (ii) to give rise to distinct types of neurons. Here we show that postnatal astroglia can be reprogrammed towards distinct neuronal subtypes, such as glutamatergic and GABAergic neurons, by forced expression of the dorsal and ventral fate determinants neurogenin-2 (Neurog2) and Dlx2, respectively. Bona-fide non-neurogenic astroglia were cultured from the P5-P7 postnatal cortex of mice expressing a tamoxifen-inducible Cre recombinase under the control of the astroglia-specific glutamate and aspartate transporter (GLAST) for fate mapping cells or astroglial origin (GLAST::CreERT2/Z/EG). Following expansion and passaging, these cells were then transduced with retroviral vectors encoding Neurog2-IRES-DsRed or Dlx2-IRES-DsRed for neuronal reprogramming. Virtually all GFP reporter-positive fate-mapped cells acquired a neuronal identity following forced Neurog2 expression and exhibited vesicular glutamate transporter expression by two weeks of age indicative of synapse formation. Consistent with this, Neurog2-reprogrammed astroglia formed functional glutamatergic synapses. In contrast, following transduction with Dlx2, roughly 30 % of the transduced cells acquired a neuronal identity. More importantly, these never gave rise to glutamatergic neurons, but exhibited classical features of GABAergic neurons, such as a fast-spiking action potential discharge pattern. Consistent with a GABAergic identity, these cells formed GABAergic synapses. This data indicates that single transcription factors can trigger transdifferentiation of postnatal astroglia into functional neurons exhibiting distinct neuronal identities.

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ß-Lactones – from activity based probes to a novel strategy to combat bacterial infections

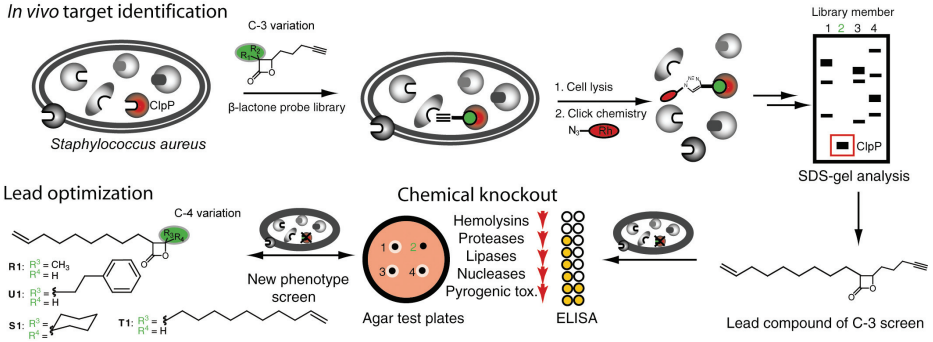
Thomas Böttcher, Stephan A. Sieber
LMU, Department of Chemistry and Biochemistry

After decades of successful treatment of bacterial infections with antibiotics, formerly treatable bacteria now are rapidly developing drug resistances and consequently pose a major threat to public health. Since many antibiotics in clinical development and application still target only a limited set of cellular functions such as cell wall, DNA and protein synthesis, it is a desirable goal to expand the number and breadth of therapeutic targets combined with a deeper knowledge about their mechanism. To address this goal, we applied a chemical proteomic strategy termed activity-based protein profiling (ABPP) that is designed to globally profile enzyme activities in complex proteomes. To identify novel targets for the treatment of multidrug resistant S. aureus (MRSA) strains, we utilized small biomimetic ß-lactones [1, 2, 3] which were modified with a small tag for the visualization and identification of dedicated targets in complex proteomes by SDS-gel-electrophoresis and mass spectrometry **(Figure 1)**. Structural variations in side chains of selected molecules led to an increased affinity for certain enzymes, one of which played a crucial role in virulence. The general utility of this approach was demonstrated by the chemical inhibition of ClpP by structurally optimized ß-lactones. Chemical knockout of this central virulence regulator in S. aureus resulted in a drastically decreased expression of major virulence factors which are key players in e.g. sepsis, tissue necrosis, inflammation and toxic shock. Since this virulence regulator is highly conserved in many pathogens, our strategy could represent a global approach for the treatment of infectious diseases by disarming the bacterial virulence repertoire. Disarmed pathogens could then be easily eliminated by the human immune response. A drug based on this concept displays many advantages over conventional antibiotics, such as preserving the useful, cooperating microorganisms e.g. in the digestive tract, and exerting less selective pressure on pathogens, which may result in decreased resistance development.

Literature:

[1] T. Böttcher, S. A. Sieber, *Angew. Chem. Int. Ed. Engl.* **2008**, 47, 4600.
[2] T. Böttcher, S. A. Sieber, *J. Am. Chem. Soc.* **2008**, 130, 14400.
[3] T. Böttcher, S. A. Sieber, *ChemBioChem* **2009**, 10, 663.
[4] T. Böttcher, S. A. Sieber, *ChemMedChem* **2009**, InPress.

Figure 1: Identification of cellular targets of ß-lactones in living cells, chemical knockout of ClpP and lead optimization.



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Neural processing of optic flow in flies

Alexander Borst
MPI of Neurobiology, Department of Systems and Computational Neurobiology

Motion of an animal in space causes the images of the environment on the observer's retina to move. The resulting distribution of motion vectors on the retina is called optic flow. Since different types of motion cause different optic flows, the latter can be used to guide the animal visually through space. In the fly, the processing of optic flow is split in two steps: First, local motion vectors are calculated from the changing retinal images in parallel by local motion detectors according to the Reichardt model. Since the output of a Reichardt detector does not necessarily reflect the local retinal velocity, the resulting 'neural optic flow' can substantially deviate from the optic flow as determined in technical applications. In a second step, the local output signals of Reichardt are further processed by large field motion sensitive neurons in the fly's lobula plate. These neurons pool, via their large dendrites, the incoming signals and additionally interact amongst themselves thereby enhancing their selectivity for particular optic flows as well as making the processing robust against various perturbations resulting from inhomogeneous contrast distribution in natural images [1,2,3]. While all of the above has been studied extensively in the blowfly Calliphora, not much was known until recently about the corresponding set of neurons in Drosophila. Our group has therefore started to investigate the lobula plate tangential cells also in Drosophila. Studying their receptive field properties via whole cell patch recording, we found them to be strikingly similar to the ones of their large cousins [4]. Most importantly, responses in these cells show all characteristics that are indicative for receiving input from Reichardt-type local motion detectors. Using the large repertoire of genetic tools including blockers of synaptic transmission and genetically encoded Calcium indicators, we now investigate which of the columnar neurons presynaptic to the lobula plate tangential cells constitute the neural substrate of the Reichardt detector.

Literature:

[1] Haag J, Borst A (**2004**). Neural mechanism underlying complex receptive field properties of motion-sensitive interneurons. *Nature Neurosci 7: 628-634.*
[2] Cuntz H, Haag J, Foerstner F, Segev I, Borst A (**2007**): Robust coding of flow-field parameters by axo-axonal gap junctions between fly visual interneurons. *PNAS 104: 10229-10233.*
[3] Elyada Y, Haag J, Borst A (**2009**): Different receptive fields in axons and dendrites underlie robust coding in motion-sensitive neurons. *Nature Neurosci, 12: 327-333.*
[4] Joesch M, Plett J, Borst A, Reiff DF (**2008**): Response properties of motion-sensitive visual interneurons in the lobula plate of Drosophila melanogaster. *Current Biology 18: 368-374.*

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Pre- and post-transcriptional mechanisms for suppressing promiscuous transcription

Stephen Buratowski and colleagues
Harvard, Department of Biological Chemistry and Molecular Pharmacology

The minimal sequences to specify transcription initiation sites for RNA polymerase II (for example, the TATA box or Initiator element) appear frequently by chance throughout eukaryotic genomes. Furthermore, enhancers can stimulate transcription over large distances and in both directions. This raises the obvious question of how cells prevent transcription from initiating throughout the genome.

Nucleosomes are clearly important for suppression of widespread initiation. However, the act of transcription itself displaces nucleosomes, at least transiently. We and others have characterized histone methyltransferases that associate with the elongating RNApII. Methylation of histone H3 lysine 36 (H3K36) recruits the Rpd3S histone deacetylase complex, and this methylation/deacetylation pathway represses initiation from cryptic promoters within the 3′ areas of transcribed genes. More recently, we found that dimethylation of H3K4 by the Set1 protein recruits a second histone deacetylase. The Set3 complex deacetylates histones in 5′ transcribed regions.

Despite these mechanisms to sequester cryptic promoter elements in repressive chromatin, it is inevitable that some background initiation will still occur, particularly around "real" active promoters that typically have low nucleosome density. We characterized a transcription termination pathway linked to early transcription elongation via an interaction with RNApII. This Nrd1/Sen1 termination complex functions at non-polyadenylated transcripts (such as snoRNAs) and is associated with the exosome, the major 3′ to 5′ exonuclease in cells. This association allows snoRNA termination to be linked to 3′ end trimming. Interestingly, the Nrd1 complex is also used to terminate and degrade short, non-coding transcripts. Recent sequencing studies have found that these cryptic unstable transcripts (CUTs) appear frequently around gene promoter regions. In at least some cases, transcription of the CUT can regulate nearby promoters or other DNA elements such as recombination enhancers.

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Chemical communications

Ryan Chiechi
University of Groningen, Stratingh Institute for Chemistry

Looking at the intersection between chemistry and information, nature shows us examples of storage (DNA), communication (concentration gradients), and power (ATP), but to find computation one has to look to larger, more complex networks (nervous systems). However chemists tend to focus their research on computation--what about communication? This presentation will discuss our research efforts in to self-powered chemical systems that can encode and transmit information as pulses of light in the visible to near infrared using combustion. The first approach uses fuses of nitrocellulose that have been patterned with emissive salts. As the flame front propagates down the fuse each spot emits light at a wavelength that is specific to the composition of that spot **(Figure 1)**. The information content of these "Infofuses" can then be read from as far as 50 m. The second approach leverages meta-stable states of small "flamelets" on a surface in which they spatially oscillate at regular frequencies. By viewing them through an opaque mask that is patterned with holes, the spatially encoded information of the mask is transduced into pulses of near-infrared light **(Figure 2)**.

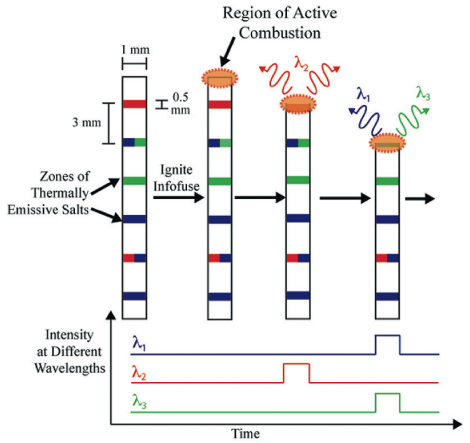

Figure 1. A cartoon of an Infofuse in which patterns of emissive salts transmit information as pulses of light of different wavelengths.

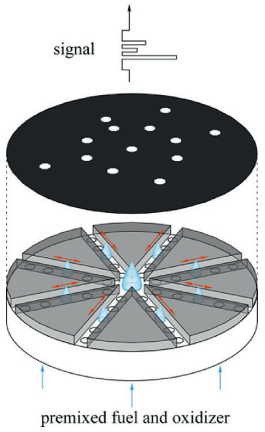

Figure 2. A schematic of a device that transmits information that is encoded spatially as pulses of light.

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Chemistry and biology of insect-bacteria mutualisms

Jon Clardy
Harvard, Department of Biological Chemistry and Molecular Pharmacology

Chemical biology addresses biological questions using chemical techniques, and in that spirit, this lecture will explore one of the most widespread biological strategies on the planet – mutualisms – using the tools of small molecule chemistry. Mutualisms, symbiotic relationships in which two partners reciprocally benefit from their association, dominate all realms of biology. Familiar examples include terrestrial plants and the mycorrhizal symbionts that provide them with inorganic compounds and trace elements or flowering plants and their pollinators.
In the past few years, the laboratory has been exploring insect-bacteria mutualisms from a chemical and biochemical perspective. These mutualisms involve the interplay of an insect-bacteria mutualism, their microbial predators, survival, and small molecules, but the roles of small molecules and the genes that made them had received relatively little attention. The lecture will emphasize three lessons about these mutualisms: 1) they are widespread in the natural world, 2) real world symbioses involve multiple players with no clear winners and losers, and 3) identifying the molecular basis these mutualisms provides a path to biologically important small molecules, their biosynthetic enzymes, and the evolution of resistance, sensitivity, and gene collectives.
Examples will be drawn from: 1) the Southern pine beetle (SPB) system, 2) ambrosia beetle systems, 3) fungus-farming ants of the Neotropics, 4) mud daubers and honeybees, and possibly others.

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The distribution of neurotransmitter receptors in neurons of the medial superior olive

Kiri Couchman[1,2], Benedikt Grothe[1,2,3], Felix Felmy[1]
[1]LMU, Division of Neurobiology
[2]Graduate school of systemic neurosciences
[3]Bernstein Center for Computational Neuroscience

In mammals the location of low frequency sounds in the horizontal plane is initially processed in the medial superior olive (MSO) by a neuronal coincidence mechanism. These coincidence detector neurons are highly sensitive to the timing and relative strengths of binaural excitatory and inhibitory inputs. The contribution of a synaptic input will depend also on its location. Therefore, spatial arrangements of synapses and neurotransmitter receptors will affect the integration of these inputs, thus shaping the neuronal coincidence precision. However, the distributions of synaptic inputs and postsynaptic receptors are as yet unclear.
We investigated the distribution of pharmacologically isolated receptor currents with whole-cell voltage recordings from MSO neurons of mature Mongolian gerbils. Brief, focal, glycine puffs were used to probe the cellular glycine receptor distribution. The AMPA and NMDA receptor distributions were investigated using fast UV-laser uncaging of MNI-glutamate.
We find that glycine receptors are located mainly on the soma and proximal dendrites of MSO neurons, and that this distribution is developmentally invariant. The distribution of NMDA receptors is similarly biased to somatic regions. The AMPA receptor distribution is highly variable between cells, however on average they are relatively evenly distributed over the cell surface. In order to determine the functional significance of these distributions i.e. the location of synapses relative to these receptors, we locally applied a high potassium concentration to elicit presynaptic vesicle release. These experiments show that glycine synapses are mainly located on the soma and proximal dendrite. AMPA mediated release could be evoked along the full extent of cell. Thus for the main neurotransmitter receptors the distribution matches the functional location.

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Odor coding based on spike timing with respect to sniff-triggered oscillations during active sampling

Kevin Cury
Harvard, Department of Molecular and Cellular Biology

Odors are first encoded by a pattern of activation across ~1,000 odorant receptors. Therefore, the problem of odor discrimination can be regarded as the recognition of an "olfactory image" whose "pixels" correspond to the analog input from each odorant receptor type. To achieve rapid and robust odor recognition, Hopfield (1995) proposed a model in which the timing of spikes with respect to an ongoing population oscillation encodes "pixel" intensity.
Olfaction is an active sense and rodents exhibit stereotypical high frequency respiration behavior ("sniffing", 7-9Hz). We hypothesized that respiration-coupled oscillations might provide a basis for temporal coding. We monitored the activity of single neurons in the olfactory bulb, the first relay center, simultaneously with respiration behavior while rats performed an odor discrimination task. First, we found that inhalation induces a stereotypical pattern of spikes, following the respiration rhythm like a clock. Although respiration frequency varies dramatically during natural behavior, this clock-like activity is consistent across the broad range of respiration frequencies. Second, upon odor stimulation, neurons responded with a temporal shift from these baseline patterns. This often occurred without a change in overall firing rate. Third, to estimate how much information such temporal patterns contain, we quantified how accurately an observer can classify odors based on neural activity with or without taking into account fine scale activity. Classification success was greatly improved (~50%) when considering fine temporal patterns. Finally, the same analysis in the piriform cortex suggested that olfactory bulb output is transformed into a more general rate based code in the cortex. In sum, we show that the timing of spikes with respect to sniff-triggered oscillations can play a substantial role in odor information coding in the early olfactory circuit.

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The worms crawl in, the worms crawl out: locomotory transitions as a window into the C. elegans motor circuit

Christopher Fang-Yen
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The nematode C. elegans moves by propagating dorso-ventral sinusoidal bending waves along its body. Although the principal interneurons, motor neurons, and muscles responsible for locomotory behavior have been identified, relatively little is known in detail about how undulatory waves are generated and propagated by these circuit elements.
To gain insight into the functional organization of the C. elegans motor circuit, we studied transitions between its two described locomotory gaits. C. elegans exhibits very different behaviors when swimming in fluids and crawling on a solid substrate. Compared with crawling, swimming is characterized by undulations of much larger wavelength and higher frequency. By immersing worms in solutions of varying viscosity, we show that this swimming-to-crawling transition is continuously modulated by mechanical loading. Swimming and crawling can be seen as low-load and high-load limits of a continuum of locomotory patterns.
Next we analyze the behavior of worms undergoing spatial transitions between swimming and crawling behaviors, such that mechanical loading is nonuniform over the length of the worm. The analysis of such transitions gives insights into the generation and propagation of the sinusoidal bending wave, and the mechanisms of modulation of locomotory gaits. We show that the worm behavior follows neither strictly local nor strictly global modulation of locomotory patterns. Rather, our results indicate that the head plays a central role in generating oscillatory bending waves during forward movement, while propagation of these waves to the rest of the body depends on local mechanical loading. We discuss these results in terms of the strong constraints they place on theoretical models of motor circuit function.

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The insect visual system as a model system for peripheral image processing

Jan Grewe
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The nervous system evaluates sensory inputs in order to adapt an animal's behavior to the current conditions. The induced behavior then, in turn, influences the input that has to be evaluated. Starting point of this action-perception cycle is the evaluation of the sensory input and during its evaluation the nervous system is confronted with different problems. The visual system for example receives input signals of an extremely wide range. Light intensities occurring during the course of the day or even within a sunlit scene can easily vary over more than 5 orders of magnitude. This, by far, exceeds the working range of the photoreceptors. Furthermore, neuronal signals are inherently noisy due to stochastic processes involved in any neuronal computations and, in case of the visual system, to the quantal nature of light. Thus the system also has to deal with uncertainties arising from internal as well as external noise sources.
Here I will present findings from the insect visual system, in particular recordings of fly photoreceptors, and present outdoor recordings demonstrating the system's ability to adapt to extremely high light levels such as direct sunlight illumination which would saturate or even damage human photoreceptors. Further, I'll demonstrate that synaptic filtering as occurring at the first synapse in the system can be a mechanism increasing the discriminability of visual input signals by rejecting internal noise. This noise is induced by large low-frequency modulations as are characteristic of natural stimuli.

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Modulation of gap junction signaling by intrinsic membrane currents

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Electrical synapses, mediated by gap junction channels, are widespread in both vertebrate and invertebrate neuronal systems. In mature mammals, electrical synapses are most often comprised of connexin 36, and couple inhibitory neurons of similar functional and biochemical profiles (Galaretta and Hestrin, 2001). The thalamic reticular nucleus (TRN), which provides the main source of feedback inhibition to thalamocortical projections, is composed exclusively of GABAergic inhibitory neurons(Nagai, McGeer, and McGeer, 1983) and has an extremely high density of gap junctions (Landisman et al., 2002). Like other thalamic neurons, TRN cells have two voltage-dependent firing modes: burst and tonic spiking, which correspond to different states of alertness (Steriade, Domich, and Oakson, 1986).
Using dual whole-cell recordings of in vitro rodent TRN neurons, we demonstrate that the amplitude of signals relayed by gap junctions depends strongly on the baseline postsynaptic membrane potential. Our results indicate that activation and inactivation of intrinsic persistent sodium currents (INaP) are responsible for the observed voltage scaling, which can cause up to four-fold changes in electrical synaptic signaling strength and can be eliminated by the sodium channel blocker TTX. This amplification is most effective in synchronizing tonic spikes, and it occurs over membrane voltages similar to the range in which thalamic neurons shift between tonic and burst firing states. This modulation of coupling strength can exert great influence on the nature and synchrony of postsynaptic inhibition provided by these neurons to their target relay cells, and thus it provides another mechanism to refine the flow of information from the periphery to cortex.

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Single cell morphology of retinal ganglion cell axon terminals in the mouse brain

Y. Kate Hong
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Retinal ganglion cells (RGCs), the sole output neurons of the retina, extend long axons to brain target regions where they send sensory information about the environment. Since the first anatomical studies by Ramon y Cajal over a century ago, much work has been dedicated to classifying RGCs based on their size, shape and laminar position of their dendrites within the retina. Moreover, these morphologically distinct subtypes of RGCs are thought to have distinct functional properties. Altogether, there are over a dozen RGC subtypes in mammalian retina, each with a unique structure and function. Because most studies of RGC subtypes have focused on the retina, little is known about the diversity of their axon terminals in the brain. We are using viral gene delivery methods as well as transgenic approaches to sparsely label RGC subsets in mouse, so we can image their terminal arbors in lateral geniculate nucleus and superior colliculus. We image fluorescently labeled cells in the retina, and retinotopically pair them to their axon terminals in the brain. Our work indicates that different RGC cell types also have different axon terminal morphologies and laminar locations suggesting that, anatomically, parallel processing streams are maintained in the mouse visual system.

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Dual roles of the vomeronasal organ in conspecific and heterospecific odor detection

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The vomeronasal organ is an accessory olfactory organ that widely exists among vertebrates and largely believed to serve as a major interface for the detection of pheromones, conspecific chemosignals that communicate species-specific information such as gender, social status as well as reproductive states.
In house mice, approximately 300 distinct chemoreceptors are expressed in the epithelium of the vomeronasal organ. Despite our detailed understanding of pheromone detection via vomeronasal pathways, we know surprisingly little about the functions of individual pheromone receptors and the molecular logic underlying receptor utilization for a variety of potential chemosignals. Here, we describe novel experimental approaches to identify vomeronasal receptors responsible for detecting different classes of semiochemicals. We found that not only are some vomeronasal receptors utilized for pheromone detection, but also a large number of vomeronasal neurons in fact appear to be utilized for the detection of heterospecific odors, especially those of predators. Importantly, receptors utilized for detecting heterospecific chemicals appear to be distinct from those detecting conspecific chemicals. Moreover, behavioral analyses using TrpC2 null mice lacking vomeronasal organ functions suggest that TrpC2 mutant mice fail to discriminate heterospecific odor cues. Taken together, our study implicates roles of the vomeronasal organ in recognition of both conspecific and heterospecific chemosignals and illustrate how rodents might have evolved specific molecular mechanisms to detect various environmental cues necessary for their fitness and survival.

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TFIIF binds the RNA polymerase II funnel and stimulates mRNA cleavage

Anass Jawhari
LMU, Department of Chemistry and Biochemistry

RNA polymerase (Pol) II initiation and elongation require the general transcription factor TFIIF. We present cryo-electron microscopic structures of the complete Pol II in complex with TFIIF, TFIIF and a DNA-RNA elongation scaffold, TFIIF and TFIIS, and with a core variant of TFIIF that comprises the Tfg1-Tfg2 dimerization module and the adjacent Tfg1 charged region. TFIIF binds on the Rpb2 side of the cleft and the TFIIF core binds the funnel. The charged region may protrude into the pore and is required for a novel mRNA cleavage-stimulatory activity of TFIIF that is specific for backtracked complexes. These results suggest the molecular basis for TFIIF functions, including stimulation of initial transcription and elongation, stabilization of the post-translocated state, and suppression of pausing and TFIIS-induced mRNA cleavage. The TFIIF core and a related module in Pol I and Pol III emerge as the key elongation stimulatory activities for all eukaryotic transcription.

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A combined electrophysiological and neurogenetic approach towards the cellular dissection of motion computation in Drosophila

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MPI for Neurobiology, Department of Systems and Computational Neurobiology

Visual motion detection is fundamental for orientation and navigation through the environment and ultimately to the survival of most animals with eyes. Remarkably, from humans to flies, uniform neuronal computations and algorithmic rules seem to underlie visual motion detection. However, the neuronal implementation of these rules has not been fully revealed in any organism until today.
We established the fruit fly Drosophila melanogaster as a genetically amenable experimental animal to investigate this classical problem in neuroscience at the cellular level. We report the first in vivo whole cell recordings from genetically identified visual interneurons during visual stimulation. A population of motion-sensitive large-field neurons is described that form a network consisting of individually identifiable, directionally-selective cells most sensitive to vertical (VS-) or horizontal (HS-cells) image motion. Their response properties to visual motion stimuli exhibit the fundamental characteristics indicative of presynaptic input from correlation-type motion detectors. Similarly, the main network properties match the ones previously described in Calliphora and other fly species.
Based on these findings we started to analyze the motion processing circuit in the lamina and medulla by expressing Tetanus-Neurotoxin in columnar interneurons and recording from the postsynaptic VS-cells in these flies. Blocking vesicle release in lamina monopolar cells L1 and L2 identified these two cell types as major input pathways to the motion detection circuitry. The L1 and L2 pathways interact to enhance contrast sensitivity and to detect the motion of edges with decreasing brightness. These results represent the first steps towards uncovering the cellular implementation of the correlation-type motion detector.

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Chemical warfare in the hive: symbiotic bacteria protect honeybees from devastating pathogen

Renee Kontnik
Harvard, Department of Biological Chemistry and Molecular Pharmacology

Many, if not all, animals participate in symbiotic relationships with microbial partners. These relationships can be varied and complex, including interactions that may be pathogenic or mutually beneficial. Honeybees, for example, live in close association with both mutualistic and pathogenic bacteria. Infection of honeybees by the bacterium Paenibacillus larvae leads to American Foulbrood, a disease that can destroy entire hives and costs the bee industry millions of dollars annually. Sampling healthy, uninfected beehives led to the isolation of an actinomycete strain which inhibits growth of the pathogenic P. larvae. Laboratory cultures of this protective actinomycete were extracted and fractionated to uncover the specific compound responsible for activity. Structural characterization of the purified compound revealed a new macrocyclic polyene lactam antibiotic.

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Role of GAD67 in the function and plasticity of GABAergic synapses

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Harvard, Department of Molecular and Cellular Biology

Interneurons that utilize gamma-aminobutyric acid (GABA) as neurotransmitter control network function by maintaining synaptic excitation-inhibition balance. Although ample evidence demonstrates that glutamic acid decarboxylase 67 (GAD67) synthesizes the majority of GABA in the brain, it is unclear how GAD67 regulates GABAergic function and circuit formation. We investigated the role of GAD67 using a mouse in which GAD67 expression is abrogated by the insertion of EGFP into the genomic locus. Since GAD67-/- animals die shortly after birth, we examined synaptic properties of GABAergic transmission in dissociated hippocampal neurons cultured from P0 pups. Electrophysiological recordings from non-green, pyramidal neurons showed that homozygous deletion of GAD67 significantly reduced miniature inhibitory postsynaptic current (mIPSC) amplitudes. Application of (1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA), a low affinity, competitive GABAa receptor antagonist, reduced mIPSC amplitudes in wild-type cultures; the fractional blockade by TPMPA was higher in GAD67-lacking neurons, suggesting GABA concentration in vesicles is lower despite unaltered levels of the second GABA synthetic enzyme GAD65 levels in synaptic terminals. This result was corroborated by reduced somatic GABA staining in GAD67-deleted neurons. Immunostaining for excitatory and inhibitory markers revealed no obvious defects in synapse formation. Chronic suppression of activity by application of tetrodotoxin (for 2 days) reduced GAD67 and GABA levels, as revealed by immunofluorescent staining. Importantly, homeostatic downregulation of mIPSC amplitude was significantly impaired in GAD67 knockout neurons, suggesting that GAD67 is a key regulatory target of activity. Taken together, these findings suggest that activity-driven expression of GAD67 critically determines synthesis of GABA and, thus, vesicular loading of the transmitter.

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On the biogenesis of cyclopiazonic acid

Xinyu Liu
Harvard, Department of Biological Chemistry and Molecular Pharmacology

Cyclopiazonic acid (CPA) is a potent neurotoxin with a highly constrained pentacyclic indole tetramic acid scaffold that acts as a nanomolar inhibitor of sarcoplasmic reticulum Ca2+ ATPase. Recently, we and others identified the biosynthetic gene clusters of CPA from Aspergillus sp. In this talk, we will describe the detailed biochemical characterizations of two novel aspects on CPA biosynthesis: 1) formation of the indole tetramic acid scaffold by an unusual non-ribosomal peptide synthetase with a C-terminal redox-incompetent reductase; 2) tailoring of the indole tetramic acid scaffold by a promiscuous dimethylallyl-transferase.

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Multiquantal release underlies the distribution of synaptic efficacies in the neocortex

Alex Loebel
LMU, Department Biology II

Inter-pyramidal synaptic connections are characterized by a wide range of EPSP amplitudes. Although repeatedly observed at different brain regions and across layers, little is known about the synaptic characteristics that contribute to this wide range. In particular, the range could potentially be accounted for by differences in all three parameters of the quantal model of synaptic transmission, i.e. the number of release sites, release probability and quantal size. Here, we present a rigorous statistical analysis of the transmission properties of excitatory synaptic connections between layer-5 pyramidal neurons of the somatosensory cortex. Our central finding is that the EPSP amplitude is strongly correlated with the number of estimated release sites, but not with the release probability or quantal size. In addition, we found that the number of release sites can be more than an order of magnitude higher than the typical number of synaptic contacts for this type of connection. Our findings indicate that transmission at stronger synaptic connections is mediated by multiquantal release from their synaptic contacts. We propose that modulating the number of release sites could be an important mechanism in regulating neocortical synaptic transmission. The analysis of multineuron patch-clamp recordings that support this prediction is presented.

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A strategy to investigate the regulation of the immune response against cancer through multiphoton intravital microscopy

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The immune system has the potential to reject tumors, with a critical involvement of tumor antigen-specific CD8+ cytotoxic T cells. However, CD4+CD25+ natural T regulatory cells (nTregs) often hamper this beneficial response. We set up a combined approach of multiphoton intravital microscopy (MP-IVM) and viral vector-mediated gene transfer to study the cellular crosstalk by which nTregs convey immunosuppressive signals to antitumor CD8+ T cells in vivo.
Tumors were generated in mice by subcutaneous injection of the colon cancer cell line CT26 expressing the model antigen influenza hemagglutinin (HA) and a fusion of the fluorescent protein Cerulean with the histone H2B. The latter allowed us to monitor tumor cell mitosis as well as nuclear disintegration accompanying tumor cell killing by MP-IVM. To test our hypothesis that nTregs alter T cell receptor (TCR) signaling in CD8+ T cells, we used two fusion proteins changing subcellular localization after TCR stimulation. We introduced eGFP-fusions with the pleckstrin homology domain of Akt (recruited to the membrane) or the consensus regulatory domain of NFAT proteins (shuttling to the nucleus) into HA-specific CD8+ T cells by retroviral transduction. This allowed us to monitor TCR signaling in CTLs by MP-IVM after adoptive transfer into tumor-bearing mice.
HA-specific nTregs disabled the rejection of CT26-HA tumors by CD8+ T cells, yet we did not observe contacts between nTregs and CD8+ T cells in vivo. We are now hypothesizing that nTregs convey suppressive signals through shared antigen-presenting cells, possibly tumor-associated macrophages (TAMs). To visualize TAMs, we created a lentiviral vector expressing mCherry controlled by the myeloid-specific SP146 promoter. This vector allowed for myeloid-specific expression of mCherry in bone marrow chimeric mice, and will be valuable to study putative cellular interactions of TAMs with nTreg and CD8+ T cells in tumors by MP-IVM.
In conclusion, we describe a novel and state-of-the-art technology to investigate the regulation of an antitumor immune response directly in living animals. Understanding how nTregs hamper the antitumor immunity will be crucial to design effective protocols for tumor immunotherapy.

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Eyes smarter than scientists believed: Neural computations in the retina

Markus Meister
Harvard, Center for Brain Science

Starting with the raw optical image projected on the photoreceptors, the retina performs a great deal of visual processing to compute about a dozen parallel neural images, which are transmitted to various areas of the brain. In this talk, I will discuss three of these neural computations identified only recently, all related to the processing of image motion. In each case, I hope to demonstrate the overall network function, explain how it is performed in terms of circuits with neurons and synapses, and discuss what purpose it serves in the overall context of vision.

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Magnetic levitation as a platform for density-based analysis and separations

Katherine Mirica
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We developed a simple and inexpensive method that uses magnetic levitation to analyze and separate diamagnetic particles based on density and differences in density. The basis for the analysis and separation is the balance of the magnetic and gravitational forces acting on diamagnetic particles suspended in a paramagnetic medium. The method requires only a capillary tube (or a vial) that contains a paramagnetic solution (Mn2+ or Gd3+ in water), and two permanent NdFeB magnets oriented with like poles facing one another. We apply this method to monitor chemical reactions on solid supports and to detect binding of proteins to resin-bound small molecules.

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Structure and in vivo requirement of the yeast Spt6 SH2 domain

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LMU, Department of Chemistry and Biochemistry

During transcription elongation through chromatin, the Ser2-phosphorylated C-terminal repeat domain of RNA polymerase II binds the C-terminal Src homology 2 (SH2) domain of the nucleosome re-assembly factor Spt6. This SH2 domain is unusual in its specificity to bind phosphoserine, rather than phosphotyrosine and because it is the only SH2 domain in the yeast genome. Here, we report the high-resolution structure of the SH2 domain from Candida glabrata Spt6. The structure combines features from both structural subfamilies of SH2 domains, suggesting it resembles a common ancestor of all SH2 domains. Two conserved surface pockets deviate from those of canonical SH2 domains, and may explain the unusual phosphoserine specificity. Differential gene expression analysis reveals that the SH2 domain is required for normal expression of a subset of yeast genes, and is consistent with multiple functions of Spt6 in chromatin transcription.

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Connecting cellular signals to gene expression: the “mediator phosphorsubprote ome”

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The Mediator complex is one of the key-players in regulation of expression of genes transcribed by RNA-Polymerase II (Pol II). In Saccharomyces cerevisiae, the Mediator complex is required for activator-dependent stimulation of Pol II transcription, which suggests a function in connecting cellular signals to the general Pol II transcription machinery. Cells exposed to high salinity rapidly respond by switching on the HOG-pathway, which leads to rapid activation of the gene expression program for immediate glycerol production to antagonize osmotic pressure. The HOG-pathway is centered on the mitogen activated protein (MAP) kinase HOG1, which translocates into the nucleus after phosporylation by upstream kinases and interacts directly with the Pol II transcription machinery. Although it has been shown that HOG1 phosphorylates the C-terminal domain of Pol II and interacts with SAGA, it is still unclear if HOG1 phosporylates other factors required for transcription initiation, particularly the Mediator complex. In order to obtain detailed information on signal processing at the level of Mediator, we used stable isotope labeling with amino acids in cell culture (SILAC) together with mass spectrometry (MS) analysis to investigate phosphorylation events upon osmotic stress. With this strategy, we were able to identify a large number of phosphoserine, -threonine and –tyrosine residues in Mediator subunits. Our preliminary results suggest a dynamic Mediator phosphorylation pattern upon osmotic stress stimuli.

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Neurotrophic function of transcription factor Pax6 in the mature dopaminergic olfactory bulb interneurons

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Transcription factors acting as fate determinants trigger the expression of specific transcript sets at certain phase in the neural progenitor specification necessary for the generation of different types of neural cells and neuronal subtypes. However, some transcription factors such as Pax6 remain expressed in the postmitotic, fully functional neurons. Here we show that the transcription factor Pax6 has a neurotrophic function in the mature dopaminergic olfactory bulb interneurons. Specific loss of Pax6 function in the mature dopaminergic neurons causes apoptosis. We further show that Pax6 in the mature dopaminergic neurons controls the expression of small heat-shock protein crystallin (CryAA). Moreover, virus mediated overexpression of CryAA rescues the loss of dopaminergic neurons in Pax6 mutant animals. Our data suggest that CryAA prevents activation of pro-caspase3 and therefore promotes survival of dopaminergic neurons. Interestingly, CryAA expression in the olfactory bulb is controlled by the Pax6 homeodomain and the animals lacking the homeodomain have reduced number of mature, functional dopaminergic neurons at adulthood. These data are in the line with the model where the homeodomain containing transcription factor controls the function of the molecular chaperone, CryAA, and therefore prevents the activation of the apoptotic pathway. This is up to our knowledge the first report of the neurotrophic function of the molecules acting as a fate determinants and the possible indication of more general mechanism controlling survival of postmitotic neurons.

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Expanding the repertoire of ABPP

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LMU, Department of Chemistry and Biochemistry

The goal of activity based protein profiling (ABPP) is to develop and apply chemicals probes that monitor the activity of many enzymes in parallel within the confines of proteomes in which they are naturally expressed. These probes consist of at least two chemical elements: 1) a reactive group for binding and covalently labeling the active sites of many members of a given enzyme class (or classes), and 2) a reporter tag for the rapid and sensitive detection and isolation of labeled enzymes from proteomes. This tag can be attached by the Huisgen cycloaddition between an alkyne and an azide. The labeled proteomes can be visualized and compared by SDS PAGE analysis and subsequent fluorescent scanning. Activity profiles can be used to identify disease associated enzymes as therapeutic targets or diagnostic markers.
Till today the variety of chemical probes used in ABPP is limited. In order to address new enzyme classes, we developed a whole new set of chemical probes for their application in ABPP in the past few years. This was achieved by derivatising diverse natural compounds. Those chemical probes were tested in ABPP and specificly labeled various enzyme classes. Another goal of our work was to improve the isolation technique of already labeled proteins. Currently solutions of isolated proteins contain minor protein impurities. Those create false positive results in MS analysis. To prevent this in the future we developed the basics of a purification system that enables mild and selective purification of probe labeled proteins without major impurities.

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A zebrafish model of Alzheimer's disease

Dominik Paquet, Ratan Bhat, Eva-Maria Mandelkow, Reinhard Köster,
Bettina Schmid, Christian Haass
LMU, Adolf-Butenandt-Institut

Our ageing society is confronted with a dramatic increase of patients suffering from Tauopathies, which include Alzheimer's disease and certain Frontotemporal Dementias. These disorders are characterized by typical neuropathological lesions including hyperphosphorylation and subsequent aggregation of Tau protein and neuronal cell death. Currently, no mechanism-based cures are available. Genetically modified animals are invaluable models to understand the molecular mechanisms of pathology and screen for disease-modifying compounds. We have generated the first Tau-transgenic zebrafish, which rapidly recapitulate key pathological features of Tauopathies including phosphorylation and conformational changes of human Tau protein, tangle formation, as well as neuronal and behavioral disturbances and cell death. In contrast to existing vertebrate models zebrafish larvae are ideally suited for both in vivo imaging and drug development due to their optical transparency and small size. Therefore, the transgenic fish are important tools better understand the pathology of Tauopathies and develop treatment approaches. We could already demonstrate for the first time that neuronal cell death can be imaged by time-lapse microscopy in vivo. Furthermore, we used our fish-model to identify new compounds targeting the Tau-Kinase GSK3,, since phosphorylation of Tau is believed to be a trigger for disease progression. We identified a novel highly active GSK3, inhibitor, which we developed by rational drug design and validated for in vivo activity in the transgenic fish model.

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Receptor reprogramming and photoswitchable agonist for the iGluRs

Alwin Reiter, Philipp Stawski
LMU, Department of Chemistry and Biochemistry

For all living organisms, the sensing of extracellular molecules and transmission of that signal into the cytoplasm is a very important process. In the mammalian brain, the majority of excitatory neurotransmissions are mediated by glutamate receptors. Due to its overwhelming complexity however, there is a constant need for new tools that help to dissect the neuronal circuitry in order to gain a detailed understanding for example in signaling pathways or how behavior is formed on a neuronal level.
Despite the fact that our understanding of cellular processes in neurons is quite good, our ignorance of network activity is bliss. Or not? It has been said that 'if the brain were so simple we could understand it, we would be so simple that we couldn't'.
In our attempt to participate in the unraveling of the mystery 'brain' we seek to develop photoswitchable ligands that are selective for certain iGluR subtypes. In addition to that, we aim at synthesizing artificial transmitters that only activate populations of genetically defined iGluRs.

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Coupling splicing and chromatin in S.cerevisiae

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Eukaryotic gene expression includes several steps that must be coordinated to allow organisms to survive, develop and adapt to environmental changes. The first step in gene expression involves the transcription of genes with the production of mRNAs that are later translated into proteins. Between the initial and final events of gene expression the mRNA undergoes several processing steps that affect its ability to be translated. One of the most important mRNA processing events is the removal of non-coding sequences from the RNA, a process called splicing.
Recent evidence shows that transcription and splicing are not temporarily separated and must be connected in order to achieve correct gene expression.
Using S.cerevisiae as a model system we studied the recruitment of splicing factors during transcription and how this recruitment correlates with chromatin modifications. Our results show that particular histone modifications can modulate the recruitment of splicing factors during transcription and therefore affect the efficiency of RNA processing. Taking advantage of the fact that splicing occurs co-transcriptionally we employed chromatin immunoprecipitation of specific splicing factors to pinpoint the specific stages in the splicing process that are coupled to chromatin modifications. The results obtained argue that transcription and splicing are coupled by several different mechanisms both direct and indirect, in some cases involving other mRNA processing events.
While only a small fraction of the total number of genes in S.cerevisiae are spliced, the defects in splicing we observed in particular cases seem to have global effects in gene expression even of genes not spliced. Our data not only allows a better understanding of the coupling between transcription and RNA processing but also provides additional details to explain the regulation of each individual mechanism.

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Activation of melanoma differentiation-associated gene 5 causes rapid involution of the thymus

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LMU, Division of Clinical Pharmacology

In the course of infection, the detection of pathogen-associated molecular patterns by specialized pattern recognition receptors in the host leads to activation of the innate immune system. Whereas the subsequent induction of adaptive immune responses in secondary lymphoid organs is well described, little is known about the effects of pathogen-associated molecular pattern-induced activation on primary lymphoid organs.
Here we show that activation of innate immunity through the virus-sensing melanoma differentiation-associated gene 5 (MDA-5) receptor causes a rapid involution of the thymus. We observed a strong decrease in thymic cellularity associated with characteristic alterations in thymic subpopulations and microanatomy. In contrast, immune stimulation with potent TLR agonists did not lead to thymic involution or induce changes in thymic subpopulations, demonstrating that thymic pathology is not a general consequence of innate immune activation. We determined that suppression of thymocyte proliferation and enhanced apoptosis are the essential cellular mechanisms involved in the decrease in thymic size upon MDA-5 activation. Further, thymic involution critically depended on type I IFN. Strikingly however, no direct action of type I IFN on thymocytes was required, given that the decrease in thymic size was still observed in mice with a selective deletion of the type I IFN receptor on T cells. All changes observed were self-limiting, given that cessation of MDA-5 activation led to a rapid recovery of thymic size.
We show for the first time that the in vivo activation of the virus-sensing MDA-5 receptor leads to a rapid and reversible involution of the thymus.

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Controlling neuronal activity with molecular switches

Dirk Trauner
LMU, Department of Chemistry and Biochemistry

The ion channels, transporters and metabotropic receptors that underlie neuronal activity can be seen as molecular machines that are amenable to functional manipulation. One of the most rewarding functions to add is sensitivity toward light. Our group has succeeded in rendering glutamate receptors and voltage-gated potassium channels light-sensitive using a combination of synthetic chemistry and protein engineering. I will discuss the principles that underlie the design of artificial photoreceptors and show their applications in cultured neurons, brain slices, and intact animals (e.g. zebrafish, leeches and mice). The usefulness use of these systems in the elucidation of neural circuitry and the restoration of vision will be discussed as well.

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Isolation of multipotent astroglia from the adult stem cell niche and the injured brain

Pratibha Tripathi, Marcos Costa, Ruth Beckervorder Sandforth-Bonk, Jovica Ninkovic, Magdalena Götz
Helmholtz Zentrum, Department of Biology

Adult neural stem cells, as the source of life-long neurogenesis, reside in the subependymal zone (SEZ) lining the lateral wall of the lateral ventricles and in the dentate gyrus of the hippocampus. In both neurogenic regions, a subset of glial fibrillary acidic protein (GFAP) protein expressing astrocytes functions as stem cells, but it is not known how to distinguish these stem cell astrocytes from other astrocyte populations. We have identified a sub-population of adult SEZ astrocytes that expresses the CD133. Using fluorescence-activated cell sorting to isolate these distinct populations of astrocytes from hGFAP/eGFP mice, we show that the exclusively SEZ astrocyte populations isolated from hGFAP/eGFP mice that expresses CD133 receptor are able to form self-renewing multipotent neurospheres. Further transcriptional profiling of these multipotent astrocytes revealed specific charecte-ristics of these neurogenic astrocytes compared to non-neurogenic parenchymal astroglia. Strikingly, we found a similar population of dedifferentiated astrocytes in non-neurogenic brain regions after following stab wound injury in the cerebral cortex. Moreover, these astrocytes isolated from the injured cortex, also reveal the stem cell properties of self-renewal and multipotency. Also we were able to show that Shh is one of the endogeneous signals that causes the de-differenciation or reprograming of cells to stem cell state upon injury in vitro. Taken together, our work allows, for the first time, the identification and characterization of the astrocyte subtypes acting as neural stem cells.

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Regulation of the alkyl-dihydrothiazole-carboxylates (ATCs) by iron and the pyochelin gene cluster in Pseudomonas aeruginosa

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As genome sequencing projects of bacteria and fungi reveal uncharacterized biosynthetic gene clusters there emerges a need for approaches that accelerate the discovery and characterization of the secondary metabolites regulated by these clusters. Here, using the pyochelin (pch) gene cluster as an example, we demonstrate the utility of untargeted metabolomics in this endeavor. Comparison of the extracellular metabolomes of pch gene cluster mutants to the wild-type Pseudomonas aeruginosa (strain PA14) identified 198 ions regulated by the pch genes. In addition to known metabolites, we characterized the structure of a pair of novel metabolites regulated by the pch gene cluster as 2-alkyl-4,5-dihydrothia-zole-4-carboxylates (ATCs), using a combination of mass spectrometry, chemical synthesis, and stable isotope labeling. Subsequent assays revealed that ATCs bind iron and are regulated by iron levels in the media in a similar fashion to other metabolites associated with the pch gene cluster. Overall, these studies highlight the ability of untargeted metabolomics to reveal regulatory connections between gene clusters and secondary metabolites, including novel metabolites.

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