LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

HARVARD UNIVERSITY

LMU–Harvard Young Scientists' Forum

From Molecules to Organisms V
Munich, July 8 – 12, 2013

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 YSF was initiated as a yearly event in 2009 and is held alternately in Munich and Cambridge.

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■ **LMU-Harvard Young Scientists' Forum at the Center for Advanced Studies (CAS^{LMU}), LMU:** From Molecules to Organisms, Munich, July 8 – 12, 2013
■ **Under the auspices** of Prof. Dr. Ulrich Pohl, Vice-President for International Affairs, LMU
■ **Academic board:** Prof. Dr. Benedikt Grothe, Munich Center for Neurosciences, MCN, Prof. Dr. Dirk Trauner, Center for Integrated Protein Science Munich, CIPSM
■ **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)
■ **Institutional responsibility:** LMU International Affairs, Center for Advanced Studies (CAS^{LMU})

Conference agenda

Monday, July 08

Arrival	Individually arranged
19:30 – open	**Welcome dinner** (**Seehaus, Kleinhesselohe 3, 80802 Munich**; Stefan Lauterbach; Head LMU International Office)

Tuesday, July 09

CAS, Seestraße 13, 80802 Munich

09:00 – 09:30	**Welcome address** (Ulrich Pohl; LMU Vice President for International Affairs)
09:30 – 10:30	**Lecture 1** – **Martha Merrow**: "Molecular mechanisms of the circadian clock" (Intro: Stefan Lichtenthaler)
	Coffee break (catered)
11:00 – 13:00	**Session 1** – "Molecular methods" **Schneider/ Derbyshire/ Xie/ Masserdotti** chaired by Stefan Lichtenthaler
	Lunch break (catered)
14:30 – 16:30	**Session 2** – "Development" **Santoro/ Perera/ Murphy/ Krol** chaired by Magdalena Götz
	Coffee break (catered)
17:00 – 18:00	**Lecture 2** – **Venkatesh Murthy**: "Odor information coding and transformation in mammalian neural circuits" (Intro: Alexander Mathis)
18:00 – open	Conference BBQ (**CAS, garden**)

Wednesday, July 10

CAS, Seestraße 13, 80802 Munich

09:00 – 10:00	**Lecture 3** – **Martin Kerschensteiner**: "Cellular and molecular in-vivo imaging of the diseased nervous system" (Intro: Benedikt Grothe)
	Coffee break (catered)
10:30 – 12:30	**Session 3** – "Neural circuits" **Wienisch/ Morgan/ Bahl/ Ucpunar** chaired by Benedikt Grothe
	Lunch break (catered) & YSF faculty meeting (oval office)
14:30 – 16:30	**Session 4** – "Neural coding" **Tang/ Pecka/ Myoga/ Chen** chaired by Andreas Herz
	Coffee break (catered)

17:00 – 18:00	**Lecture 4** – **David Cox**: "Reverse-engineering visual object recognition in cortex" (Intro: Andreas Herz)
18:00 – open	At free disposal (student representative activities)

Thursday, July 11

Biocenter Martinsried, Grosshadernerstr. 2, 82152 Martinsried

08:15 – 09:00	Pre-arranged transfer hotel – biocenter
09:00 – 10:00	**Lecture 5** – **Ilona Kadow**: "The sense of smell: neural circuits of olfaction guided behaviour" (Intro: Stylianios Michalakis)
	Coffee break (catered; seminar room D00.013)
10:30 – 12:30	**Session 5** – "Signalling" **Kobold/ Freier/ Hassan/ Grassmann** chaired by Stylianios Michalakis
	Lunch break (catered) *& YSF poster session (foyer at D00.003)*
	Cantley/ Direnberger/ Heydenreuter/ Keplinger/ Koch/ Michalakis/ Murenu/ Pătîrniche/ Raps/ Thornton
14:30 – 16:30	**Session 6** – "Chemical biology" **Schönberger/ Koussa/ Dahmen/ Gaglia** chaired by Dirk Trauner
	Coffee break (catered)
17:00 – 18:00	**Lecture 6** – **Ralph Mazitschek**: "Chemical biology of HDAC inhibitors" & closing remarks (Intro: Dirk Trauner)
18:00 – 18:30	*YSF wrap-up session* *(faculty; seminar room D00.013)*
18:30 – open	BBQ faculty of biology, biocenter (Pre-arranged transfer biocenter – hotel)

Friday, July 12

Hotel Cosmopolitan, Hohenzollernstr. 5, 80801 Munich

09:00 – 19:00	Pick-up, excursion to Petersberg, Kufstein

Saturday, July 13

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

- **Kenneth Blum**, Executive Director, Harvard Center for Brain Science
- **Alexandra Cantley**, PhD Student, Department of Biological Chemistry and Molecular Pharmacology, Laboratory of Jon Clardy
- **Xiuye Chen**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Florian Engert
- **David Cox**, Professor, Department of Molecular and Cellular Biology
- **Emily Derbyshire**, Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Laboratory of Jon Clardy
- **Giorgio Gaglia**, PhD Student, Systems Biology, Laboratory of Galit Lahav
- **Richard Gregory**, Professor, Harvard Stem Cell Institute
- **Mounir Koussa**, PhD Student, Harvard Medical School, Laboratory of Wesley Wong
- **Sasha Krol**, PhD Student , Department of Neurobiology, Laboratory of Lisa Goodrich
- **Ralph Mazitschek**, Professor, Harvard Center for Systems Biology
- **Josh Morgan**, Postdoctoral Fellow, Department of Neurobiology, Laboratory of Jeff Lichtman
- **Alexander Murphy**, Ph.D. Student, Harvard University Program in Neuroscience, Laboratory of Jeffrey Macklis
- **Venkatesh Murthy**, Professor, Department of Molecular and Cellular Biology
- **Stephen Santoro**, Research Associate, Department of Molecular and Cellular Biology, Laboratory of Catherine Dulac
- **Hanlin Tang**, PhD Student, Harvard Graduate Program in Biophysics, Laboratory of Gabriel Kreiman
- **James Thornton**, PhD Student, Department of Medicine, Laboratory of Richard Gregory
- **Martin Wienisch**, Postdoctoral Fellow, Department of Molecular and Cellular Biology, Laboratory of Venkatesh Murthy
- **Ting Xie**, PhD student, Department of Chemistry and Chemical Biology, Laboratory of Nathanael Gray

Ludwig-Maximilians-Universität München (LMU)
Helmholtz Zentrum München – German Research Center for Environmental Health (HMGU)
Max Planck Institute of Neurobiology (MPIN)
Technische Universität München (TUM)

- **Armin Bahl**, PhD student, MPIN, GSN, Department of Systems and Computational Neurobiology, Laboratory of Alexander Borst
- **Oliver Baron**, Managing Director, Center for Integrated Protein Science Munich (CIPSM)
- **Oliver Behrend**, Managing Director, Munich Center for Neurosciences – Brain & Mind (MCN)
- **Martin Biel**, Professor, Department of Chemistry and Pharmacology
- **Lena Bouman**, Academic Coordinator (Natural Sciences and Medicine), Center for Advanced Studies (CASLMU)
- **Maria Dahmen**, PhD student, TUM, CIPSM, Laboratory of Stephan Sieber
- **Stephan Direnberger**, Postdoctoral Fellow, LMU, Neurobiology, Department Biology II, Laboratory of Lars Kunz
- **Stefan Endres**, Professor, LMU, Division of Clinical Pharmacology
- **Christoph Freier**, PhD student, LMU, Division of Clinical Pharmacology, Laboratory of Stefan Endres
- **Simone Glasl**, Project Manager, International Cooperation LMUexcellent
- **Magdalena Götz**, Professor, LMU, HMGU
- **Simon Grassmann**, PhD student, LMU, Division of Clinical Pharmacology, Laboratory of Stefan Endres
- **Benedikt Grothe**, Professor, LMU, Department Biology II
- **Sami Hassan**, PhD student, LMU, Department of Pharmacy – Center for Drug Research, Laboratory of Martin Biel
- **Andreas Herz**, Professor, LMU, Computational Neuroscience, Department Biology II
- **Wolfgang Heydenreuther**, PhD student, CIPSM, TUM, Laboratory of Stephan Sieber
- **Ilona Kadow**, PI, MPI of Neurobiology, Sensory Neurogenetics Group
- **Stefan Keplinger**, PhD student, LMU, Neurobiology, Department Biology II, Laboratory of Lars Kunz
- **Martin Kerschensteiner**, Professor, LMU, Institute of Clinical Neuroimmunology

- **Sebastian Kobold**, Postdoctoral Fellow,
  LMU, Division of Clinical Pharmacology, Laboratory of Stefan Endres
- **Max Koch**, PhD student,
  CIPSM, TUM, Laboratory of Stephan Sieber
- **Lars Kunz**, PI, LMU, Neurobiology, Department Biology II
- **Christian Leibold**, Professor,
  LMU, Neurobiology, Department Biology II
- **Stefan Lauterbach**, Head of LMU International Office
- **Stefan Lichtenthaler**, Professor,
  TUM, German Center for Neurodegenerative Diseases
- **Giacomo Masserdotti**, Postdoctoral Fellow,
  LMU, HMGU, Laboratory of Magdalena Götz
- **Alexander Mathis**, Postdoctoral Fellow,
  LMU, Computational Neuroscience, Department Biology II
- **Martha Merrow**, Professor,
  LMU, Institute of Medical Psychology
- **Stylianos Michalakis**, PI,
  LMU, CIPSM, Laboratory of Martin Biel
- **Elisa Murenu**, PhD student,
  LMU, HMGU, Laboratory of Magdalena Götz
- **Mike Myoga**, Postdoctoral Fellow,
  LMU, Department Biology II, Laboratory of Benedikt Grothe
- **Dinu M. Pătîrniche**, PhD Student
  LMU, Neurobiology, Department Biology II, Laboratory of Andreas Herz
- **Michael Pecka**, Postdoctoral Fellow,
  LMU, Department Biology II, Laboratory of Benedikt Grothe
- **Arshan Perera**, PhD student,
  LMU, Department of Pharmacy – Center for Drug Research, Laboratory of Martin Biel
- **Ulrich Pohl**, Vice-President for International Affairs, LMU
- **Johannes Raps**, PhD student,
  LMU, Division of Clinical Pharmacology, Laboratory of Stefan Endres
- **Simon Rothenfusser**, Professor,
  LMU, Division of Clinical Pharmacology
- **Andreas Schmidt**, Postdoctoral Fellow,
  LMU, Department of Molecular Biology, Laboratory of Stefan Endres
- **Sarah Schneider**, PhD student, LMU, HMGU, Laboratory of Leda Dimou
- **Matthias Schönberger**, PhD student,
  LMU, Department of Chemistry and Pharmacy, Laboratory of Dirk Trauner
- **Stephan Sieber**, Professor, CIPSM, TUM
- **Dirk Trauner**, Professor, LMU, Department of Chemistry and Pharmacy
- **Habibe Ucpunar**, PhD student,
  MPIN, Laboratory of Ilona Kadow

Abstracts of lectures and posters

Object tracking in motion-blind flies

Armin Bahl, Georg Ammer, Tabea Schilling, Alexander Borst
MPI of Neurobiology, Department of Systems and Computational Neurobiology

Different visual features of an object, such as its position and direction of motion, are important elements for animal orientation, but the neural circuits extracting them are generally not well understood. We analyzed this problem in *Drosophila*, focusing on two wellstudied behaviors known as optomotor response and fixation response. In the neural circuit for motion computation, columnar T4 and T5 cells are thought to play a pivotal role1. We found that blocking these neurons resulted in a complete loss of the optomotor response rendering the flies motion-blind. Nevertheless, these flies were still able to fixate a black bar, although at a reduced performance level. Further analysis revealed that flies in which T4 and T5 cells were blocked possess an intact position circuit that is implemented in parallel to the motion circuit.
The position circuit generates a turning response toward the local luminance changes created by the moving object and thereby leads to its fixation. In control flies, the motion circuit induces turning in the direction of motion of the object. This response is stronger for front-to-back than for back-to-front motion, which further improves object fixation.
We conclude that the optomotor response is exclusively controlled by the motion circuit, whereas the fixation response is supported by both the position and the motion circuit.

[1] Schnell, B., Raghu, S. V., Nern, A. & Borst, A. Columnar cells necessary for motion responses of wide-field visual interneurons in *Drosophila*. *J. Comp. Physiol.* A 198, 389–395 (2012).

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Elucidating the role of secondary metabolites in a novel *D. discoideum* – bacterial symbiosis

Alexandra Cantley
Harvard, Department of Biological Chemistry and Molecular Pharmacology

The study of symbiotic relationships can reveal a wealth of interesting evolutionary adaptations, unique biological phenomena and novel modes of chemical communication. In this study we investigate a symbiotic relationship between the social amoeba *Dictyostelium discoideum* and the environmental bacterial species that it farms. Interestingly, while about half of the farmed species serve as a food source for the amoeba, the role of the other half remains unknown. We hypothesize that the study of secondary metabolite exchange between non-food source bacteria and their amoeba host may uncover small molecules that promote host growth or provide some other ecological advantage. Our study of the non-food source bacteria *Pseudomonas fluorescens Dd-2*, led to the isolation of a novel chromene that can both increase spore production of the farmer *D. discoideum* and decrease that of the non-farmer. Genomic comparisons of the non-food source *P. fluorescens- Dd2* with *P. fluorescens-Dd3*, a close relative that *is* a food source, revealed characteristics that differentiate food source bacteria from non-food source bacteria. Ongoing investigations are exploring the chemical communication between the lesser-studied non-food source bacteria *Burkholderia xenovorans*, and its *D. discoideum* host.

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History-dependent modulation of turning underlies phototactic behavior in larval zebrafish

Xiuye Chen[1], **Florian Engert**[1,2]
1 Harvard, Department of Molecular and Cellular Biology
2 Harvard, Center for Brain Science

Understanding how the brain transforms sensory input into complex behavior is a fundamental question in systems neuroscience. Here we study the temporal component of phototaxis in the larval zebrafish, that is, orientation decisions that are based on comparisons of light intensity at successive moments in time.
We developed a novel "Virtual Circle" assay where a whole-field illumination is abruptly turned off when the fish swims out of a virtually defined circular border, and turned on again when it returns into the circle. Under these conditions the animal receives no direct spatial cues, experiencing only whole-field temporal fluctuations in light. Remarkably, the fish spends most of its time within the invisible virtual border. Behavioral analyses of swim bouts in relation to lightdark and dark-light transitions were used to develop four discrete temporal algorithms that transform the binary visual input (uniform light/uniform darkness) into the observed behavior. Many aspects of these algorithms are based on the specific few-step behavioral history before individual turning events. Computer simulations show that these algorithms almost completely recapture the behavioral swim statistics of real fish.
We discovered that turning properties in larval zebrafish are distinctly modulated by temporal step functions in light intensity in combination with the specific motor history preceding these turns. Several aspects of the behavior suggest memory usage over up to 10 bouts (~10 sec). Thus, we show that a complex behavior that might appear to require an internal representation of space can actually emerge from only a small number of relatively simple temporal rules.

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Reverse-engineering visual object recognition in cortex

David Cox
Harvard, Department of Molecular and Cellular Biology

Humans are able to interpret complex visual scenes with such ease that it is easy to overlook what an impressive computational feat this represents. Any given object in the world can cast an effectively infinite number of different images onto the retina, depending on its position relative to the viewer, the configuration of light sources, and the presence of other objects in the visual field. Biological visual systems are able to effortlessly account for all of this variation while remaining sensitive to the subtle variations that distinguish similar, but different objects –- a feat that no current artificial
system can come close to achieving. In my talk, I will give a tour of our efforts to tackle this problem both from a „reverse"-engineering standpoint -- studying how neuronal circuits achieve this computation, as well as using a „forward"-engineering approach -- building computer models of these networks. Along the way, I'll describe our work establishing and taking advantage of new rodent models in a traditionally primate-dominated field, and outline new opportunities to bring powerful tools to bear on this problem.

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The architecture of the ClpXP protease derived from *Staphylococcus aureus* and *Listeria monocytogenes*

Maria Dahmen, Stephan A. Sieber
TUM, CIPSM, Organic Chemistry Department

The caseinolytic protease P (ClpP) is a virulence regulator in bacterial pathogens like *Staphylococcus aureus* or *Listeria monocytogenes*. To gain proteolytic activity, the ClpP multimer associates with one or two hexameric rings of Clp ATPases like ClpX, forming a proteolytic complex. Repetitive cycles of ATP hydrolysis by ClpX unfold ssrA-tagged substrates *in vivo*. We monitor the degradation of ssrA-tagged green fluorescent protein (GFP) to study this system *in vitro* and gain insight in this proteolytic activity.

Although most organisms possess a single ClpP protein, some organisms like *L. monocytogenes* encode two ClpP isoforms. Interestingly, LmClpP2 shares a high-sequence homology with ClpP enzymes of various organisms that feature one ClpP. LmClpP1 exhibits only 41% sequence identity with LmClpP2, raising the question of how these two distinct isoforms interact and how they differ functionally. The *in vitro* GFP-ssrA degradation assay was used to understand the architecture and different functions of the two LmClpP isoforms.

The GFP-ssrA degradation is used as a tool to study proteolytic activity which is closer to the natural environment instead of the more artificial peptidase activity. Thus the GFP-ssrA degradation assay was used to validate and compare several inhibitors like β-Lactones, fluorophosphonates and sultams which have been found to bind the *S. aureus* ClpP based on activity based protein profiling (ABPP).

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Discovering molecules to probe and treat malaria

Emily Derbyshire
Harvard, Department of Biological Chemistry and Molecular Pharmacology

Discovering new malaria inhibitors is critical for the development of chemical tools to advance our biological understanding of *Plasmodium* parasites, the causative agents of the disease, and is requisite for the design of new drugs. The life cycle of malaria parasites includes an obligatory asymptomatic liver stage in a human host, which enables progression to the lifethreatening blood stage. The liver stage of malaria remains relatively unexplored despite the fact that these parasites contain potential targets for prophylaxis. Using a high-throughput phenotypic screening approach several small molecules were identified that inhibit liver stage malaria, including several FDA-approved drugs. This presentation will include a summary of screening hits and identification of putative targets.

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Calcium imaging and optical manipulation of vestibular neurons in the Axolotl

Stephan Direnberger[1], Roberto Banchi[1], Christian Seebacher[2], Rainer Uhl[2], Felix Felmy[1], Hans Straka[1], Lars Kunz[1]
1 LMU, Division of Neurobiology, Department Biology II
2 LMU, BioImaging Center, Department Biology I

The combination of several imaging methods, such as calcium imaging and optical manipulation of neuronal networks becomes a progressively important feature in neuroscientific studies. Here we introduce a new epi-fluorescence microscope concept which enabled us to implement two spatially and temporally separated illumination pathways using lost-cost, high-power LEDs. These two optical pathways can be illuminated by LEDs of various wavelengths and are independently adjustable in size and position. Image detection is realised by the latest generation of a CMOS camera providing image capturing at high resolution and speed-rates of 100 Hz at maximum resolution. Moreover, we implemented a voice-coil driven high NA objective to ensure z-movement at maximum speed and precision. The multiple LED arrangement and the separation of excitation pathways allow us to accomplish different imaging approaches simultaneously. Combining these optical features with electrical stimulations becomes a powerful tool to investigate function and connectivity of neuronal pathways. The performance of the microscope was tested using in vitro whole head preparations of axolotl (Ambystoma mexicanum) larvae. This preparation allows studying neuronal systems with all sensory pathways intact and can be maintained up to one week. Thus, we were able to use calcium imaging to record sensory evoked neuronal responses of central vestibular neurons elicited by electric stimulation of specific semicircular canals. Further, to quantify the glutamate uncaging efficiency we calculated the required light intensity and duration to optically evoke action potentials by patching those neurons. Finally, we could show that by means of spatially separating calcium imaging and glutamate uncaging we were able to manipulate ipsilateral semicircular canal evoked calcium responses by optically activating contralateral inhibitory/excitatory pathways.

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Characterization of regulatory T cells attracting chemokines as targets for cancer therapy

Christoph Freier
LMU, Division of Clinical Pharmacology

Different types of immune cell can be found in malignant tumors among them a population named regulatory T cells (Tregs). Tregs are a subpopulation of T cells with the capacity of down-regulating the immune response, thus promoting tumor-induced immune suppression. Infiltration of tumors with Treg is associated with bad prognosis in many patients. Several chemokines were described to be responsible for Tregs accumulation in tumors and therefore, represent potential targets or cancer therapy. A systematic analysis that evaluates the capacity of all so far known chemokines to attract Tregs would help to identify more of these targets. We quantified the expression of all known human chemokine receptors on Tregs and performed migration assays with the respective chemokines. Our results show that only a very few chemokines are able to specifically attract Tregs. Among CC chemokines we identified CCL22, CCL1 and CCL27. These chemokines specifically recruit Tregs and thus maybe involved in tumor-induced immune suppression. It will be interesting to evaluate these chemokines for tumor therapy.

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Dynamics of p53 oligomerization in single living cells

Giorgio Gaglia[1], Yinghua Guan[2], Jagesh V Shah[2], Galit Lahav[1]
1 Harvard Medical School, Department of Systems Biology
2 Brigham and Women's Hospital, Department of Medicine

Homo-oligomerization, the formation of a protein complex out of identical components, is extremely common in nature; in E. Coli it is estimated that 35% of proteins form homo-oligomers with an average of 4 subunits per complex. In yeast and human cells many transcription factors undergo homo-oligomerization, which has been shown to be crucial for their function. The molecular dynamics of oligomerization have been studied for some proteins *in vitro*, but no study has measured a dynamic oligomerization process in cells and quantified discrete number of oligomers.

One such transcription factor is the stress response regulator p53, which orchestrates cell fate decisions such as cell-cycle arrest, senescence and apoptosis. Homo-tetramerization of p53 is required for its direct binding to DNA. Mutations in the p53 tetramerization domain (326-356 aa) lead to a reduction in, or loss of, its transcriptional activity in cells and were shown to cause early cancer onset. *In vitro* evidence suggests that p53 should be primarily dimeric in basal conditions and that it forms tetramers in stressed conditions. However there is no experimental evidence for this in live cells.

We used fluorescence correlation spectroscopy (FCS) to quantify the fraction of p53 monomers, dimers and tetramers in living single cells in a basal state and after DNA damage. We then combined these measurements to mathematical modeling to study the dynamical changes in p53 homo-oligomerization and its effect on p53 transcriptional function.

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A new PD1-CD28 chimeric receptor overcomes PD-1-mediated immunosuppression in adoptive T cell therapy

S. Grassmann[1], P. Peters[1], Y. Zeng[1], Jan Schmollinger[1], Stefan Endres[1] and Sebastian Kobold[1]
1 LMU, Division of Clinical Pharmacology

Background: Although tumor specific, cytotoxic T cells are capable of killing tumor cells both *in vitro* and *in vivo*, treatment of immunocompetent hosts with adoptive T cell transfer does not lead to sufficient tumor regression without adjuvant therapy. This lack of efficacy has been proposed to be due to tumor-promoted T cell exhaustion and anergy. We and others have previously shown that programmed death receptor-1 (PD-1) upregulation is a hallmark of tumor infiltrating, adoptively transferred T cells. PD-1 and its ligand (PD-L1) constitute a major immunosuppressive axis driven by tumor cells. Disruption of this axis may hit an Achilles he*E*l heal of tumor immune escape.

Methods: A PD1-CD28 chimeric receptor was cloned into the retroviral vector pMP71 and expressed in primary murine T cells specific for the model antigen ovalbumin (OT-1 cells). Functionality was addressed *in vitro* using ELISA and flow cytometry. *In vivo* ovalbumin and PDL1 overexpressing Panc02 tumor cells were inoculated subcutaneously in immunocompetent female C57Bl/6 mice. Mice (n = 6 per group) were treated twice i.v. with chimeric receptortransduced T cells or controls. Primary endpoint was tumor growth.

Results: *In vitro*, PD-1-CD28 chimeric receptor-transduced primary T cells released 130 fold more interleukin-2 (IL-2) and more than 300 fold more interferon-γ than untransduced and control-transduced T cells when stimulated with CD3 and PD-L1, demonstrating the functionality of the chimeric receptor ($p < 0.01$). In co-culture experiments with the tumor cell line Panc02, effective co-stimulation through PD1-CD28 was only seen in the presence of the TCRrecognized antigen OVA and PD-L1. Upon blockade of MHC or PD-1, co-stimulation through the receptor was abrogated. Culture of transduced T cells in the presence of CD3 and PD-L1 increased cell numbers 4 fold and significantly increased viability of cells compared to untransduced or control-transduced T cells ($p < 0.01$). *In vivo*, treatment of mice with an established (OVA and PDL1 expressing) Panc02 subcutaneous tumor (mean tumor size at treatment onset 26.2 mm3) with PD1-CD28-transduced OT-1 slowed tumor growth compared to treatment with with control-transduced OT-1 cells ($p < 0.01$). This demonstrates the functionality of the chimeric receptor in an immunocompetent organism.

Conclusions: Adoptive T cells therapy with PD-1-CD28 chimeric receptor-transduced T cells is a promising approach to overcome PD-1/PD-L1-mediated tumor-induced anergy and immunosuppression.

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The role of Two-pore-Channels in vesicular fusion and neuronal function

Sami Hassan
LMU, Department of Pharmacy, Center for Drug Research

Regulation of ionic homeostasis in intracellular organelles critically depends on intracellular ion channels. There is initial evidence that two pore channels (TPCs) are specifically expressed in endo/lysosomes and are involved in regulating membrane trafficking, signal transduction and vesicular ion homeostasis. Recently, we and two other groups demonstrated that two pore channels (TPCs) are lysosomal Ca^{2+} release channels that are activated by a distinct second messenger, nicotinic acid adenine dinucleotide phosphate (NAADP). It is currently unclear whether activation by NAADP is conferred by direct binding of this ligand to the TPC channel or, alternatively, to a not yet identified NAADP binding protein that is tightly assembled with TPCs. Moreover, TPC channel activators different from NAADP may also exist in the cell. So far, there is very limited information about the physiological processes which are regulated by TPCs. It has been suggested that TPCs regulate the secretion of hormones, smooth muscle contraction and the development of skeletal muscle. Here, I will present the role of TPCs for vesicular fusion processes and neurotransmitter release in the hippocampus.

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Synthetic studies towards activity based probes for evaluation of protein reactivity of the highly bioactive natural products (-)-Nitidon and Pestalone

Wolfgang Heydenreuter, Stephan A. Sieber
TUM, CIPSM, Organic Chemistry Department

(-)-Nitidon
The fungal metabolite (-)-Nitidon, isolated from *Junghuhnia nitida*, is characterised by a pyronediyne structure unique to this class of natural products. The compound itself displays a variety of interesting biological activities, e.g. cell differentiation, antibacterial and antifungal properties and cytotoxicity in the nanomolar range. Although different derivatives were synthesized and tested in a preclinical antitumor screening,[1] there is no biological target known to date. As the electrophilic diyne system might be susceptible to a nuclephilic attack of activated amino acid moieties, we investigate the possibility to synthesize alkyne bearing probes for *activity* based *protein profiling*. Pyrones are furthermore capable of forming high reactive ketene intermediates upon irradiation with UV-light. This should enable target identification *via* cross linking reactions in proteoms.[2]

Pestalone
The highly functionalized benzophenone Pestalone, isolated from the marine fungus Pestalotia, exhibits strong antibacterial activity against drug resistant strains of *Staphylococcus aureus* and *Enterococcus faecium*. Although two total syntheses were published in recent years,[3,4] studies towards the target identification of this compound are not reported so far. We managed to introduce an alkyne moiety into the core structure and proceed to elucidate structure/target relations by UV-crosslinking/*affinity based protein profiling* methodology.

[1] Bellina, F.; Carpita, A.; Mannocci, L.; Rossi, R. *Eur. J. Org. Chem.* 2004, 2610-2619.
[2] Battenberg, O. A.; Nodwell, M. B.; Sieber, S.A. *J. Org. Chem.* 2011, 76, 6075–6087.
[3] Iijima, D.; Tanaka, D.; Hamada, M.; Ogamino, T.; Ishikawa, Y.; Nishiyama, S.; *Tetrahedron. Lett.* 2004, 45, 5469–5471.
[4] Slavov, N.; Cvengros, J.; Neudörfl, J.-N.; Schmalz, H.-G. *Angew. Chem. Int. Ed.* 2010, 49, 7588 –7591.

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The sense of smell: neural circuits of olfaction guided behavior

Ilona Kadow
MPI of Neurobiology, Sensory Neurogenetics Group

The focus of my presentation will be the formation and function of the olfactory nervous system. Research in my lab combines Drosophila genetics with behavioral studies, anatomy, electrophysiology and functional imaging. Specifically, I will speak about two main directions in our lab. First, because odors are detected and translated by specific receptor neurons into an odor-specific connectivity map in the brain, we have identified several genetic factors in olfactory circuit formation. Second, we ask how the wiring of the olfactory nervous system relates to the behavior it controls. In particular, I will talk about our approaches aiming at understanding how positive and negative cues are encoded in the brain, and how valence decisions depend on internal and external context.

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An optogenetic approach to the auditory system of the Mongolian gerbil

Stefan Keplinger[1], Fred Koch[2], Stylianos Michalakis[2], Martin Biel[2], Benedikt Grothe[1], and Lars Kunz[1]
1 LMU, Division of Neurobiology, Department Biology II
2 LMU, CIPSM, Department of Pharmacy – Center for Drug Research

An optogenetic approach to the auditory system of the Mongolian gerbil
Stefan Keplinger1, Fred Koch2, Stylianos Michalakis2,Martin Biel2, Benedikt Grothe1, and Lars Kunz1
1LMU, Division of Neurobiology, Department Biology II
2LMU, CIPSM, Department of Pharmacy – Center for Drug Research

The Mongolian gerbil (*Meriones unguiculatus*) represents an apt model organism for human hearing due to its similar hearing range and ability to localise low frequency sound sources. However, electrophysiological recordings in auditory nuclei reach their limit in specificity when it comes to electrical stimulation within these nuclei heavily intermingled with massive fibre bundles passing through. In contrast, utilising optogenetic techniques would allow for specific control of electrical activity of neurones in individual auditory nuclei by light and would circumvent off-target responses from passing fibres where electrical stimulation lacks precision. However, by choosing the Mongolian gerbil as model we currently lack genetic tools already established in mouse or rat, which has so far hampered the use of optogenetics in this species.
To establish optogenetics in the Mongolian gerbil, we performed stereotactic injection of adeno-associated virus (AAV) into auditory nuclei of anaesthetised animals followed by an expression period of 2-3 weeks. Animals were sacrificed and brain slice preparations were performed. Whole-cell patch-clamp recordings from targeted auditory nuclei in acute brain slices were used to characterise kinetic parameters of the expressed channelrhodopsins. The spread of the AAV infection and the co-localisation of fluorescence-tagged channelrhodopsin with a neuronal marker (microtubule-associated protein 2, MAP2) were determined by immunohistochemistry and confocal scanning of paraformaldehyde-fixed brain slices.
AAV mediated gene delivery reliably drove neuronal-specific expression of channelrhodopsins in the inferior colliculus (IC) and other auditory nuclei. Viral transduction of neurons in these nuclei with channelrhodopsin variants enabled us to control neuronal activity with light of appropriate wavelengths. Performing current-clamp recordings we determined maximum firing rates, firing reliability and jitter of elicited action potentials. Transitions from peak to stationary current (τin), current kinetics after light-off (τoff) and maximum photocurrents were measured in voltage-clamp mode. Our results represent a first step to establish optogenetics in the auditory system of the Mongolian gerbil and might aid to decipher the intricate connectivity of auditory nuclei.

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Cellular and molecular *in vivo* imaging of the diseased nervous system

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In my lecture I want to discuss how modern *in vivo* microscopy techniques can improve our understanding of the cellular, subcellular and molecular mechanisms that mediate neuroinflammatory tissue damage. To illustrate this approach I will use our recent insights into the *in vivo* pathogenesis of immune-mediated axon damage as an example. Immune-mediated axon damage plays a crucial role in inflammatory diseases of the central nervous system (CNS) like multiple sclerosis (MS), as the number of axons damaged by immune cells critically determines the clinical disability of MS patients. However we still understand very little about the process that leads to axon damage. Recently, we have used an *in vivo* imaging approach to investigate the pathogenesis of immune-mediated axon damage in an animal model of multiple sclerosis. By time-lapse imaging of fluorescently labeled axons we could follow the slow and spatially restricted degeneration of axons in inflammatory CNS lesions. This "focal axonal degeneration" appears to be a novel type of axonal degeneration that is characterized by intermediated stages that can persist for several days and progress either to the degeneration or full recovery of the affected axons. *In vivo* imaging approaches now allow us to address the following key aspects of the axon degeneration process: First, to identify the molecular mechanisms that drive axonal degeneration, we now reveal the actions of key damage mediators, in particular the influx of calcium and the release of reactive species, *in vivo*. Second, to better understand the relation between structural and functional axon damage in neuroinflammatory lesions, we directly measure axonal transport in neuroinflammatory lesions. Using these examples, I hope to illustrate how recent advances in light microscopy can help us to reveal and mechanistically dissect neuroinflammatory processes as they happen in the living nervous system.

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A new EGFR – EpCAM bispecific antibody enhances the efficacy of adoptive T-cell therapy in a murine gastric tumor model

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Background: A limiting step for adoptively transferred tumor-specific T cells is their recruitment from the blood circulation to the proximity of tumor cells and subsequent engagement in direct tumor cell contact. We hypothesized that a bispecific antibody recruiting T cells to a target antigen on tumor cells could enhance T-cell-tumor interaction and thus increase the efficacy of adoptively transferred T cells.

Methods: A new bispecific murine IgG2a antibody (BsAb) was generated that recognizes EpCAM as a tumor antigen and truncated EGFR (delta-EGFR) as an inert surface marker protein on transduced T cells. T cells from transgenic mice for TCR specific for the SV40 large T antigen (TCR-1) were retrovirally transduced with delta-EGFR. S.c. tumors were induced in C57Bl/6 mice by injecting mGC8 cells derived from a syngeneic large T antigen expressing EpCAM-positive gastric tumor.

Results: *In vitro*, the BsAb increased (4-fold) binding of transduced T cells to EpCAM positive tumor cells. In the presence of the BsAb, tumor-directed T cells efficiently lysed EpCAM-positive cells (83 % at a 10:1 effector to target ratio). *In vivo*, the antibody reached EpCAM+ tumor cells as evidenced by immunofluorescence. mGC8 tumor-bearing mice were treated twice with a combination of the BsAb and transduced TCR-I T cells. Tumor growth was significantly reduced for over 30 days (n=12) compared with control groups (transduced T-cells or BsAb alone) and survival was prolonged by > 30 days ($p<0.001$).

Conclusions: Co-administration of a BsAb bridging adoptively transferred tumor-specific T cells via an inert surface molecule to a tumor-associated surface antigen enhances the efficacy of therapeutic T cell transfer.

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Untargeted metabolite profiling as a tool for target validation in ABPP

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Nature offers a vast diversity of molecular motifs that exhibit promising potency against severe diseases such as cancer. Thus, biological target identification of these compounds represents a prominent goal in chemical biology research. A recent and remarkable proteomic method is activity-based protein profiling (ABPP), which facilitates the target identification of small molecules in intact biological organisms.

In course of our work, we identified the enzymatic target of an acivicin-derived 3-haloisoxazol probe. *In situ* experiments in Gram-positive bacteria *Bacillus subtilis* revealed an aldehyde dehydrogenase as the major targeted enzyme. Further investigation of the probe's influences on the complex metabolic network is performed via metabolomic studies. Thereby untargeted metabolite profiling on a sophistical LC-HRMS based analytical platform is established.

Combining these two powerful "-omic" approaches, we aim to establish a proof of concept for further investigations of natural product-derived small molecules. *In vivo* studies of various biological systems verify new targets in drug therapy and demonstrate their possible potency as therapeutic agents.

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A novel and robust technique for creating protein-DNA hybrids

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DNA has become the scaffold of choice for the construction of self-assembling nanosturctures. As techniques for the programmed patterning of these structures have advanced, it is now possible to create functional structures. Such structures have proved very useful in biophysical studies of proteins. Antibodies, molecular motors, and cytoskeletal proteins have been studied at great length using such techniques. Many of the proteins of interest to neuroscientists, which are often less amenable to harsh linking-chemistries, have not. The barrier is the development of a successful means of coupling a protein of interest to DNA. Chemistries often used for linking proteins to oligonucleotides include di-sulfide linkage and Thiol-primary amine linkages. Although these chemistries are often effective, they react with functional groups common in biology. These techniques can thus not be used when the protein of interest has endogenous cysteines or when one wants to couple specifically to the N or C terminus. For this reason we have developed a new and robust technique that frontloads all non-enzymatic chemistry to an oligonucleotide and small synthetic peptide, which are far more tolerant of non-physiological conditions. This technique utilizes a combination of commercially available purification resins and an evolved version of the enzyme sortase, isolated from *Staphylococcus aureus*, to yield the product of interest pure of any side products and reactants.

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The Fat3 signaling pathway in neuronal morphogenesis

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Neurons need to be precisely oriented with respect to other neurons and their environment to make the correct connections vital for nervous system function. To orient themselves correctly, neurons must interpret extracellular cues and translate them into a polarized cytoskeleton. In the retina, amacrine cells extend a single dendritic arbor into the inner plexiform layer (IPL). Migrating amacrine cells retract trailing processes present during migration following contact with the IPL. A good candidate to receive an orienting cue from the IPL is Fat3, a cell surface receptor expressed by amacrine cells and localized to the IPL. Amacrine cells lacking Fat3 fail to reliably retract extra processes and form an extra dendritic arbor. We propose that asymmetric Fat3 signaling helps instruct amacrine cell morphogenesis.

The requirement of Fat3 for normal amacrine cell morphology suggests that Fat3 guides the organization of the cytoskeleton. However nothing is known about the Fat3 signaling pathway. We have found that the Fat3 ICD binds Ena/VASP proteins, major regulators of the actin cytoskeleton and have identified the Ena/VASP binding site in the Fat3 ICD. The localization of Fat3 to the elaborated amacrine cell dendrite in the IPL suggests that asymmetric Fat3 signaling is critical to guide amacrine cell morphology. We propose that the absence of Fat3 leads to less asymmetric Ena/VASP localization which disrupts the retraction of inappropriate dendritic processes. Supporting this, uniform Ena/VASP localization in amacrine cells led to extra processes reminiscent of those formed in the absence of Fat3 signaling. Linking Ena/VASP signaling to the extracellular environment through Fat3 provides a novel mechanism for neurons to polarize relative to each other.

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Investigating the molecular mechanisms of astroglia-to-neuron conversion by forced expression of proneural genes

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Direct conversion of differentiated cells into neurons is emerging as a promising alternative for cell-based therapies of neurodegenerative diseases, and represents a novel approach to tackle fundamental questions of developmental biology regarding cell plasticity, cell fate specification, and differentiation. For instance, we showed that astroglia cultures obtained from postnatal mouse cerebral cortex could be efficiently converted into functional neurons upon overexpression of two proneural genes, *Neurog2* and *Ascl1*.

To understand the molecular mechanisms underlying astroglia-to-neuron conversion, we generated a fusion construct in which the cDNA of the proneural gene (either *Neurog2* or *Ascl1*) is fused to a modified estrogen receptor-binding domain (ER^{T2}), thus rendering transcription factor activity dependent upon tamoxifen addition to the culture medium. Unbiased transcriptome analysis of RNA extracted from astroglial cultures transduced with control viruses or *Neurog2*- (or *Ascl1*)-ER^{T2} expressing viruses and treated with tamoxifen for 24 hours identified a small subset of genes regulated by both transcription factors, suggesting that the two proneural proteins activate different neurogenic programs already at a very early stage of reprogramming. Among the shared targets, we investigated the role of some transcription factors in the execution of a generic program for neurogenesis, and found that some of them – NeuroD4, Insm1, Prox1, Sox11 and others – not only are important during Neurog2-induced reprogramming, but also could generate neurons from astroglia when co-expressed.

These data provide first insights into the early stages of direct reprogramming towards neurogenesis, highlighting the early activation of a neurogenic program, and identifying key factors required for the neuronal generation from postnatal astroglial cells.

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Chemical biology of HDAC inhibitors

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HDACs are master regulators of chromatin structure and function. Beyond controlling the acetylation state of histones they are widely recognized as regulators of non-histone proteins. HDAC inhibitors have been instrumental to study basic biology of HDACs and recognized as promising drugs for the treatment of cancer and beyond.

Our group has developed novel HDAC inhibitors and assays to better understand the function of individual HDAC isoforms and to identify lead candidates for the development of novel therapeutics.

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Molecular mechanisms of the circadian clock

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The circadian clock is a temporal program that coordinates all levels of biology from genes to behaviour. Accordingly, the clock is important for health and performance. A similar molecular mechanism involving transcriptional feedback loops as well as metabolic oscillators is apparently present in organisms from unicellular cyanobacteria to mammals. Major questions in circadian biology include how clock 'outputs' are regulated and how the circadian system synchronises with the environment (entrainment). We use simple clock systems such as *Neurospora crassa*, *Caenorhabditis elegans* and human tissue culture cells to answer these questions.

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Mapping neural circuits in mouse visual thalamus using high throughput electron microscopy

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Broadly speaking, neural circuits are often thought of in terms of the connections that link brain regions or link neuronal cell types. However, much of the activity dependent development of the nervous system is devoted to generating a different kind of neural circuit in which particular patterns of connectivity among neurons of a single type are elaborated by the gain and loss of connections. The present understanding of these patterns of connectivity within a class of cells is quite limited. Much of the problem stems from the technical challenges of mapping the synaptic connectivity of large numbers of neurons in a single piece of brain tissue. Using new techniques for automating both brain sectioning and electron microscope imaging, I have imaged a volume of an activity regulated neural circuit (the mouse visual thalamus) that is larger than any previous high resolution reconstruction of mammalian brain tissue. Now, using a combination of manual and computer automated segmentation tools I am tracing a set of overlapping retinal projection axons to map their connectivity to a network of thalamic target cells.

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Role of Polycomb Repressive Complex 1 in neuronal differentiation

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Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) are known to maintain cell stemness by repressing key developmental genes. In the canonical model, PRC2 would tri-methylate histone 3 on lysine 27 (H3K27me3) and PRC1 would bind to this mark and promote chromatin compaction by mono-ubiquitylating histone 2A on lysine 119 (H2AK119Ub). However, many different PRC1-like complexes were described to act independently of PRC2 and to regulate transcription of genes that are partly different from the one bound by canonical PRC1. Despite these differences, the ubiquitin-ligase Ring1B was found in all PRC1 complexes so far characterised.

In the neural lineage, *in vitro* experiments indicated that genes required for neural differentiation are repressed by PRC2 in proliferating embryonic stem cells. Moreover, at developmental stages, PRC1 catalytic protein Ring1B has been demonstrated to be crucial for the correct onset of gliogenic phase and for the repression of genes implicated in the neuronal glutamatergic lineage. However, little is known about PRC1 activity at adult stages in progenitors from the sub-ependymal zone (SEZ) and in their progeny.

Here we investigate the role of PRC1 in lineage choices of adult neural stem cells, by analysing their differentiation potential *in vitro* and *in vivo* after deletion of Ring1B protein.

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Investigating the coordination of cellular identity programs with molecular wiring mechanisms

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During axonal wiring of the mammalian neocortex, many distinct subtypes of projection neurons (PNs) extend axons to reach a variety of specific intracortical, subcortical, and subcerebral targets. These diverse targets are reached through tightly coordinated, regulated expression of receptors that interpret sequential, molecular guidance cues presented in the extracellular environment. Previous work from our lab and others has identified several combinatorial transcription factor programs that specify distinct PN subtypes. However, given the large suites of gene loci typically regulated by such transcription factor programs, their downstream effectors remain largely unidentified, particularly those that implement subtypespecific axonal wiring.

Our lab has previously identified the zinc finger transcription factor Ctip2 as a critical regulator over the subcerebral extension of an axon by corticospinal motor neurons (CSMN) to the spinal cord (Arlotta *et al, Neuron*, 2005). To discover the networks through which Ctip2 instructs the specification of CSMN connectivity, we are undertaking a dual profiling approach to delineate gene loci actively transcribed by the Ctip2 identity program and the translational fate of these transcripts at particular developmental time points during CSMN axonal extension.

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Odor information coding and transformation in mammalian neural circuits

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Precise timing of glycinergic inhibition modulates coincidence detection in the auditory brainstem

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Spike generation by precisely-timed synaptic inputs is a hallmark of neuronal computations. Unlike many types of neurons throughout the central nervous system, neurons in the brainstem nucleus of the medial superior olive (MSO) detect the coincidence of only a few excitatory and inhibitory inputs from both sides and encode interaural time differences (ITDs) with microsecond precision. Synaptic inhibition has been implicated in tuning ITD functions, but the cellular mechanisms underlying its specific role in coincidence detection remain elusive. In the present study we measured excitatory and inhibitory synaptic responses in adult MSO slices and investigated the influence of inhibition on coincidence detection using conductance clamp. We found that fast hyperpolarizing inhibition shifted the peak timing of excitation, thereby biasing the timing of best coincidence detection in a manner that is consistent with the role of inhibition observed *in vivo*. Shifts were sensitive to the strength, timing, and balance of excitation relative to inhibition and modestly to low-threshold potassium (K_{LTA}) channels. Our results provide evidence that interactions between inhibition and excitation on microsecond timescales are indeed important for binaural processing.

**These authors contributed equally to this work.*

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Linking Geometry-Dependent Nonlinear Current Conduction Characteristics to Synaptic Efficacy

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Protruding from the dendrites of several neuronal types are subcellular compartments known as spines, which are targeted by synapses. The morphology of spines is highly dynamic, likely affecting the strength of the synapse — a key aspect of neuronal plasticity. 3D spine reconstructions at nanometer resolution reveal a dense assembly of supramolecular structures that create a spatially intricate network of intracellular components. Actin filaments, microtubuli, endoplasmic reticulum, spine apparati, and the cellular membrane have polar surfaces that contact the aqueous phase. Within this tight ultrastructural mesh, slits measuring less than 100 nm are not uncommon, thus favoring the emergence of nonlinear current conduction characteristics. In order to appreciate how the geometry affects current conduction within highly confined spaces, the Poisson-Nernst-Planck system needs to be solved for spatial complex geometries. Here we describe how different functional and computational properties of synapses can arise from current conduction characteristics induced by specific geometrical constraints and compare these to engineering applications in nanofluidics.

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Adaptation in sound localization: from GABAB receptor-mediated synaptic modulation to perception

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Across all sensory modalities, the impact of context-dependent neural adaptation can be observed at every level from receptors to perception. Nonetheless, it has long been assumed that processing of interaural time differences, our primary cue for sound localization, is nonadaptive, as its outputs are directly mapped onto a hard-wired representation of space. Here we present evidence derived from *in vitro* and *in vivo* experiments in gerbils, which indicates that the coincidence-detector neurons in the medial superior olive modulate their sensitivity to interaural time differences via a rapid, GABAB receptor-mediated feedback mechanism. We show that this mechanism provides a gain control in the form of output normalization, which influences the neuronal population code of auditory space. Furthermore, psychophysical tests show that the paradigm used to evoke neuronal GABAB receptor-mediated adaptation causes the perceptual shift in sound localization in humans expected on the basis of our physiological results in gerbils.

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Role of 5-hydroxymethylcytosine during postnatal retinal maturation

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5-hydroxymethylcytosine (5hmC), also known as the sixth base of the genome, is a recently discovered oxidative product of 5-methylcytosine (5mC) generated by the enzymatic action of Ten Eleven Translocation (TET) family members. The functional role of 5hmC is by and large unknown. However, studies in embryonic stem cells and cancer tissues suggest that TET enzymes and 5hmC may be involved in gene regulation. Here, we analyzed the role of 5hmC during postnatal retinal maturation in the mouse. Eye opening at postnatal week 2 is a key time point during mouse retinal maturation. Maturation of retinal cells as well as the formation of the retinal network is ongoing after eye opening and is completed only one to two weeks later. To examine a possible contribution of cytosine hydroxymethylation to this process we performed stainings for 5hmC in retinal slices and quantified global 5hmC levels in the mouse retina at week 2 and week 3 using ultra high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Moreover, we mapped 5hmC to the retinal genome by hydroxymethylated DNA immunoprecipitation (hMeDIP) and subsequent next generation sequencing to correlate 5hmC marks with retinal gene expression data obtained from microarray experiments. At postnatal week 2, 5hmC weakly localized to nuclei of cells within the ganglion cell layer and the inner nuclear layer of the retina. The levels of 5hmC in these cells increased at week 3. In addition, 5hmC was now detectable in retinal photoreceptors were it co-localized with histone marks of the euchromatin. hMeDIP experiments revealed a developmentally programmed acquisition of 5hmC during retinal maturation at gene-rich regions and in genes containing activating histone marks. We conclude that 5hmC is dynamically regulated during postnatal retinal maturation and is capable to elevate the gene expression of retina-specific genes.

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Modulation of virus recognition by the innate RNA receptor Lgp2

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Viruses are recognized by the innate immune system via conserved structures in their nucleic acids. The RIG-I-like helicase (RLH) family does this in the cytoplasm of infected cells by binding to 5'-triphosphorylated double-stranded RNA which is absent from uninfected cells. Binding of the appropriate ligand triggers a signaling cascade that results in the production of type I interferons and cytokines that limit viral propagation. Two members of this receptor family, RIG-I itself and MDA-5, are directly involved in coupling recognition of viral RNA to signaling. A third member called Lgp2 lacks domains important to trigger signaling directly, but has been shown to be involved in modulating the RLH pathway. One hypothesis is that Lgp2 facilitates virus recognition by altering the protein-covered viral genomic particles called RNPs to make the RNA accessible for recognition by RIG-I or MDA-5. We use a combination of biochemical and cell biological methods to investigate the effects of Lgp2 on viral RNPs. We show that viral RNPs are substrates of Lgp2 in vitro and that loss of Lgp2 expression in cells leads to a reduced secretion of cytokines which is consistent with the hypothesis stated above. Taken together we are beginning to gain mechanistic insight into the role of Lgp2 in virus recognition.

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An olfactory-specific histone variant: we are what we smell?

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Abstract: We identified a replication-independent histone variant called H2B.E that in mice is expressed exclusively by olfactory chemosensory neurons. The nuclear levels of H2B.E protein were found to be heterogeneous among olfactory neurons, but stereotyped according to the identity of the co-expressed olfactory receptor gene. Sensory deprivation and stimulation experiments revealed that H2B.E levels are inversely related to neuronal activity. Knockout and overexpression experiments demonstrated that high H2B.E levels modulate olfactory receptor expression frequencies in the adult olfactory epithelium by promoting neuronal cell death. Our findings indicate a model in which inactive olfactory neurons display higher levels of the variant, have shortened life spans, and become relatively depleted within the neuronal population compared to active neurons. Post-translational modifications of H2B.E differ from those of the canonical H2B, consistent with a role for H2B.E in altering transcription. We propose a physiological function for H2B.E in modulating olfactory neuron population dynamics to adapt the olfactory receptor repertoire to the environment.

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Probing for oligodendrocyte progenitor cell function by limiting their proliferation in the adult brain

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NG2+ cells, also known as oligodendrocyte progenitor cells (OPCs), are the only proliferating cells outside the neurogenic niches in the adult brain and constitute a major fraction (5-10%) of the brain cells at this age. Despite their high numbers and substantial characterization, their actual function in the adult brain is still wildly unknown. To address this important question, we used conditional genetic deletion of the Esco2 protein, in the inducible Sox10iCreERT2xCAGeGFPxEsco2fl mouse line (Simon et al. 2012) to specifically ablate OPCs, as the absence of Esco2 is forcing dividing cells during M-phase into apoptosis (Whelan et al. 2012), As the proliferation of OPCs is tremendously increased after stab wound injury (Simon et al. 2011), we reasoned this lesion might give a deeper insight in this context. Preliminary results indicate that the Esco2 deletion in OPCs leads to a transient reduction of OPC numbers resulting in alterations of other glial cell populations. Interestingly, even without injury, the temporary depletion of proliferating OPCs leads to behavioral deficits which will be described.

To identify the molecular mechanisms by which NG2+ cells may react and influence other cells after brain injury, we isolated Sox10-GFP+ cells by FACS either from the intact or the lesioned cerebral cortex at three days after stab wound injury. Comparative genome-wide expression profiling revealed exciting candidates for OPC function in the physiological and pathological brain.

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Developing photochromic tools for transmembrane proteins

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Transmembrane receptors allow a cell to communicate with its environment in response to a variety of input signals such as ligand concentration, temperature, pressure, transmembrane potential, or light intensity. Our group has shown how voltage-gated and ligand-gated ion channels can be endowed with synthetic photoswitches and that the resulting artificial photoreceptors can be used to optically control biological systems with exceptional temporal and spatial precision. We now demonstrate how our approach can be applied to new protein targets such as the epithelial sodium channel, which is a key target for treating hypertension and cystic fibrosis, and the μ-opioid receptor, which is a prime target for anesthetics.

Epithelial sodium channels (ENaCs) are heterotrimeric transmembrane proteins assembled from a pool of four different subunits – αβγ and δ. While classical αβγENaC is primarily found in tight epithelial where its role is well investigated, δENaC has been found in non-epithelial tissues, including the central nervous system of primates, where its role is still not understood. We therefore equipped the canonical ENaC blocker amiloride with a photoswitchable handle to enable the optical control of ENaC channels. Interestingly photoswitching of our compound primarily occurs on the δ-containing isoform, which could be instrumental for unraveling its role in the primate central nervous system.

Furthermore, we are applying the concept of azobenzene containing photoswitchable ligands to G-Protein-Coupled-Receptors (GPCRs). These seven transmembrane helix receptors constitute the largest membrane signaling protein family and are targeted by a majority of pharmaceutical drugs. We now show that the μ-opioid receptor, which is natively activated by endorphins and pharmaceutically targeted by anesthetics including morphine, can be activated by light through the action of a photoswitchable azobenzene ligand. Our attempts may not only yield a highly valuable tool for deciphering opioid receptor function in complex system, but also demonstrate a general way to turn other GPCRs into light-gated receptors.

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A role for the terminal uridyl transferases Zcchc11 and Zcchc6 in developmental miRNA uridylation

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MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate gene expression by binding to the 3'UTRs of coding genes and disrupting translation or causing mRNA destabilization. miRNAs have recently been found to undergo extensive non-templated modifications at their 3' ends, yet many of the factors responsible for these modifications and the outcomes of such modifications remain unclear. We have recently identified a family of Terminal Uridyl Transferases (TUTases) that are responsible for the turnover of immature miRNAs of the let-7 family in embryonic stem cells, by catalyzing an oligo uridine tail that signals the miRNAs for degradation. Here we describe the ability for these same TUTases, Zcchc11 and Zcchc6, to specifically uridylate a subset of mature miRNAs in a sequence-dependent manner. Zcchc11 and Zcchc6 specifically bind to and uridylate miRNAs with a defined sequence motif *in vitro*, and depletion of these TUTases reduces non-templated uridylation of the same miRNAs in cultured cells. Upon TUTase depletion there is a concomitant increase in non-templated adenylation, suggesting the presence of a terminal(A) adding enzyme. Interestingly, the only miRNAs that contain the sequence motif are developmentally crucial miRNAs, suggesting that mature miRNA uridylation plays an important role in mammalian development.

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Smell the ripened one: ethylene detection in *Drosophila*

Habibe Ucpunar, Ilona Grunwald-Kadow
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As their common name indicates, fruit flies prefer to feed on fruits and especially on ripened / over-ripened ones. As a contrary to their living condition in laboratories, they have to scan a vast area in their natural environment to find a food source. More importantly, they have to distinguish a high-quality food source from a low-quality one without wasting time with trialerrors. To do that, flies follow chemical cues released from the food sources. In natural conditions ripening or rotting fruits that flies are attracted are frequently fall into the category of climacteric fruits. Those fruits release high amounts of ethylene (C_2H_4), which is the only known gaseous plant hormone, during the ripening process. A family of five receptors mediates responses to ethylene in Arabidopsis and nothing much known about the relevance to animal / insect physiology.

We recently found that the ethylene is a strongly attractive gaseous odor cue for flies. So far no receptor for ethylene had been identified in flies/animals. Using a combination of behavioral analysis and genetics, we excluded the classical ORs to be the putative receptor. By using the same methods we have narrowed down our search on a candidate receptor which abolishes the attraction for ethylene when mutated. However, the same receptor is known as mediating response for an aversive odor, too. Further experiments are underway to determine the neurophysiological mechanisms that lead to integrate two opposing olfactory signals through one receptor.

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Spatiotemporal dynamics underlying recognition of occluded objects in human visual cortex

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Natural vision often involves recognizing objects from partial information due to occlusion. Recognition of occluded objects presents a significant challenge for feed-forward theories of vision because it requires extrapolation from prior knowledge. Here we recorded intracranial field potentials from 1,699 electrodes in 18 subjects to measure the location and timing of selective neurophysiological responses along the human visual cortex during recognition from partial information. Signals from the ventral visual stream, particularly the Inferior Occipital and Fusiform Gyri, remained visually selective despite strong occlusion (75-90%). However, these visually selective signals emerged ~100 ms later for occluded versus whole objects. This latency difference persisted when controlling for changes in contrast, signal amplitude, and the strength of selectivity. These results argue against a feed-forward only explanation of recognition from partial information, and provide spatiotemporal constraints on computational models of object recognition that involve recurrent processing.

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Visualizing functional integration of newborn neurons into the adult brain

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The vertebrate olfactory bulb (OB) is the primary processing center for odor information from the sensory epithelium. Interestingly, two types of OB interneurons, granule and periglomerular cells undergo continuous turnover and replacement throughout adulthood. Progenitor cells are generated in the subventricular zone, migrate through the rostral migratory stream, and differentiate upon arrival in the OB. The need to integrate these newborn cells into a working neural system while maintaining full functionality represents a unique challenge. The morphological maturation of these cells over time has been described in some detail and the development of their electrical properties has been examined at the single cell level using ex vivo slice preparations. However, virtually nothing is known about how the circuit properties of adult born neurons and their sensory-evoked responses develop. We sought to visualize the integration of individual adult-born cells functionally over extended time periods *in vivo*. To specifically track and measure neuronal activity from newborn cells, we injected a lentiviral vector genetically encoding a red fluorescent protein and a green fluorescent calcium indicator into the rostral migratory stream of adult mice. We then installed chronic cranial windows over the OB allowing us to repetitively image identified cells and dendrites over several weeks using two-photon microscopy. Using this approach, we successfully imaged for the first time odorevoked activity of integrating adult-born cells in living mice. Surprisingly, newborn cells were easily excited by odorant stimuli as soon as they reached their final position in the OB, long before being morphologically mature. Furthermore, newly integrating cells often displayed strong odor responses to specific stimuli that decreased in time while the overall width of the odor receptive field stayed relatively constant. These results support the hypothesis that adultborn cells might undergo a functional critical period and that adult neurogenesis contributes to plasticity mechanisms of the neural circuitry in the OB.

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Targeting pseudo-kinase ErbB3 with covalent inhibitors that induce protein degradation

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Her3 (ErbB3) is a member of the epidermal growth factor receptor tyrosine kinases that has been well credentialed as a potential anti-cancer target in breast cancer, ovarian cancer and non-small cell lung cancer. Several therapeutic antibodies directed against the extracellular domain are undergoing clinical testing.

To-date there have been no reported small molecule inhibitors directed against the kinase domain of Her3 primarily because Her3 has been classified as a 'pseudokinase' due to its exceptionally low kinase activity. An important question for Her3 and the additional 60 mammalian pseudokinases is whether ATP-competitive small molecules are capable of blocking their biological functions. Here we report the development of the first selective Her3 binder, TX1-85-1 that form a covalent bond with Cys721 located in the ATP-binding site of Her3 and demonstrate that covalent modification of Her3 in cells does not interfere with Her3-dependent signaling or proliferation of cancer cell lines.

We further derivatized these covalent Her3 ligands with a hydrophobic adamantane moiety and demonstrate that the result bivalent ligand TX2-121-1 can selectively induce Her3 degradation thereby inhibiting Her3-dependent signaling and proliferation. Mechanistic studies demonstrate that ligand induced degradation involves Hsp90 and the proteosome. Further optimization of the compounds may provide the first pharmacological means of antagonizing Her3-dependent functions in preclinical and clinical models.

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