LUDWIG-
MAXIMILIANS-
UNIVERSITÄT
MÜNCHEN

HARVARD UNIVERSITY

LMU-Harvard Young Scientists' Forum

From Molecules to Organisms III
Munich, June 26 – 30, 2011

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.
The first conference of the series was held at LMU in June 2009, followed by a conference at Harvard University in 2010.
LMU-Harvard YSF 2011 will be held at the Center for Advanced Studies (CAS[LMU]) of the LMU Munich.

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- LMU-Harvard Young Scientists' Forum at the Center for Advanced Studies (CAS[LMU]), LMU: From Molecules to Organisms III, Munich, June 26 – 30, 2011
- Under the auspices of Prof. Dr. Ulrich Pohl, Vice-President for International Affairs, LMU
- Academic Board: Prof. Dirk Trauner (Center for Integrated Protein Science Munich, CIPSM), Prof. Dr. Benedikt Grothe (Munich Center for Neurosciences, MCN)
- Program Management: Simone Glasl (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), Dr. Nina Mäusbacher, Alexander Mathis, Alvaro Tejero Cantero (Ph.D. / Post-doctoral representatives)
- Institutional Responsibility: LMU International Affairs, Center for Advanced Studies (CAS[LMU])

Conference agenda

June 26th

Sunday	
	Arrival in Munich (Transfer to the Hotel individually arranged) Hotel Cosmopolitan, Hohenzollernstr. 5, 80801 München, Phone: +49 (0) 89 383810, www.cosmopolitanhotel.de
15.00 – 16.30	**Optional Program: Guided city tour** (meeting point: hotel lobby)
19.00	meeting point: hotel lobby, walk to the restaurant
19.30	**Welcome Dinner** Seehaus im Englischen Garten, Kleinhesselohe 3, 80802 München, Phone: +49 (0) 89 381 613 0

June 27th

Monday	
	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 218072080
08.30	meeting point: hotel lobby, walk to the CAS
09.00 – 09.10	**Opening Remarks: Ulrich Pohl**, Vice President, LMU
09.10 – 10.10	lecture 1 – **Christian Haass**: "The molecular clockwork of Alzheimer's disease"
10.10 – 10.30	coffee break (catered)
10.30 – 12.30	session 1 "Pathologies" (Chair: Christian Haass) **Dorothee Dormann** – "ALS-associated FUS mutations disrupt Transportin-mediated nuclear import" **Stylianos Michalakis** – "Gene replacement therapy for retinal CNG channelopathies" **Michael McKeown** – "Development of direct acting inhibitors of epigenetic targets" **Moritz Rapp** – "Toll-like receptor agonists prevent regulatory T cell infiltration of tumors by specifically inhibiting migration of FoxP3+ cells"
12.30 – 13.30	lunch (catered)

13.30 – 14.30	lecture 2 – **Catherine Dulac**: „Sex Battles in the Brain: a Genome-Wide Analysis of Genomic Imprinting“
14.30 – 14.50	coffee break (catered)
14.50 – 16.50	session 2 “Development and Neural Circuits” (Chair: Markus Meister)
	Sylvia Cappello – “A new mouse model of ‘double cortex’ – the role of RhoA in cortical development”
	Patrick Müller – “The distribution of Nodal and Lefty signals is determined by differential diffusivity and clearance”
	Yong Li – “Color Vision Circuitry in the Outer Retina of Zebrafish”
	Jonathan Garst Orozco – “Learning-related synaptic reorganization in the motor control circuits of songbirds”
16.50 – 17.10	coffee break (catered)
17.10 – 18.10	lecture 3 – **Matthias Mann**: “High resolution, quantitative proteomics turbo-charges biochemistry”
18.30 – 22.00	**Conference Dinner** Reitschule, Königinstr. 34, 80802 München, Phone: +49 (0) 89 3888760

June 28th

Tuesday	
	Center for Advanced Studies (CAS^LMU^) Seestraße 13, 80802 München, Phone: +49 (0) 89 218072080
09.00 – 10.00	lecture 4 – **Tobias Bonhoeffer**: “How activity changes synapses in the mammalian brain”
10.00 – 10.20	coffee break (catered)
10.20 – 12.20	session 3 “Neural circuits” (Chair: Felix Felmy)
	Hubert Eichner – “Two separate motion detectors for on and off signals”
	Risa Kawai – “The Role of Motor Cortex in the Production of Complex Motor Sequences”
	Aditi Deshpande – “Rabies virus mediated tracing of synapses onto adult generated neurons”
	Arpiar Saunders – “Balance of direct/indirect pathway activity governs corticostriatal synaptogenesis”
12.20 – 13.30	lunch (catered) & **YSF future session** *(faculty only: CAS ‘oval office’)*
13.30 – 14.15	bus transfer CAS – HighTechCampus
	Munich Center for Neurosciences MCN^LMU^ Department Biology II Neurobiology Großhadernerstraße 2, 82152 Planegg – Martinsried
	Munich Center for Integrated Protein Sciences, CIPSM Department of Chemistry and Biochemistry Butenandtstraße 5-13, 81377 München
14.30 – 16.30	session 4 “Molecular methods” (Chair: Jon Clardy) *LMU Dept. Chemistry and Pharmacy, building C, seminar room C2.003*
	Alwin Reiter – “Structure of the ligand binding domain of an iontropic glutamate receptor with an optical switch”
	Peter Duewell – “Tri-functional siRNA combining TGF-beta silencing, RIG-I activation and apoptosis induction induces effective antitumor responses in pancreatic carcinoma”
	Gaëtan Bellot – “Malleable DNA-Nanostructures as Alignment Media for Membrane Protein NMR”
	Johannes Kreuzer – “Acivicin as a probe for ABPP (Activity-Based Protein Profiling)”
	session 5 “Neural Coding” (Chair: Christian Leibold) *LMU Dept. Biology II, BioCenter, seminar room D00.013*
	Alexander Mathis – “Optimal Distribution of Spatial Periods for Grid Cells Ensembles on Linear Track”
	Margarida Agrochao – “Visual Cortex in Motion”
	Alice Yiqing Wang – “Neural basis of decision making: choosing what to do and how quickly to act”
16.30 – 16.50	coffee break (catered)
16.50 – 17.50	lecture 5 – **William Shih**: “Self-assembled DNA-nanostructure tools for molecular biophysics”; *LMU Dept. Pharmacy; building F; lecture hall FU1.013*
	lecture 6 – **Venkatesh Murthy**: “Feedback control of olfactory processing in mammals”; *LMU Dept. Biology II; BioCenter; lecture hall B01.019*
18.00 – 18.45	bus transfer HighTechCampus – CAS

	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 218072080
19.00 – 19.30	**CAS Reception**
19.30 – 21.00	Parmenides Lecture – **Antonio Damasio (assoc. YSF event)**: “Onto- and phylogenesis of selfhood”

June 29th

Wednesday	
	Center for Advanced Studies (CAS[LMU]) Seestraße 13, 80802 München, Phone: +49 (0) 89 / 218072080
09.00 – 10.00	lecture 7 – **Thomas Carell**: “The chemistry of genome maintenance”
10.00 – 10.20	coffee break (catered)
10.20 – 12.25	session 6 “Signaling” (Chair: Benedikt Berninger) **Pia Johansson** – “Long distance signalling from the hindbrain choroid plexus to forebrain stem cells via WNT modulators secreted into cerebrospinal fluid” **Jason Zhang** – “Domain-level essentiality of the Mycobacterium tuberculosis genome” **Falko Hampel** – “FLRT2 and FLRT3 act as repulsive guidance cues for Unc5-positive neurons” **Laura Strittmatter** – “A Systems Analysis of Mitochondrial Metabolism” **Alvaro Tejero-Cantero** – “Coherent phasic excitation during hippocampal ripples”
12.25 – 14.00	lunch break (catered) & YSF posters **Johannes Broichhagen** – “Optochemical genetics: Photocontrolling enzymes” **Felix Felmy** – “Comparing the synaptic transmission and postsynaptic integration of large synapses in the auditory brainstem of mongolian gerbils” **Thomas Gerling** – “Re-building evolution: toward synthetic nucleotide-dependent switch structures assembled with DNA origami” **Thomas Martin** – “Custom DNA-origami apertures for solid-state nanopores”
14.00 – 16.00	session 7 “Chemical Biology” (Chair: Dirk Trauner) **Kevin Esvelt** – “A system for the continuous directed evolution of biomolecules” **Nina Mäusbacher** – “Natural product-inspired novel antibiotics” **Anna Lone** – “A substrate-free activity-based protein profiling screen for the discovery of selective PREPL inhibitors” **Laura Stone** – “A screen for compounds that select against antibiotic resistance”
16.00 – 16.20	coffee break (catered)
16.20 – 17.20	lecture 8 – **Eric Rubin**: “Making mistakes in translation, accidentally on purpose”
17.20 – 17.30	closing remarks and comments
17.30 – 18.15	wrap-up session (faculty only; CAS ‘oval office’)

July 30th

Thursday	
08.30 – 19.00	Excursion to Berchtesgaden (hiking) *(meeting point: hotel lobby)*

July 1st

Friday	
	Departure, individually arranged

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

- **Margarida Agrochao**
  Harvard, Harvard Center for Brain Science, laboratory of Markus Meister
- **Gaëtan Bellot**
  Harvard, Department of Cancer Biology, laboratory of William Shih
- **Kenny Blum**, Executive Director
  Harvard Center for Brain Science
- **Jon Clardy**, Professor
  Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology
- **Catherine Dulac**, Professor
  Harvard, Department of Molecular and Cellular Biology
- **Florian Engert**, Professor
  Harvard, Department of Molecular and Cellular Biology
- **Kevin Esvelt**
  Harvard, Department of Chemistry and Chemical Biology, laboratory of David Liu
- **Jonathan Garst Orozco**
  Harvard, Department of Organismic and Evolutionary Biology, laboratory of Bence Ölveczky
- **Risa Kawai**
  Harvard, Department of Organismic and Evolutionary Biology, laboratory of Bence Ölveczky
- **Michael McKewon**
  Harvard, Harvard Medical School, laboratory of James E. Bradner
- **Yong Nathan Li**
  Harvard, Department of Molecular and Cellular Biology, laboratory of John Dowling
- **Anna Mari Lone**
  Harvard, Department of Chemistry and Chemical Biology, laboratory of Alan Saghatelian
- **Markus Meister**, Professor
  Harvard, Department of Molecular and Cellular Biology, Harvard Center for Brain Science
- **Patrick Mueller**
  Harvard, Department of Molecular and Cellular Biology, laboratory of Alex Schier
- **Venkatesh Murthy**, Professor
  Harvard, Department of Molecular and Cellular Biology
- **Eric Rubin**, Professor
  Harvard, Department of Immunology and Infectious Diseases
- **Arpiar Saunders**
  Harvard, Department of Neurobiology, laboratory of Bernardo Sabatini
- **William Shih**, Professor
  Harvard, Department of Cancer Biology
- **Laura Stone**
  Harvard, Department of Systems Biology, laboratory of Roy Kishony

- **Laura Strittmatter**
  Harvard, Department of Systems Biology, laboratory of Vamsi Mootha
- **Alice Yiqing Wang**
  Harvard, Department of Molecular and Cellular Biology, laboratory of Naoshige Uchida
- **Jason Zhang**
  Harvard, Department of Immunology and Infectious Diseases, laboratory of Eric Rubin

Ludwig-Maximilians-Universität München (LMU)

Technische Universität München (TUM)
Max Planck Institute of Neurobiology (MPI of Neurobiology)
Max Planck Institute of Biochemistry (MPI of Biochemistry)
Helmholtz Institute of Stem Cell Research

- **Oliver Baron**, Managing Director
  of the Center for Integrated Protein Science Munich (CIPSM/LMU)
- **Oliver Behrend**, Managing Director
  of the Munich Center for Neurosciences – Brain & Mind (MCN/LMU)
- Benedikt Berninger, Post-doctoral fellow
  LMU, Institute of Physiology, Department of Physiological Genomics, laboratory of Magdalena Götz
- **Martin Biel**, Professor
  LMU, Pharmacology for Natural Sciences, Department of Pharmacology
- **Tobias Bonhoeffer**, Professor
  MPI of Neurobiology, Department of Cellular and Systems Neurobiology
- **Axel Borst**, Professor
  MPI of Neurobiology, Department of Systems and Computational Neurobiology
- **Lena Bouman**, Academic Coordinator (Natural Sciences and Medicine)
  Center for Advanced Studies (CAS[LMU])
- **Johannes Broichhagen**, PhD student
  LMU, Department of Chemistry, laboratory of Dirk Trauner
- **Silvia Capello**, Post-doctoral fellow
  Helmholtz Zentrum München, Institute of Stem Cell Research, laboratory of Magdalena Götz
- **Thomas Carell**, Professor
  LMU, Department of Chemistry
- **Patrick Cramer**, Professor
  LMU, Department of Biochemistry
- **Aditi Deshpande**, PhD student
  LMU, Department of Physiology, laboratory of Magdalena Götz
- **Dorothee Dormann**, Post-doctoral fellow
  DZNE/ LMU, Adolf Butenandt Institute, Department of Metabolic Biochemistry, laboratory of Christian Haass
- **Peter Düwell**, Post-doctoral fellow
  LMU, Medical Clinic, Clinical Pharmacology, laboratory of Stefan Endres
- **Hubert Eichner**, PhD student
  MPI Neurobiology, Department of Systems and Computational Neurobiology, laboratory of Axel Borst
- **Stefan Endres**, Professor
  LMU, Medical Clinic, Clinical Pharmacology
- **Felix Felmy**, Post-doctoral fellow
  LMU, Department of Biology II, Neurobiology, laboratory of Benedikt Grothe
- **Thomas Gerling**, PhD student
  TUM, Physics Department
- **Simone Glasl**, Project Manager
  International Cooperation LMUexcellent
- **Magdalena Götz**, Professor
  Helmholtz, Institute of Stem Cell Research
- **Benedikt Grothe**, Professor
  LMU, Department of Biology, Division of Neurobiology
- **Matthias Hadesbeck**, Deputy Director
  International Office, LMU
- **Christian Haass**, Professor
  DZNE/ LMU, Adolf Butenandt Institute, Metabolic
- **Falko Hampel**, PhD student
  MPI of Neurobiology, Department of Molecular Neurobiology, laboratory of Rüdiger Klein
  Biochemistry
- **Andreas Herz**, Professor
  LMU, BCCN, Department of Biology II
- **Pia Johansson**, Post-doctoral fellow
  Helmholtz, Institute of Stem Cell Research, laboratory of Magdalena Götz
- **Ulrich Pohl**, Professor
  Vice-President of LMU for International Affairs
- **Johannes Kreuzer**, PhD student
  TUM, Department of Chemistry, laboratory of Stephan Sieber
- **Rüdiger Klein**, Professor
  MPI of Neurobiology, Department of Molecular Neurobiology
- **Christian Leibold**, Professor
  LMU, Neurobiology, Department of Biology II

- **Nina Mäusbacher**, Post-doctoral fellow
  TUM, Department of Chemistry, laboratory of Stephan Sieber
- **Matthias Mann**, Professor
  MPI of Biochemistry, Department of Proteomics and Signal transduction
- **Thomas Martin**, PhD student
  TUM, Physics Department, CIPSM
- **Alexander Mathis**, PhD student
  LMU, BCCN, Department of Biology II, laboratory of Andreas Herz
- **Stylianos Michalakis**, Post-doctoral fellow
  LMU, Department of Pharmacology, laboratory of Martin Biel
- **Moritz Rapp**, PhD student
  LMU, Medical Clinic, Clinical Pharmacology, laboratory of Stefan Endres
- **Alwin Reiter**, PhD student
  LMU, Department of Chemistry, laboratory of Dirk Trauner
- **Stephan Sieber**, Professor
  TUM, Department of Chemistry
- **Alexandra Stein**, Program Coordinator
  Graduate School of Systemic Neurosciences
- **Alvaro Tejero Cantero**, PhD student
  LMU, Neurobiology, Department of Biology II, laboratory of Christian Leibold
- **Dirk Trauner**, Professor
  LMU, Department of Chemistry
- **Sylvia Zehner**, Assistant
  Munich Center for Neuroscience – Brain & Mind (MCN)

Abstracts of lectures and posters

Visual cortex in motion

Margarida Agrochao
Harvard, Harvard Center for Brain Science

What brain area would you choose to monitor if you wanted to flag the times when an animal moves? Here we argue that the visual cortex would be a good choice.
We recorded extracellular action potentials from neurons in the visual cortex of rats chronically implanted with tetrodes. The animals participated in laboratory-bound experiments, both freely moving and anesthetized, for the purpose of measuring receptive fields. In addition, we took the rats outside into a wooded area and recorded brain signals during unconstrained free-ranging behavior in this natural environment. Along with the tetrode drive, the animals also carried a miniature wireless video camera used to capture the direction and speed of the animal's motion during free behavior.
Here we report the initial but robust observation that a significant number of visual cortex neurons fire in a manner strongly correlated with locomotion of the animal. Using movies from the head-mounted camera, we searched for parameters of the visual image motion experienced by the rat that best explain the modulation in firing rate. Our findings stress the strong coupling between motor actions and sensory inputs, and the resulting importance of analyzing brain function under natural operating conditions.

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Malleable DNA-Nanostructures as Alignment Media for Membrane Protein NMR

G. Bellot[1,2], JJ. Chou[2], WM. Shih[1,2,3]
1 Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston
2 Department of Cancer Biology, Dana-Farber Cancer Institute, Boston
3 Wyss Institute for Biologically Inspired Engineering at Harvard, Cambridge

Biological systems create very complex devices with nanometer-scale dimensions and precisely controlled three-dimensional architectures. A few years ago, the scientific community started to design artificial nanostructures that mimic nature's architecture. One recent field is DNA nanotechnology, which uses DNA as a molecular engineering material to create nanostructures with controlled geometries, topologies, and periodicities of nanoscale objects and arrays of increasing complexity. The William Shih group is a pioneer within the field that generalized a strategy to build custom-shaped, three-dimensional objects with precisely controlled dimensions and a high level of complexity in shape and size *[1,2]*. We are developing DNA nanotechnology as a tool for structural NMR studies of membrane proteins and single-molecule biophysics *[3]*. Membrane proteins are encoded by 20–35% of genes but represent <1% of known protein structures to date. Thus, improved methods for membrane-protein structure determination are of critical importance. Residual Dipolar Couplings (RDCs), commonly measured for biological macromolecules weakly aligned by liquid- crystalline media, provide important global angular restraints for NMR structure determination. For alpha-helical membrane proteins >15 kDa in size, Nuclear-Overhauser effect-derived distance restraints are difficult to obtain, and RDCs could serve as the main reliable source of NMR structural information. In many of these cases, RDCs would enable full structure determination that otherwise would be impossible. However, none of the existing liquid-crystalline media used to align water-soluble proteins are compatible with the detergents required to solubilize membrane proteins. We generated detergent-resistant liquid crystals of 0.8-µm-long DNA nanotubes that enable weak alignment of detergent-reconstituted membrane proteins. This DNA-nanotube liquid crystal will introduce the advantages of weak alignment to NMR structure determination for a number of membrane proteins. We are applying our DNA-nanotools towards structure determination and mechanistic analysis of mitochondrial membrane proteins and GPCRs. Furthermore, to generalize the method to be compatible with positively-charged protein-micelle complexes and to facilitate measurement of linearly independent restraints to get more structural information, we generated additional NMR sample conditions and DNA-nanostructure based alignment media.

[1] Douglas, S. M. & Shih, *W. M. Nature 459, 414–418 (2009).*
[2] Dietz, H, Douglas, S. M. & Shih, *W. M. Science 325, 725–730 (2009).*
[3] Douglas, S. M. Chou, J. J. & Shih, *W. M. Proc Natl Acad Sci 104, 6644–6448 (2007).*
distinct neuronal identities.

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How activity changes synapses in the mammalian brain

Tobias Bonhoeffer
MPI of Neurobiology, Department of Cellular and Systems Neurobiology

One of the most fundamental properties of the brain is its ability to adapt rapidly to environmental changes. This is achieved mainly by changes in the connectivity between individual nerve cells. Synapses can be modulated in their strength by a variety of different mechanisms. We have investigated a number of these mechanisms, ranging from homeostatic control of synaptic efficacy to morphological manifestations of synaptic strengthening or weakening, and the role of calcium in these processes. Yet, while we are beginning to understand the cellular mechanisms underlying synaptic changes, it is important to consider the functional implications of synaptic plasticity in the intact brain. We are therefore applying new imaging methods to investigate the effects of experience on synaptic changes in cortical circuits. In particular, in vivo two-photon microscopy has enabled us to study morphological as well as functional plasticity at the level of individual neurons in the neocortex. These experiments are beginning to close the gap between traditional cellular and systems studies, and they will enable us to obtain a much more comprehensive understanding of the phenomenon of synaptic plasticity and its role in cortical function and ultimately behavior.

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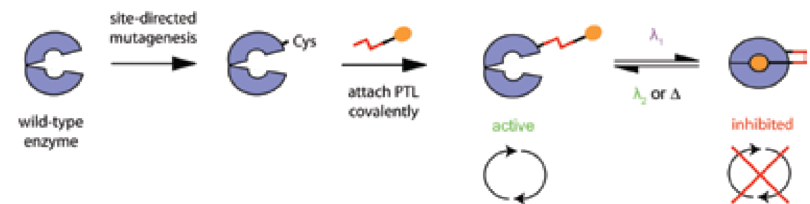
Optochemical genetics: Photocontrolling enzymes

Johannes Broichhagen and Dirk Trauner
LMU, Department of Chemistry

Optochemical Genetics merges the known and established optogenetic protocols with chemical tools. While the ultimate goal of controlling biological function with light remains, synthetic photoswitches are implemented for this purpose inplace of hijacking and genetically modifying nature's light-responsive proteins. Here we present this approach with two well-studied enzymes, namely human carbonic anhydrase II (hCAII) and acetylcholin esterase (AchE), serving as models for this novel approach. A native agonist is designed as a photochromic tethered ligand (PTL) and chemically, *i.e.* covalently, attached to the enzyme in close proximity to the active center. The particular attachment site is a mutated cystein amino acid and its location is chosen using a computer model. The resulting chemically modified enzyme can be modulated to the active or inhibited state by choice of an appropriate wavelength of light. Advantages are more wild type like proteins that only have to be genetically engineered in one position. This minor modification will conceivably affect activity and structure less than using state-of-the-art chimeric approaches.

Graphical Abstract:



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A new mouse model of 'double cortex' – the role of RhoA in cortical development

Cappello S.[1], Böhringer C.[1], Giulio Srubek Tomassy[2], Paola Arlotta[2], Marco Mainardi[3], Manuela Allegra[3], Matteo Caleo[3], Jolanda van Hengel[4], Brakebusch C.[5] and Götz M.[1,6]
1 Institute of Stem Cell Research, Helmholtz Center Munich
2 Harvard Medical School, Harvard University
3 CNR Neuroscience Institute, Pisa, Italy
4 Department of Biomedical Molecular Biology, Ghent University, Belgium
5 Institute of Molecular Pathology, University of Copenhagen, Denmark
6 LMU, Institute of Physiology

The events that organize brain structure during development include neurogenesis, cell migration as well as axon projections and guidance. A hallmark of the mammalian neocortex is the arrangement of functionally distinct neurons in six horizontal layers which possess distinct properties in different sensory or motor areas. In human patients mislocalisation of neurons often result in profound neurological and cognitive defects, but the cause of these malformation is still poorly understood. Here we examined the role of the small GTPase RhoA, a key regulator of cytoskeleton dynamics, for the first time in brain development in vivo. Conditional deletion of RhoA in the developing cerebral cortex results in a prominent formation of a heterotopic cortex underlying the normotopic cortex as well neurons protruding beyond layer I (type II cobblestone lissencephaly). The heterotopic cortex is comprised of neurons from all layers, but mostly contains upper layer neurons, while layering of the normotopic cortex is normal. Notably, while sensory arealization of both cortices is preserved, processing of visual information is severely impaired. Transplantation experiments into wildtype cerebral cortices revealed that RhoA-/- neurons migrate normally and do not settle at heterotopic positions. The only defects occurred at the end of neuronal migration with RhoA-/- neurons protruding beyond layer I. Conversely, wildtype cells transplanted into the RhoA-/- cerebral cortex settled in an ectopic band below the normal cortex, thereby revealing a non-cell-autonomous cause for the heterotopic cortex formation. This is due to the severe alterations in the radial glial scaffold in the RhoA-/- cerebral cortex due to increased levels of G-actin and tyrosinated tubulin, i.e. destabilization of both the actin and tubulin cytoskeleton. Thus, our results demonstrate that RhoA is largely dispensable for neuronal migration, but is necessary for formation and maintenance of radial glia processes. These data further showed that defects in the radial glia cytoarchitecture are sufficient for formation of a 'double cortex' thereby providing a novel concept of the etiology of this 'neuronal migration disorder', implying glia rather than neurons as the cause.

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The chemistry of genome maintenance

Thomas Carell
LMU Munich, Department of Chemistry

Our genome is constantly damaged by various exogenous and endogenous events. 50'000 to 100'000 DNA lesions are generated each day per cell. The formed chemical DNA modifications interfere with normal DNA transcription and replication events causing mutations and cell death. In the lecture I will describe the chemical synthesis of oxidative DNA lesions, DNA lesions, which are formed upon UV-irradiation of cells, and of lesions formed by cisplatin in the course of typical anticancer therapy. I will discuss how these lesions are synthetically incorporated into oligonucleotides using either solid phase chemistry or direct chemistry on DNA. DNA double strands containing a defined (6-4) lesion at a defined site were e.g. used to create co-crystal structures with the (6-4) DNA photolyase from *Drosophila melanogaster*[1]. From this structure and correlated biochemistry we could develop a new "ribonucleotide reductase like" DNA repair mechanism used by the protein to achieve a light induced repair reaction. Furthermore, co-crystal structures of cisplatin lesion containing DNA in complex with polymerase-eta allowed us to decipher step-by-step the mechanism of translesion synthesis[2] which establishes lesion tolerance in human cells. Finally I will discuss new results in the area of epigenetic research especially on hydroxymethyl-cytosine.[3]

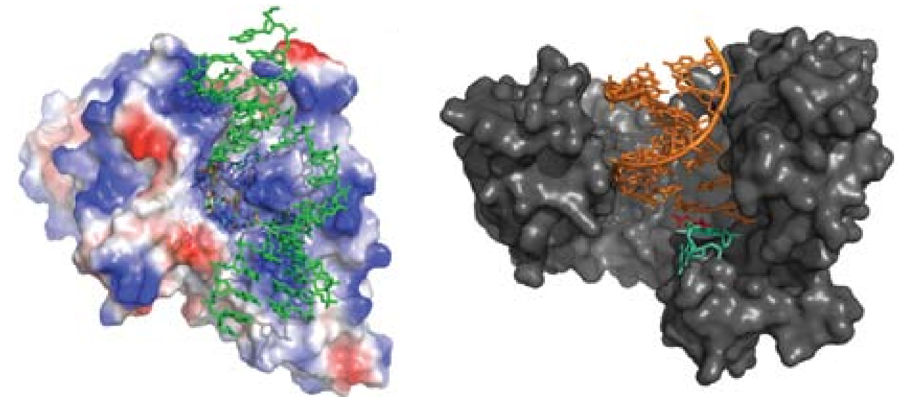

Figure 1: (6-4) DNA photolyase (left)[1] and polymerase-eta (right)[2] in complex with DNA containing synthetic DNA lesions.

[1] a) M. J. Maul, T. R. M. Barends, A. F. Glas, M. J. Cryle, T. Domratcheva, S. Schneider, I. Schlichting, T. Carell, *Angew. Chem. Int. Ed. 2008, 47,* 10076-10080. „Crystal Structure and Mechanism of a DNA (6-4) Photolyase"
b) A. F. Glas, M. J. Maul, M. Cryle, T. R. M. Barends, S. Schneider, E. Kaya, I. Schlichting, T. Carell, *Proc. Natl. Acad. Sci. USA, 2009, 106,* 11540-11545. "The Archaeal Cofactor F_0 is a Light Harvesting Vitamin in Eukaryotes"
[2] A. Alt, K. Lammens, C. Chiocchini, A. Lammens, J. C. Pieck, D. Kuch, K.-P. Hopfner, T. Carell, *Science 2007, 318,* 967-970. „Bypass of DNA lesions generated during anticancer treatment with cisplatin by DNA polymerase eta"
[3] M. Münzel, D. Globisch, T. Brückl, M. Wagner, V. Welzmiller, S. Michalakis, M. Müller, M. Biel, T. Carell *Angew. Chem. Int. Ed.* **2010**, *49, 5375-5377*. „Quantification of the Sixth DNA Base Hydroxymethylcytosine in the Brain"

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Rabies virus mediated tracing of synapses onto adult generated neurons

Aditi Deshpande[1], Karl-Klaus Conzelmann[3], Magdalena Götz[1,2], Benedikt Berninger[1,2]
1 LMU, Department of Physiological Genomics, Institute of Physiology
2 Institute of Stem Cell Research (Helmholtz Center Munich)
3 LMU, Max von Pettenkofer Institute and Gene Center

Adult neurogenesis has been well established as an inherent property of the mammalian brain, being confined to two neurogenic niches - the subgranular zone of the dentate gyrus (DG) of the hippocampus and the subependymal zone (SEZ) of the lateral ventricle. Although it is known that the newborn neurons intergrate into preexistent networks, there remains an ambiguity about the precise nature of this functional integration as well as cellular specificity of the pre- and postsynaptic targets. Here we aimed at identifying the presynaptic partners of adult generated neurons by modifying a recently developed method of retrograde tracing of monosynaptic connections using an EnvA pseudotyped reporter-expressing rabies virus (Wickersham et al., 2007). In our approach, we modified a Moloney based retrovirus that selectively transduces proliferating cells, to express two key proteins – TVA, required for infection by the EnvA-pseudotyped rabies virus and a glycoprotein, required for transsynaptic spread by the rabies virus. Thus only progenitors transduced with the retrovirus will get infected by the replication competent pseudotyped rabies virus and can subsequently transport the virus to their presynaptic partners. In embryonic cortical cultures, infection with the rabies virus 2-3 weeks after transduction with the retrovirus, we observed double (retro- and rabies virus)-transduced cells, surrounded by a cluster of RV only infected cells. By paired recordings from double (postsynaptic) and single (presynaptic) labeled cells, we could demonstrate that these cells are indeed synaptically connected.
To identify presynaptic partners of adult-generated neurons in vivo, we performed stereotaxic injections of the TVA and glycoprotein expressing retrovirus into the dentate gyrus of adult C57B/L6 mice, followed by a second injection of the EnvA-pseudotyped rabies virus. At earlier timepoints we observed both, double labeled granule neurons as well as cells in the hilus and DG labeled with rabies virus alone. Neurochemical marker expression, location of the cell body as well as the pattern of their axonal and dendritic arborisation suggest that these cells comprise of distinct types of local interneurons. These data are consistent with the idea that GABAergic interneurons are amongst the first to form synapses onto newly generated granule cells. At late timepoints following rabies virus injection, we also observed distinct labeling of pyramidal neurons in the entorhinal cortex, the primary excitatory input to granule neurons via the perforant path.

We performed similar studies in the olfactory bulb where we injected the retrovirus into the SEZ to label progenitors, followed (4 days later) by the rabies virus injection into the rostral migratory stream to hit migrating cells expressing the retroviral transgenes. Upon their arrival in the olfactory bulb we observed a heterogeneous population of cells infected with rabies virus alone in the olfactory bulb itself and interestingly, also pyramidal neurons in the anterior olfactory nucleus (AON) which are known to form projections onto bulbar granule cells. This data shows that some of the earliest excitatory synaptic input onto new-born neurons in the olfactory bulb originates in the AON.

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ALS-associated FUS mutations disrupt Transportin-mediated nuclear import

Dorothee Dormann
LMU, German Center for Neurodegenerative Diseases (DZNE) and Adolf-Butenandt-Institute, Biochemistry

Mutations in the *fused in sarcoma (FUS)* gene cause familial amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease. Patients carrying FUS mutations show a characteristic accumulation of FUS within neuronal cytoplasmic inclusions, whereas in healthy individuals FUS is predominantly nuclear. Cytoplasmic FUS inclusions also have been identified in a subset of patients with frontotemporal lobar degeneration (FTLD-FUS), a related disorder. We demonstrate that a non-classical PY nuclear localization signal (NLS) in the C-terminus of FUS is necessary for nuclear import. The majority of ALS-associated mutations occur within the NLS and impair nuclear import to a degree that correlates with the age of disease onset. This presents the first case of disease-causing mutations within a PY-NLS. Nuclear import of FUS is dependent on Transportin, and interference with this transport pathway leads to cytoplasmic redistribution and recruitment of FUS into stress granules. Moreover, proteins known to be stress granule markers co-deposit with inclusions in ALS and FTLD-FUS patients, implicating stress granule formation in disease pathogenesis. We propose that two pathological hits, namely nuclear import defects and cellular stress, are involved in ALS/FTLD pathogenesis.

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Tri-functional siRNA combining TGF-beta silencing, RIG-I activation and apoptosis induction induces effective antitumor responses in pancreatic carcinoma

Peter Duewell, Jonathan Ellermeier, Jiwu Wei, Sabine Hoves, Stefan Endres, Max Schnurr
LMU, Division of Clinical Pharmacology

Background: Increasing evidence suggests that immune responses are critical in the control of tumors. Continued tumor growth in the presence of a lymphocytic infiltration indicates a failure of immune control. The cytokine TGF-beta plays a critical role in the immunosuppressive network generated by tumors and increased serum levels correlate with poor prognosis. Hence, silencing of TGF-beta with siRNA is a promising strategy to break immune suppression. Retinoic acid inducible gene I (RIG-I) is a ubiquitously expressed cytosolic helicase detecting viral RNA by its 5'-triphosphate (3p) end. Activation of RIG-I leads to a type I IFN response and pro-apoptotic program in virally infected cells. In our study, we modified siRNA targeting TGF-beta as 3p-siRNA to combine gene silencing with RIG-I activation in one single molecule.

Results: We could show that human and murine pancreatic cancer cells express functional RIG-I that, upon 3p-RNA-stimulation, leads to phosphorylation of IRF3. Bifunctional 3p TGF-beta reduced TGF-beta production of pancreatic carcinoma cells both in vitro and in vivo. In addition, it induced type I IFN production, upregulation of MHC class I molecules and apoptosis of both, murine and human pancreatic tumor cells. In an orthotopic murine model of pancreatic cancer systemic therapy with 3p-TGF-beta mediated control of tumor growth and prolonged survival. Histological analysis showed potent induction of apoptosis in pancreatic tumors. Cytokine analysis revealed downregulation of TGF-beta and a Th1-biased immune response in tumor tissue.

Conclusion: Our work demonstrates that the combination of gene silencing, immune activation and apoptosis induction in one single, bi-functional RNA molecule is an innovative and promising strategy for the treatment of pancreatic cancer.

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Sex Battles in the Brain: a Genome-Wide Analysis of Genomic Imprinting

Catherine Dulac
Harvard, Department of Molecular and Cellular Biology

We are interested in the phenomenon of genomic imprinting in the brain, and the role of this mode of epigenetic modification in brain development and adult brain function. Genomic imprinting results in preferential expression of the paternally, or the maternally inherited allele of certain genes. My group recently used a genome-wide approach to characterize the repertoire of imprinted genes in the mouse embryonic and adult CNS. This study uncovered over 1000 new loci with imprinted features. Imprinting appears to preferentially affect neural systems associated with social, motivational and homeostatic brain functions. Comparison of the imprinted gene repertoire in the adult hypothalamus and cortex, and in the developing brain demonstrates a complex spatiotemporal, species-, sex- and isoform-specific regulation. Genomic imprinting thus emerges as a major and dynamic mode of epigenetic regulation of brain function, with direct implications for the understanding of evolution and diseases.

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Two separate motion detectors for on and off signals

Hubert Eichner[1], Maximilian Joesch[2], Bettina Schnell[1], Dierk F Reiff[1], and Alexander Borst[1]
1 MPI of Neurobiology, Department of Systems and Computational Neurobiology
2 Harvard, Department of Molecular and Cellular Biology

Motion detection in Drosophila starts with splitting the visual input into two parallel channels encoding brightness increments (ON) or decrements (OFF). This suggests that the internal structure of the subsequent motion detection circuit is comprised of either two (ON-ON, OFF-OFF) or four (for all pair wise interactions) separate, independent motion detectors. To distinguish these two alternatives, we stimulated flies using apparent motion stimuli, consisting of sequences of short ON and OFF brightness pulses, while recording from motion sensitive tangential cells. In both fly species tested, we observed direction-selective responses to sequences of same sign (ON-ON, OFF-OFF) but not of opposite sign (ON-OFF, OFF-ON), refuting the existence of four separate detectors. Inspired by further measurements, we propose a model that reproduces a variety of additional experimental data sets, including ones that were previously interpreted as support for four separate detectors. These experiments and the derived model mark an important step in guiding the ongoing dissection of the fly motion detection circuit.

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A system for the continuous directed evolution of biomolecules

Kevin Esvelt
Harvard, Department of Chemistry and Chemical Biology

Laboratory evolution has generated many proteins and nucleic acids with desired properties, but a single round of directed evolution typically requires days or longer with frequent human intervention. Because evolutionary success is dependent on the total number of rounds performed, a means of performing laboratory evolution continuously and rapidly could dramatically enhance its effectiveness. We have developed a system enabling the continuous directed evolution of gene-encoded molecules that can be linked to the production of a protein in E. coli. During phage-assisted continuous evolution (PACE), evolving genes are transferred from host cell to host cell through a modified bacteriophage life cycle in a manner that is dependent on the activity of interest. Dozens of rounds of evolution can occur in a single day of PACE without human intervention. Using PACE, we continuously evolved T7 RNA polymerase to recognize a distinct DNA promoter and to initiate transcripts with nucleotides other than G, with all activities emerging in less than a week of evolution. By greatly accelerating laboratory evolution, PACE may provide solutions to otherwise intractable directed evolution problems and address novel questions about molecular evolution.

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Comparing the synaptic transmission and postsynaptic integration of large synapses in the auditory brainstem of mongolian gerbils

Felmy F.[1], Meyer E.M.M.[1], Grothe B.[1,2]
1 LMU, Department Biology II, 2 LMU, Computational Neuroscience

In the auditory brainstem large excitatory terminals are present. The auditory nerve fibre gives rise to the endbulb of Held in the cochlear nucleus (CN), the bushy cells to the calyx of Held in the medial nucleus of the trapezoid body (MNTB) and the octopus cells to large terminals in the ventral nucleus of the lateral lemniscus (VNLL). In general such large synapses are believed to allow for a faithful information transfer. Synaptic transmission has been well studied at the synapses in the CN and the MNTB, but presynaptically, only the calyx of Held has been investigated. The features of synaptic transmission of large terminals in the VNLL are largely unknown, and thus no physiological comparison of auditory presynaptic terminals is available so far.

To compare the membrane physiology, the synaptic transmission and the postsynaptic integration of synapses in the MNTB and VNLL we used pre- and postsynaptic whole-cell current- and voltage-clamp recordings and ratiometric fura Ca^{2+}-measurements from visually identified compartments in brain slices of Mongolian gerbils of postnatal day 9-11 at 34°C.

We find that both terminals have resting membrane potentials of -68 mV that the input resistance is lower and the membrane time constant faster in terminals in the VNLL. Action potential (AP) half width is not different, but its size was ~20 mV smaller in terminals in the VNLL. The resting Ca^{2+} concentration and the endogenous calcium binding ratio are slightly larger in the VNLL. The VNLL synapse generates AMPA and NMDA currents (3.1 nA and 2.3 nA respectively) of about half the amplitude of clayx of Held synapses. Both AMPA and NMDA currents are faster in their kinetic profile in VNLL. The AMPA current profile of VNLL synapses is able to trigger a postsynaptic AP in the MNTB but not in the VNLL. However, pre-depolarising the postsynaptic VNLL cell with holding currents allowed for the generation of a fast onset AP similar to MNTB neurons. Under control conditions a fast transient potassium channel present in the postsynaptic VNLL somata appears responsible for the suppressing the AP generation by a single terminal in the VNLL. Activation of two synaptic terminals in the VNLL within a time window of about 7-10 ms can lead to postsynaptic AP initiation.

In contrast to the calyx of Held information transfer in the VNLL is based on coincidence detection and possibly temporal summation instead of a direct one-to-one transmission. Thus, also at large, fast glutatamtergic synapses, the postsynaptic integration contributes substantially to the pattern of information transfer.

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Learning-related synaptic reorganization in the motor control circuits of songbirds

Jonathan Garst Orozco, Bence P. Ölveczky
Harvard, Department of Organismic and Evolutionary Biology

Complex motor sequence learning is accompanied by synaptic changes in motor control circuits, yet the nature and logic of these changes are not well understood. The zebra finch, a songbird with an exceedingly well understood neural circuit underlying song, offers a unique opportunity to explore this question. Projection neurons in premotor nucleus HVC provide a temporally structured input to RA, a topographically organized motor cortex analogue, which in turn drives motor neurons innervating vocal muscles. Here we explore learning-related synaptic modifications in the descending motor circuit by probing the strength and number of HVC inputs to RA neurons in acute brain slices at different developmental time points.
Using angled parasagittal slices we were able to record from neurons in RA under whole-cell voltage clamp while preserving the bulk of HVC projections to RA. By electrically stimulating HVC axons with increasing intensity, we recruited inputs to RA neurons in an incremental fashion. Synaptic connectivity was characterized as the current elicited by activation of a single HVC inputs (single fiber current), and as the current elicited upon activating all the HVC fibers within the slice (maximal current). The ratio of single fiber to maximal currents (fiber fraction) provided an estimate of the number of HVC neurons projecting to a single RA neuron.
Developmental changes in the fiber fraction and single fiber currents showed that song learning is accompanied by a reorganization of synaptic connectivity in RA. In early development (~45 days post hatch) RA neurons receive many weak inputs from HVC, however, with learning/age, HVC inputs to RA become fewer and stronger, suggesting a process of synaptic strengthening and pruning. Our results suggest that synaptic reorganization in the HVC-RA network may underlie the changes in both the neural activity of RA neurons and the vocal output that accompany song learning in zebra finches.

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Re-building evolution: toward synthetic nucleotide-dependent switch structures assembled with DNA origami*

Thomas Gerling & Hendrik Dietz
TUM, Physics Department

Synthetic approaches open avenues for testing models for molecular motor function. Motor proteins such as kinesins or myosins are thought to have evolved from an ancient structure that was capable of switching conformation upon binding of a nucleotide. Here we report on our efforts in using molecular self-assembly with DNA origami to build a synthetic structure that can switch conformation upon binding of ATP. Our goal is to use this synthetic nucleotide switch in the future as a platform for testing models of how ATP hydrolysis can couple to and power conformational switching of motors against forces. Our structure is designed to exist in a dynamic equilibrium between a straight and a 90° twisted conformation. A number of binding pockets for ATP are modeled by single-stranded vacancies in double-helical domains that are embedded in a tightly interlinked three-dimensional multi-helix structure. Binding of ATP to the vacancies induces local twist and strain that we expect to couple to the global conformation of the synthetic nucleotide switch by shifting the equilibrium to the twisted state. TEM experiments confirm that the synthetic nucleotide switch does indeed exist in both straight and twisted conformations. Currently, we are employing fluorescence microscopy to evaluate the dynamics of switching in absence and presence of ATP.

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The molecular clockwork of Alzheimer‘s Disease

Christian Haass
LMU, Adolf Butenandt-Institute, Biochemistry, &
DZNE – German Center for Neurodegenerative Diseases, Munich

Alzheimer‘s disease (AD) is the most abundant neurodegenerative disorder and is threatening our ageing society. Pathologically, AD is invariably characterized by Amyloid β-peptide (Aß) deposition in senile plaques and tau aggregation in neurofibrillar tangles. Understanding the pathways leading to Aß generation and toxicity stands in the center of large worldwide research efforts, since reducing Aß production has a great therapeutic potential. Proteolytic Aβ generation from its precursor, the ß-Amyloid precursor protein (APP), turned out to be just one example of a general physiological mechanism now known as regulated intramembrane proteolyis (RIP). In a principal version of this process, membrane proteins first undergo a regulated shedding of their ectodomains by membrane-anchored proteases referred to as secretases or sheddases, releasing the large luminal domains into extracellular fluids. The membrane-retained stubs can then be cleaved within their transmembrane domains (TMDs) to release small hydrophobic peptides (e.g., Aβ in the case of APP) into the extracellular space and the intracellular domains (ICDs) into the cytoplasm. The free ICDs may have important functions, including the activation of nuclear signaling pathways, as in the case of the Notch ICD. In the case of APP and certain other RIP substrates, ectodomain shedding can be mediated by either of two distinct membrane-anchored proteases, α-secretase (now known to be ADAM 10) or β-secretase (BACE). The membrane-associated stub created by BACE cleavage can then undergo an intramembrane scission mediated by a unique protease complex, γ-secretase, that is composed of four proteins (presenilin-1 or 2 (PS1/PS2), Nicastrin, APH-1, and PEN-2). All secretases are targets for Aβ lowering therapeutic strategies. Data will be presented on the biological and pathological function of the two amyloidogenic secretases.

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FLRT2 and FLRT3 act as repulsive guidance cues for Unc5-positive neurons

Falko Hampel, Satoru Yamagishi[1,7],*, Katsuhiko Hata[2], Daniel del Toro[1], Manuela Schwark[3,4], Elena Kvachnina[3], Martin Bastmeyer[5], Toshihide Yamashita[2], Victor Tarabykin[3,4], Rüdiger Klein[1], Joaquim Egea[1,6]
1 MPI of Neurobiology, Department of Molecular Neurobiology
2 Department of Molecular Neuroscience, Division of Health Sciences, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan
3 MPI for Experimental Medicine, Hermann-Rein Strasse 3, 37075 Göttingen,
4 Institute of Cell Biology & Neurobiology, Charité-Universitätsmedizin Berlin, Campus Mitte, Berlin
5 Karlsruher Institut für Technologie (KIT), Zoologisches Institut Abteilung für Zell- und Neurobiologie, Haid-und-Neu-Strasse 9, 76131 Karlsruhe,
6 Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida/IRBLLEIDA, Spain
7 Present address: Department of Anatomy, Hamamatsu University School of Medicine, Shizuoka 431-3192, Japan
* Equal contribution

Netrin-1 induces repulsive axon guidance by binding to the mammalian Unc5 receptor family (Unc5A-Unc5D). Mouse genetic analysis of selected members of the Unc5 family, however, revealed essential functions independent of Netrin-1, suggesting the presence of other ligands. Unc5B was recently shown to bind Fibronectin and leucine-rich transmembrane protein-3 (FLRT3), although the relevance of this interaction for nervous system development remained unclear. Here, we show that the related Unc5D receptor binds specifically to another FLRT protein, FLRT2. During development, FLRT2/3 ectodomains are shed from neurons and act as repulsive guidance molecules for axons and somata of Unc5 positive neurons. In the developing mammalian neocortex, Unc5D is expressed by neurons in the subventricular zone (SVZ), which display delayed migration to the FLRT2-expressing cortical plate (CP). Deletion of either FLRT2 or Unc5D causes a subset of SVZ derived neurons to prematurely migrate towards the CP, whereas overexpression of Unc5D has opposite effects. Hence, the shed FLRT2 and FLRT3 ectodomains represent a novel family of chemorepellents for Unc5-positive neurons and FLRT2/Unc5D signaling modulates cortical neuron migration.

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Long distance signalling from the hindbrain choroid plexus to forebrain stem cells via WNT modulators secreted into cerebrospinal fluid.

Johansson PA[1], Irmler M[2], Acampora D[3,4], Beckers J[2,6], Simeone A[3,4] and Götz M[1,5]
1 Institute of Stem Cell Research (Helmholtz Center Munich)
2 Institute for Experimental Genetics, Neuherberg/Munich
3 CEINGE Biotecnologie Avanzate, SEMM European School of Molecular Medicine – Naples site, Italy
4 Institute of Genetics and Biophysics. Naples, Italy
5 LMU, Physiological Genomics

Cerebrospinal fluid (CSF), is in contact with all cells that are facing the ventricle, such as the stem cells (radial glia) in the developing brain, and contains important signalling molecules capable of influencing brain development. The choroid plexuses, which secrete CSF, differentiate during the period of neurogenesis (the choroid plexuses differentiate between embryonic day (E) 10 and 14 in mouse embryos). The hindbrain choroid plexus differentiates first and as such has a unique possibility to influence CSF composition along the entire neuroaxis. However, little is still known about factors regulating choroid plexus development and to which extent choroid plexus differentiation influences CSF composition. Here we set out to elucidate these functions by conditional deletion of the transcription factor Otx2 specifically in the hindbrain choroid plexus.
Conditional deletion of floxed Otx2 alleles using the roof-plate specific Gdf7-Cre mouse line lead to loss of Otx2 only in the hindbrain choroid plexus. The Otx2 deletion resulted in a severely hypomorphic choroid plexus, which was caused by an increase in apoptosis. Further, hypotrophy of the hindbrain choroid plexus resulted in changes in CSF composition and, rather unexpectedly, caused an increase in proliferation in the far distant forebrain stem cells. Transcriptome and candidate analysis showed that the changes in proliferation could be explained by altered secretion of Wnt-signalling modulators from the hindbrain choroid plexus which in turn lead to an increase in Wnt-signalling in the cerebral cortex. Taken together, our results reveal for the first time a direct role of the choroid plexus in long-distance coordination of brain development through the secretion of signalling components into CSF.

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The Role of Motor Cortex in the Production of Complex Motor Sequences

Risa Kawai
Harvard, Department of Organismic and Evolutionary Biology

Much of our behavioral repertoire consists of learned movement sequences, yet little is known about the neural mechanisms underlying their acquisition and production. We use rats to study the neural circuit mechanisms underlying complex motor sequence learning. Rats were trained to tap a lever in precise temporal sequences, a process that, within weeks, resulted in highly stereotyped and surprisingly complex motor sequences. We developed two distinct methods of training the same motor behavior. In one method, rats learned the tapping sequence through trial-and-error learning while in the other method, a light cue indicated the temporal sequence of taps. To identify the neural circuits controlling the learned behaviors, we started by lesioning motor cortex after learning. Preliminary findings show that although the motor output resulting from the two training methods are similar, the effects of motor cortex lesions are significantly different. Rats that acquired the motor sequence through trial-and-error learning were surprisingly unaffected, while rats that learned the task by responding to cues were unable to perform the learned motor sequence after lesions. Many more animals and experiments are needed to confirm these results and to continue our exploration of the neural circuit mechanisms involved in motor sequence learning. To help ourselves we are implementing a completely automated training paradigm, allowing us to train 50 animals simultaneously for long periods of time with minimal effort.

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Development of direct acting inhibitors of epigenetic targets

Michael McKeown; Ghayas Issa; Jason Marineau; Christopher Ott; Ron Paranal; Jun Qi; Katharin Shaw; William B. Smith; James Bradner
Department of Medical Oncology, Dana-Farber Medical Institute
Chemical Biology Program, Harvard Medical School

The reversible, non-covalent assembly of macromolecules is essential to gene regulation. These regulatory complexes are well validated targets in cancer, but have long been considered intractable for ligand discovery. Epigenetic pathways affect gene expression by controlling and interpreting critical molecular marks on chromatin. Cancer cells often exploit these mechanisms to generate altered epigenetic landscapes that serve an oncogenic program. As part of our interest in epigenetic targets, we have recently developed a cell-permeable, potent small-molecule inhibitor (JQ1) with specificity for the BET-family of bromodomain proteins and established the feasibility of targeting protein-protein interactions of epigenetic "readers". Furthermore, we explored this chemical scaffold to develop more potent and selective inhibitors. The emergent chemical probes provide unique opportunities to explore use of BET inhibitors in cancer therapy and in non-malignant diseases. In addition, we are investigating new biology associated with bromodomain inhibition as well as additional targets of transcriptional consequence.

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Acivicin as a probe for ABPP (Activity-Based Protein Profiling)

Johannes Kreuzer
TUM, Faculty of Chemistry

Acivicin is a natural product isolated from *Streptomyces Sviceus*. It is an irreversible inhibitor of gammaglutamyltransferase, cytosinetriphosphatesynthetase, asparagin-and glutamylsynthetase and has a high antitumor activity. Therefore acivicin has already been subject to several clinical trials against leukemia, ovarian carcinoma and several other carcinomas. However, due to its neurotoxic and gastrointestinal side effects it failed as a drug candidate. Nevertheless acivicin remains an interesting compound for cancer research because of its mode of action.
Enzyme inhibition is mediated by a chloroisoxazol moiety, which is attacked by a nucleophilic residue of the active site. In order to unravel all dedicated targets and off-targets hat contribute to its mode of action we applied a proteomics technique called activity-based protein profiling (ABPP). Results on acivicin reactivity and target preferences in living cells will be presented.

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Color Vision Circuitry in the Outer Retina of Zebrafish

Yong Li
Harvard, Department of Molecular and Cellular Biology

Many fish and other non-mammalian species have rich color vision systems. Zebrafish, for example, possess four cone types: red- (R), green- (G), blue- (B) and ultraviolet-sensitive (UV) cones. Further, they show features of color processing in the retina – double opponent bipolar cells – not seen in mammals until one reaches the cortex. In addition, some features of color processing in fish horizontal cells have not been seen as yet in any mammalian neurons – trichromatic and tetrachromatic opponency. In an attempt to understand how initial color processing is accomplished, I studied the precise connectivity of zebrafish photoreceptors with horizontal and bipolar cells. DiI was inserted into whole-mounted transgenic zebrafish retinas to label horizontal and bipolar cells. The photoreceptors that connect to these DiI-labeled cells were identified by transgenic fluorescence or their relative positions to the fluorescent cones as cones are arranged in a highly-ordered mosaic in the zebrafish retina. There are three types of cone horizontal cells: H1 cells connect to R, G and B cones; H2 cells to G, B and UV cones; H3 cells to B and UV cones. 322 bipolar cells were studied and as many as 19 connectivity types were observed: 9 connectivity types account for 90% of the bipolar cells and will be described in some detail.

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A substrate-free activity-based protein profiling screen for the discovery of selective PREPL inhibitors

Anna Mari Lone
Harvard, Department of Chemistry and Chemical Biology

Peptidases play vital roles in physiology through the biosynthesis, degradation, and regulation of peptides. Prolyl endopeptidase-like (PREPL) is a newly described member of the prolyl peptidase family, with significant homology to mammalian prolyl endopeptidase (PEP) and the bacterial peptidase oligopeptidase B (OPDB). The biochemistry and biology of PREPL is of fundamental interest due to this enzyme's homology to the biomedically important prolyl peptidases and its localization in the central nervous system (CNS). Furthermore, genetic studies of patients suffering from hypotonia-cystinuria syndrome (HCS) have revealed a deletion of a portion of the genome that includes the *PREPL* gene. HCS symptoms thought to be caused by lack of PREPL include neuromuscular and mild cognitive deficits. A number of complementary approaches, ranging from biochemistry to genetics, will be required to understand the biochemical, cellular, physiological, and pathological mechanisms regulated by PREPL. We are particularly interested in investigating physiological substrates and pathways controlled by PREPL. Here, we use a fluorescence polarization activity-based protein profiling (fluopol-ABPP) assay to discover selective small-molecule inhibitors of PREPL. Fluopol-ABPP is a substrate-free approach that is ideally suited for studying serine hydrolases for which no substrates are known, such as PREPL. After screening over 300,000 compounds using fluopol-ABPP, we employed a number of secondary assays to confirm assay hits and characterize a group of 3-oxo-1-phenyl-2,3,5,6,7,8-hexahydroisoquinoline-4-carbonitrile and 1-alkyl-3-oxo-3,5,6,7-tetrahydro-2*H*-cyclopenta[c]pyridine-4-carbonitrile PREPL inhibitors that are able to block PREPL activity in cells. Moreover, when administered to mice, 1-isobutyl-3-oxo-3,5,6,7-tetrahydro-2*H*-cyclopenta[c]pyridine-4-carbonitrile distributes to the brain, indicating that it crosses the blood-brain barrier, and may be useful for *in vivo* studies. The application of fluopol-ABPP has led to the first reported PREPL inhibitors, and these inhibitors will be of great value in studying the biochemistry of PREPL, and in eventually understanding the link between PREPL and HCS.

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Natural product-inspired novel antibiotics

Nina Mäusbacher, Max Pitscheider and Stephan Sieber
TUM, Department of Chemistry

By developing escape mechanisms to several of the currently used antibiotics, different bacterial strains become resistant and hence cause life-threatening diseases. To address this problem the generation of new and highly efficient antibacterial substances is required. We designed two probes that specifically bind to the active sites of FabH and FabF, two crucial components of the Type II fatty acid synthesis pathway in Staphylococcus aureus and Listeria monocytogenes, thus inhibiting bacterial proliferation. Herein activity-based protein profiling in combination with in gel fluorescence staining and mass spectrometry allowed the identification of probe-bound proteins. The targeting of these specific proteins was then confirmed by comparing the probes' minimal inhibitory concentrations in different populations of genetically modified bacteria expressing FabH and FabF either to a normal, increased or diminished extent, respectively. Further research on such probes may help to develop useful therapeutic drugs against resistant bacterial strains.

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High resolution, quantitative proteomics turbo-charges biochemistry

Matthias Mann
MPI BioChem, Department of Proteomics and Signaltransduction

Despite the central role of proteins in all life and disease processes, the large scale study of proteins – proteomics[1] – has until recently not been a prominent member in the ‚post-genomics' field. This was due to the technological challenges associated with measuring large numbers of proteins at the same time and dealing with their large concentration differences. Now, advances in mass spectrometric technology, sample preparation and computational tools have made the concept of ‚precision proteomics' a reality. This technology will be summarized with emphasis on recent instrumental breakthroughs. We demonstrate that proteomics can now identify almost all fragmented peptides, facilitating comprehensive proteome identification and quantitation and leading to a discovery of a plethora of post-translational modifications. A first completely identified and quantified proteome of a model species has been achieved[2] and mammalian cells can be probed in very great depths as well. Cell signaling is now routinely studied using expression proteomics, interaction proteomics and the large-scale study of post-translational modifications. Examples include quantitative proteomics and phosphoproteomics of changes induced by cytokine and growth factor treatment of cells in culture[3].
Furthermore, we describe ongoing efforts in our laboratory to make precision proteomics amenable to clinical questions. For instance, quantitative proteomics can now directly address the consequences of genome amplifications on the proteome. It turns out that gene amplification is only translated to the protein level in a gene, implying a regulation of protein amount for many genes that is post-transcriptional. In conclusion, proteomics is now a powerful technology, bringing the same depths and accuracy to the large-scale study of proteins that we otherwise associate with genomics technology.

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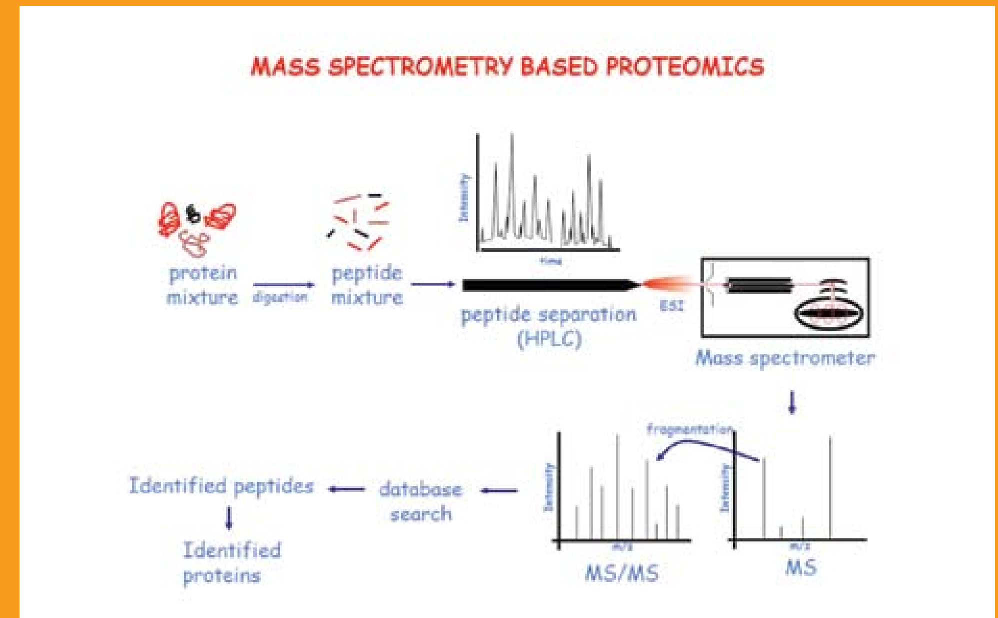

Figur 1 Proteomics workflow

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Optimal Distribution of Spatial Periods for Grid Cells Ensembles on Linear Track

Alexander Mathis[1,2,3], Martin Stemmler[1,2,3], Andreas Herz[1,2,3]
1 LMU, Division of Neurobiology
2 LMU, Computational Neuroscience
3 Graduate School of Systemic Neuroscience

A grid cell in the medial entorhinal cortex or subiculum spikes at multiple locations in the environment of a rat. Remarkably, these locations are arranged in a hexagonal lattice [1, 2]. However striking such a pattern is, the presence of multiple firing fields for each grid cell introduces ambiguity in the coding of space at the single cell level. Hence, a population of grid cells is needed to represent the entire environment and encode space precisely.

We set out to compute the resolution of grid codes, defined as the mean square error of the maximum likelihood estimate, in a realistic parameter regime. For this purpose, we assumed a finite number of spiking grid cells that encode a limited region of space using different configurations of spatial periods. To achieve the best encoding of location, we predict that there should be far more small spatial periods present than large periods. This predicted distribution largely resembles empirical observations by Brun et al. [3]. Furthermore, we predict that the area of the firing fields should cover roughly one-third of the spatial period of the hexagonal grid.

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Custom DNA-origami apertures for solid-state nanopores

Thomas Martin[1], Ruoshan Wei[2], Ulrich Rant[2], and Hendrik Dietz[1,2]
1 CIPSM, Center for Integrated Protein Science Munich
2 TUM, Physics Department, Walter Schottky Institute

Nanoscale pores in membranes offer a route for identifying translocating biomolecules by ion current blockade signatures. Applications such as molecular sorting or DNA sequencing demand pores with well-controlled diameters on the 1 to 10 nanometer scale and that include custom chemical functionalities that selectively delay the translocation of a molecule of interest. Here we meet this demand with self-assembled DNA nano-apertures in combination with silicon-nitride-based solid-state nanopores. We use molecular self-assembly with DNA origami to produce 50x50 nm wide rectangular DNA plates that can be plugged in a user-defined orientation onto 25 nm wide channels in silicon-nitride membranes. We show that the DNA plates entirely block translocation of protein and DNA molecules through the silicon-nitride channel, while only mildly affecting the ionic conductance of the SiN nanopore. By introducing rectangular apertures of varying dimensions in the center of the DNA plates we show that flexible molecules such as single-stranded or double-stranded DNA do translocate through the apertures while globular proteins with dimensions that exceed the aperture dimensions do not. Further, by including single-stranded heptanucleotides in the center of the apertures we were able to significantly delay the translocation of single-stranded DNA molecules through our hybrid nanopores. We observed step-wise translocation of DNA molecules in a sequence dependent fashion, revealing distinct off-rates for dissociation of di- to heptanucleotide duplexes formed by hybridization of segments of the translocating DNA molecule and the aperture-bound heptanucleotides. Taken together, our results open up rich prospects for applications of DNA origami / solid-state hybrid nanopores in a variety of fields such as DNA sequencing and molecular sorting.

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Gene replacement therapy for retinal CNG channelopathies

Stylianos Michalakis
LMU, Department of Pharmacy – Center for Drug Research

Cyclic nucleotide-gated (CNG) channels are crucial for phototransduction in retinal cones and rods. Mutations in the genes encoding the two cone photoreceptor CNG channel subunits, *CNGA3* and *CNGB3*, cause achromatopsia (ACHM). Mutations in the analogous rod CNG channel genes, *CNGA1* and *CNGB1*, lead to retinitis pigmentosa (RP). ACHM mutations result in congenital absence of cone photoreceptor function that is clinically characterized by strongly impaired daylight vision and loss of color discrimination. RP patients display a primary degeneration of rod photoreceptors that is followed by a secondary degeneration of cone photoreceptors, eventually leading to complete blindness. We used CNGA3$^{-/-}$ and CNGB1$^{-/-}$ mice as models to evaluate recombinant adeno-associated virus (rAAV)-mediated gene replacement as a potential treatment for ACHM and RP. Therapeutic rAAVs expressing mouse CNGA3 or CNGB1a under photoreceptor type-specific promoters were injected into the subretinal space of CNGA3$^{-/-}$ or CNGB1$^{-/-}$ mice. The treatment success was monitored using immunohistochemical, behavioral and/or electrophysiological methods.
Viral gene replacement normalized regular expression and localization of CNG channel complexes in outer segments. In contrast to untreated mice, treated CNGA3$^{-/-}$ mice became able to generate cone photoreceptor responses and to transfer these signals to bipolar and ganglion cells. Moreover, treated mice were able to solve a water-maze task designed to test for cone-mediated vision. Treated CNGB1$^{-/-}$ mice showed a significant improvement in rod photoreceptor function and morphology.
These results provide a proof-of-concept in mice for the treatment of ACHM and RP by rAAV-mediated gene replacement.

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The distribution of Nodal and Lefty signals is determined by differential diffusivity and clearance

Patrick Müller, Katherine Rogers, Joon Lee, Ben Jordan, Drew Robson, Xiaoxiao Zhang, Sharad Ramanathan and Alexander Schier
Harvard, Department of Molecular and Cellular Biology

One of the earliest patterning events during vertebrate embryogenesis is the formation of the three germ layers: endoderm, mesoderm and ectoderm. Nodal molecules are secreted proteins that induce the expression of meso- and endodermal target genes in a concentration-dependent manner. Previous studies suggested that the zebrafish Nodal signals Cyclops and Squint and their antagonists Lefty1 and Lefty2 have different activity ranges. However, it has been unclear how activity range is controlled. To visualize the distributions of these signals, we generated fluorescent fusion proteins of Cyclops, Squint, Lefty1 and Lefty2. We introduced a localized source of these proteins in zebrafish embryos and observed that Cyclops and Squint form gradients, whereas Leftys are almost uniformly distributed. To dissect the biophysical parameters contributing to the different gradient profiles, we measured the diffusion coefficients of the tagged proteins and determined that Leftys have a higher effective diffusion coefficient than Nodals. Using photoconvertible fusion proteins, we determined the extracellular half-lives of Nodals and Leftys and found that Leftys have a lower clearance rate than Nodals. Taken together, our data indicate that differential diffusivity and clearance account in large part for the distinct distribution profiles of these signaling molecules. Interestingly, the properties of Nodals and Leftys are consistent with classic reaction-diffusion models that postulate differential diffusivity of agonist and antagonist during morphogenesis.

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Feedback control of olfactory processing in mammals

Venkatesh Murthy
Harvard, Department of Molecular and Cellular Biology

Sensory processing is not a simple feed-forward process and higher brain areas can actively modulate how information is processed even in the earliest stages. In the olfactory system, odors are sampled actively by sniffing, which can be modulated by higher brain centers. In addition, strong feedback projections from the cortex as well as midbrain neuromodulatory centers are present even in the first processing stage (the olfactory bulb). In this talk, I will discuss our studies on the circuitry of these feedback projections as well as their influence on odor coding in the olfactory bulb.

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Toll-like receptor agonists prevent regulatory T cell infiltration of tumors by specifically inhibiting migration of FoxP3+ cells

Moritz Rapp
LMU, Division of Clinical Pharmacology

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Structure of the ligand binding domain of an iontropic glutamate receptor with an optical switch

Alwin Reiter
LMU, Department of Chemistry and Biochemistry

Compounds containing photoisomerizable double bonds are beginning to have a big impact on neuroscience and, double-bonded compounds can respond to light by converting between *trans* and *cis* isomers. The nitrogen-nitrogen double bond in azobenzenes converts from its *trans* to its *cis* form in response to ultraviolet light and switches back under green light.
By "caging" molecules such as glutamate with a light-activated, synthetically attached functional azobenzene group, it has been shown that neuronal signals can be modulated *in vivo*. For instance, using a light-activated glutamate-tipped switch, a zebra fish's response to touch could be controlled by selectively controlling glutamate-gated ion channels.[1] In one state of the switch (*cis* conformation), the ligand cannot reach the binding pocket, whereas in the other state (*trans* conformation), the ligand docks and stabilizes the activated conformation of the LBD. The LBD closes like a clamshell as it binds the agonist. This reversible binding and closure is allosterically coupled to the opening of the pore. The ultimate goal of such a system is to control the activity of neurons in culture and in living organisms using light.
Ion channels play a crucial role in the generation of action potentials. They open and close upon stimulation and allow ions to flow in and out of cells to propagate electrical signals. Glutamate-gated ion channels convert chemical messages into electrical signals. Here, glutamate acts as a ligand, inducing a conformational change in the ion channel that causes it to open.
Crystal structures of iGluRs LBDs in complex with glutamate and other agonists have already been solved.[2] With the structural knowledge of the LBD of iGluRs containing an optical switch it might be possible to design new photoswitches with higher selectivity and affinity. Also, this gives us detailed information about the mechanisms underlying the recognition and binding of the ligand and the associated conformational changes of iGluRs.

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Making mistakes in translation, accidentally on purpose

Eric Rubin
Harvard, Department of Immunology and Infectious Diseases

The complement of proteins in a cell is widely believed to accurately reflect the genetic code. However, at least in mycobacteria, a considerable number of proteins contain non-encoded amino acid substitutions. These bacteria have evolved to tolerate these substitutions even at high levels. Our data suggest that misincorporation of amino acids is a regulated process and, under some stressful conditions, misincorporation can be a beneficial process.

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Balance of direct/indirect pathway activity governs corticostriatal synaptogenesis

Arpiar Saunders
Arpiar Saunders*, Yevgenia Kozorovitsky* and Bernardo Sabatini
Howard Hughes Medical Institute, Harvard Medical School, Department of Neurobiology
*equal contribution

Neural activity plays a key role in wiring excitatory circuits in the brain, but the influence of inhibitory activity on neural system development is less well-understood. Here we focus on a complex inhibitory network of the basal ganglia, a group of forebrain nuclei that connect the cortex, thalamus and brainstem through a series of parallel inhibitory loops. The two main loops, direct and indirect pathways, originate from two populations of GABAergic medium spiny neurons in the striatum and exert opposite effects on basal ganglia output. We use BAC transgenic mice to target manipulations of activity to each pathway during postnatal development. Conditional knockout of the vesicular GABA transporter from MSNs completely abolishes GABA release, while MSN firing can be decreased by viral transduction of receptors activated solely by synthetic ligands. Selectively altering the output of direct and indirect pathway, whether throughout development or in the time window of excitatory synaptogenesis, exerted opposite effects on synaptogenesis and spinogenesis in the striatum. Our results support a model where basal ganglia pathway output sets the levels of glutamatergic innervation of the striatum during development. Because glutamate release was sufficient to cause the appearance of new dendritic spines with functional synapses on MSNs, the autoregulation of excitatory input into the striatum may be an important mechanism for titrating excitatory drive in the developing basal ganglia.

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Self-assembled DNA-nanostructure tools for molecular biophysics

William Shih
Harvard, Department of Cancer Biology

Our group previously solved a key challenge for nanotechnology: programmable self-assembly of complex, three-dimensional nanostructures. Our solution was to build custom three-dimensional structures that can be conceived as stacks of nearly flat layers of DNA. I will discuss applications of this technology for molecular biophysics: (1) weak-alignment media for NMR structure determination of membrane proteins such as UCP2, a 30 kDa six-transmembrane helix mitochondrial proton transporter; (2) scaffolds for determining the number of SNARE complexes required for lipid-bilayer fusion; (3) artificial actin filaments built as chimeras between protein and DNA for dissecting the mechanism of movement by processive myosin bipedal walkers.

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A screen for compounds that select against antibiotic resistance

Laura Stone
Harvard, Department of Systems Biology

Clinical use of antibiotics promotes selection for resistance, ultimately rendering the drugs ineffective. We suspect that in nature, antibiotics represent only one aspect of a complex set of secreted compounds, which also includes chemicals that select against resistance in the presence of antibiotics. We have developed a novel screening strategy, based on direct competition between antibiotic resistant and sensitive bacterial strains, for identifying compounds that select against antibiotic resistance. Such new chemical entities could be used together with their respective antibiotics as a treatment paradigm that slows or even reverses the spread of antibiotic resistance.

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A Systems Analysis of Mitochondrial Metabolism

Laura Strittmatter
Harvard, Department of Systems Biology

Compartmentalization of metabolic pathways and reactions is crucial for the regulation of metabolism. The mitochondrion is a particularly interesting compartment, given its importance in energy production and human disease, yet we are far from a complete understanding of mitochondrial metabolism. Additionally, of the 20,000 genes in the human genome, only 25% are currently well characterized. We hypothesize that some of these uncharacterized genes might be involved in mitochondrial metabolic pathways. To approach this problem, we have performed targeted metabolic profiling of ~250 polar metabolites on mitochondria isolated from mouse liver to identify those metabolites that change under various physiologic conditions. We have also used bioinformatics to prioritize mitochondrial-localized proteins with predicted enzymatic activity but no known metabolic function. Currently, we are using computation and RNAi to link metabolic enzymes of unknown function with mitochondrial metabolic activities, with the goal of identifying novel metabolic pathways that are active in mitochondria.

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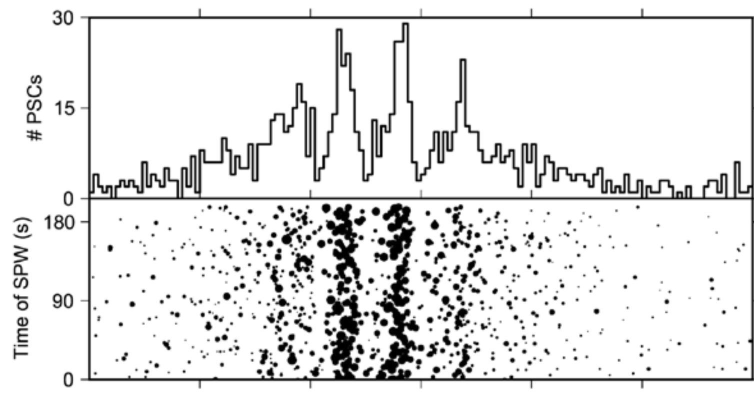
Coherent phasic excitation during hippocampal ripples

Álvaro Tejero-Cantero[1,2], Nikolaus Maier, Anja L. Dorrn, Jochen Winterer, Prateep S. Beed, Genela Morris, Richard, Kempter, James F.A. Poulet, Christian Leibold & Dietmar Schmitz.
1 LMU, Division of Neurobiology
2 Graduate School of Systemic Neurosciences

High frequency (~200 Hz) hippocampal neuronal network oscillations, or „ripples“, are thought to be involved in the formation and consolidation of episodic memory. According to current theories, a memory trace is an assembly of principal neurons that are simultaneously activated during ripple-associated network states.
First, I report on an in-vitro model [Maier et al. 2009]. The observed excitatory synaptic currents incoming into CA1 pyramidal neurons are rippled, phase-locked to the field oscillation and coherent among even distant pyramidal cells. These finding shows that assemblies of CA1 principal cells fire highly synchronized, a pre-requesite for fast replay phenomena in vivo.
Second, I also show that the capacity for the replay of such memory sequences in a hippocampal-like model network [Leibold & Kempter 2006] is strongly enhanced by introducing a global stabilizing inhibitory feedback.



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Neural basis of decision making: choosing what to do and how quickly to act

Alice Yiqing Wang
Harvard, Department of Molecular and Cellular Biology

Abstract: Animals have the ability to adjust their behavior depending on the prospects of their future outcomes. Current models propose that the process of making decisions occurs in two hierarchical stages, valuation and action selection. According to these models, certain brain areas first evaluate the utility of each potential outcome on an absolute scale (valuation) and next, downstream regions read out these absolute-values to compute the relative-values needed for the selection of the best action. Classic psychological theories, however, postulated a framework that differs from the above model and emphasized the importance of regulating one's generalized drive, or how motivation energizes a relatively non-specific set of actions. Taken together, optimal decision making thus depends on the ability to both 'energize' (regulation of performance vigor) and 'direct' actions (for action selection) appropriately. However, studies of decision-making have largely neglected this energizing process and instead focused on the ‚directing' aspect. In this talk, I will present our behavioral and neurophysiological experiments in rats that aimed to dissociate the energizing from the directing processes. We monitored the activity of single neurons in various regions of the striatum with tetrodes as rats performed a self-paced, two alternative choice task. We tease apart differentiable roles of the dorsal and ventral striatum in different stages of decision making: valuation, directing (selection), and energizing.

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Domain-level essentiality of the Mycobacterium tuberculosis genome

Jason Zhang
Harvard, Department of Immunology and Infectious Diseases

Mycobacterium tuberculosis (Mtb) has evolved to survive within a mammalian host, combating many arms of host immunity to establish infection. To understand bacterial networks that contribute to immune evasion, we developed a highly replicable and high-resolution genome-scale assessment of essentiality.
As confirmation of the method's efficacy, a pilot screen was performed. Transposon libraries were grown on standard media, and deep-sequencing of transposon-genome junctions identified the location of the transposon insertion for each surviving mutant. The number of insertions recovered in any given region was used to generate a test statistic defining the quantitative growth requirement of every 250-1000 bp region of the genome, resulting in an unbiased comprehensive map of genomic essentiality.
When overlaying gene annotations with our comprehensive growth requirement map, we discovered ~800 genes that contain regions required for growth. About 100 of these genes also contain regions not required for growth, likely due to differences in the contribution to growth between multiple domains in the same gene. Additionally, a comprehensive assessment of non-coding region growth requirement was performed, revealing 55 essential non-coding regions. We next seek to infect mice with CD4 and CD8 deficiencies to define the genes specifically required to combat those two arms of host adaptive immunity.

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For scientific enquiries, please contact:
Oliver Behrend, Managing Director, Munich Center for Neurosciences – Brain & Mind (MCN), o.behrend@lmu.de

For administrative enquiries, please contact:
Simone Glasl, Project Manager,
International Cooperation LMUexcellent,
simone.glasl@verwaltung.uni-muenchen.de