2012 LMU-Harvard Young Scientists' Forum

7 – 10 July 2012

Harvard University

SATURDAY 07 JULY

Northwest Building Auditorium, B103
52 Oxford Street
Cambridge, MA 02138

8:30AM	Depart hotel and dormitory
8:45AM	Continental breakfast at Northwest Building, B1 lobby
9:00AM	Opening Remarks
9:15AM	Faculty Seminar: Rachel Wilson
10:15AM	Break
10:30AM	Student Session 1
	Student 1: Michael Pecka (postdoc, Grothe)
	Student 2: Lasse Bräcker (student, Kadow)
	Student 3: Ashley Vonner (student, Samuel)
	Student 4: Alex Mauss (postdoc, Borst)
12:30PM	Lunch
2:00PM	Student Session 2
	Student 5: Nina Mäusbacher (postdoc, Sieber)
	Student 6: Laura Laprell (student, Trauner)
	Student 7: Bryan Dickinson (postdoc, Liu)
	Student 8: Julia Litzlbauer (student, Griesbeck)
4:00PM	Break
4:15PM	Faculty Seminar: Martin Biel
5:30PM	Poster session 1 (all posters present)
7:30PM	Blue Ribbon BBQ in Tozzer Courtyard (Rain location: Northwest)

SUNDAY 08 JULY

Northwest Building Auditorium, B103
52 Oxford Street
Cambridge, MA 02138

8:45AM	Continental breakfast at Northwest Building, B1 lobby
9:00AM	Faculty Seminar: Magdalena Götz
10:00AM	Break
10:30AM	Student Session 3
	Student 9: Graziana Gatto (student, Klein)
	Student 10: Caroline Rouaux (postdoc, Arlotta)
	Student 11: Vibnu Sahni (postdoc, Macklis)
	Student 12: Gregor Pilz (student, Götz)
12:30PM	Lunch
2:00PM	Faculty Seminar: Wolfgang Wurst
3:15PM	Poster Session 2 (all posters present)
5:30PM	Dinner on your own in Cambridge

MONDAY 09 JULY

Cannon Room
Building C
Harvard Medical School
Boston, MA 02115

8:45AM	Board shuttle for Harvard Medical School
9:45AM	Continental breakfast in Cannon Room lobby
10:00AM	Faculty Seminar: Nathanael Gray
11:00AM	Break
11:30AM	Student Session 4
	Student 13: Thomas Graham (student, Loparo)
	Student 14: James Thornton (student, Gregory)
	Student 15: Mike Myoga (postdoc, Grothe)
12:30PM	Lunch
2:00PM	Student Session 5
	Student 16: Christine Beemelmanns (postdoc, Clardy)
	Student 17: Thomas Murooka (postdoc, Mempel)
	Student 18: Ralf Jungmann (postdoc, Shih)
	Student 19: David Long (postdoc, Walter
4:00PM	Break
4:30PM	Student 20: Sally Deeb (student, Mann)
5:00PM	Faculty Seminar: George Church
6:00PM	Dinner on your own in Boston

TUESDAY 10 JULY

9:00AM	Depart Northwest Building
10:15AM	Arrive Wingaersheek Beach, Gloucester, MA
12:45PM	Depart Wingaersheek for boat dock
1:00PM	Pick up box lunches
1:30PM	Whale watch
6:00PM	Depart Gloucester
6:30PM	Dinner at Woodman's
8:30PM	Depart Woodman's
9:30PM	Arrive Northwest Building

SATURDAY 07 JULY TALKS

Sensory Processing in a Small Brain

Rachel I. Wilson
Harvard Medical School, Department of Neurobiology

The *Drosophila* olfactory system is a useful model for investigating sensory processing. The compartmental organization of the first olfactory relay of the fly brain (the antennal lobe) makes it relatively easy to map connections between neurons, and each compartment corresponds intuitively to a discrete processing channel in the network. Moreover, this circuit contains a relatively small number of neurons, and genetic tools allow us to label identified individual neurons within this network for recording. We monitor *in vivo* odor responses in these neurons using electrophysiological recordings, and we use genetic tools and pharmacology to probe their functional interactions. I will describe recent experiments from our laboratory investigating the properties of cells and synapses in this circuit, the sensory transformations that this circuit performs, and the potential adaptive functions of these transformations.

Neuronal context-sensitivity created by experience-dependent adaptation for efficient coding of natural stimuli

Michael Pecka
LMU, Division of Neurobiology

Neuronal circuits are thought to represent environmental signals efficiently in terms of information transfer and energy consumption. For this to occur, sensory circuits must be specialized for processing natural stimuli that are characterised by unique spatiotemporal statistics. It is generally assumed that the ability of sensory circuits to process natural stimuli efficiently develops by experience of environmental signals during the maturation of an individual. However, it remains unclear whether the ability to process natural stimuli efficiently is inherent to sensory circuits, or whether it develops by experience of environmental signals during the maturation of an individual (Simoncelli and Olshausen, 2001). Here, we performed *in vivo* patch-clamp-recordings from mouse V1 neurons to determine how responses to natural stimuli presented to the classical receptive field (CRF) are modified by presentation of stimuli with different spatiotemporal structure in the CRF surround (nCRF). In adult mice, response sparseness and information transmitted per spike significantly increased during full-field naturalistic stimulation compared to stimulation confined to the CRF, consistent with findings from monkey and cat V1. These effects diminished with nCRF stimuli lacking second and/or higher-order statistical regularities of natural scenes. Crucially, this specialization for representing full-field naturalistic stimuli most efficiently was neither present in infant mice immediately after eye-opening nor in adult, visually-deprived mice. These results demonstrate that the efficient representation of natural signals by neuronal circuits requires the prior experience of its unique statistical features. Conversely, these findings furthermore suggest that the function of a particular sensory circuit can only be understood in its entirety if probed with an adequate stimulus set, i.e. a stimulus set that resembles the context to which processing of the circuit is adapted. We aim to transfer these conclusions from the visual to the auditory system, specifically by investigating how stimulus context influences the processing of sound location in the auditory cortex and midbrain.

CO_2 avoidance requires mushroom body activity in a context dependent manner in Drosophila

Lasse Bräcker
Max-Planck Institute of Neurobiology, Sensory Neurogenetics Research Group

Viral infections are a constant threat to higher organisms. They not only cause severe infectious diseases but can also be a cause for cancer. The cell-autonomous detection of viral but also all other infections is based on the recognition of conserved molecular patterns that are present in the pathogen but not in the host. The innate immune system has evolved so called “pattern recognition receptors” that scan the extracellular and intracellular space for signs of infection. In the case of viral infection some of these receptors recognize structural features of foreign nucleic acids and their aberrant localisation. Recently, a new class of these receptors has been described as the RIG-I-like DEXD/H-box helicases (RLHs) RIG-I, MDA-5 and Lgp2. They are expressed in virtually all cells of the body and recognize viral RNA in the cytoplasm of infected cells. Recognition of viral RNA by these helicases leads to the production of type I interferons and inflammatory cytokines and is essential for host defence. We have characterized the ligand-binding properties of RLHs in order to understand how they distinguish self from non-self RNA and how they are activated.

Cold sensing in *Drosophila* larvae

Ashley Vonner
Harvard University, Department Physics

Systems neuroscience aims to explain complex behavior, but as the human nervous system contains billions of neurons, simpler model systems can assist in approaching this goal by providing relevant analogies. Here, *Drosophila* larvae are used to study thermotaxis, or navigation in response to changes in environmental temperature, to provide insights into decision-making behavior. This work focuses on larval cold sensing, with quantitative analysis of thermotactic behavior, *in vivo* calcium imaging at single neuron resolution on awake immobilized larvae exposed to temperature modulations, and targeted laser ablation experiments to demonstrate the necessity of specific cold-sensing neurons for cold avoidance behavior.

Optogenetic dissection of the motion vision circuitry in *Drosophila*

Alex Mauss
MPI of Neurobiology, Department of Systems and Computational Neurobiology

A fundamental function of visual systems is the detection of shifting images projected onto the photoreceptors. It may serve animals to identify moving objects or to evaluate self-motion when natural scenes are displaced over time as two-dimensional fields of motion vectors. An algorithmic model, the so-called ‘Reichardt detector’, has proven to be an accurate description for motion detection [1]. However, its neuronal implementation in any organism remains largely unknown.

We approach this issue by combining optogenetics and electrophysiology in *Drosophila*. The optic lobe of dipteran flies comprises four neuropile layers termed lamina, medulla, lobula and lobula plate all of which are built of columnar arrays in register with the ommatidial layout of the compound eye. Large tangential cells in the lobula plate (LPTCs) with broad receptive fields integrate signals from local motion detectors of yet unknown identity and, thus, display direction-selective responses: they depolarize when

images shift along their preferred direction and hyperpolarize for the opposite direction. Two anatomical pathways have been proposed to convey the information from the eye to the LPTCs [2-5]. The assumed outputs of these pathways onto the LPTCs are many hundreds of columnar T4 and T5 cells [6]. To resolve the underlying synaptic connectivity we combine optogenetic stimulation of these neuron types with pharmacology and whole-cell patch-clamp recordings from LPTCs. To address the roles of T4 and T5 in motion processing we furthermore develop strategies to alter their activity states optogenetically while simultaneously recording the visual responses of LPTCs. From these experiments, we expect to gain new insights into the cellular organisation of the Reichardt model of motion detection.

From the fluorescence label-dependent Activity based protein profiling approach to a gel free approach

Nina Mäusbacher
TU Munich, Department of Chemistry

Activity-based protein profiling aims to identify functionally active proteins. Therefore alkyne-tagged probes are synthesized that selectively bind into the active site of their target proteins. Subsequent coupling of the probe to a trifunctional linker harbouring a biotin tag and a fluorescent dye enables the enrichment of target proteins and their visualization on SDS gels followed by identification via mass spectrometry. We now aim to establish a gel-free approach which as readout is solely based on quantitative mass spectrometry. For the analysis of both approaches pretubulysin, a cytostatic peptide which binds to beta-tubulin and thereby inhibits microtubule formation, was used.

ATG - A Photochromic Agonist of NMDA Receptors

Laura Laprell
LMU, Department of Chemistry

NMDA-receptors belong to the group of non-selective ionotropic glutamate-gated receptors. They are named after their specific agonist *N*-methyl D-aspartate and play an important role in excitatory neurotransmission involved in several important processes, including synaptic plasticity, learning and behavior. NMDA-receptors are heteromeric complexes composed of four subunits, each derived from three related families (NR1, NR2A-D, NR3A-B), whereby NR1 is obligatory for receptor function and NR3 is mainly expressed in glia cells, but not in neurons. NMDARs are special in many respects. Firstly two different ligands are necessary for receptor activation (glutamate and glycine or D-serine) and secondly the channel is blocked by Mg^{2+}-ions at the resting potential of the neuron. Because of this Mg^{2+}-block, NMDARs can only be fully activated, when a presynaptic signal and a postsynaptic depolarization happen simultaneously. This so called coincidence detector serves as a fundamental component of postsynaptic plasticity.

Since NMDA receptors are involved in many different processes, it is not surprising that they are relevant drug targets and that many scientists are working to better understand the underlying activation mechanism of NMDARs. To gain further insight into their role in synaptic transmission, it would be useful to bestow NMDARs with light sensitivity, to be able to control its gating in a precise spatiotemporal fashion.

In order to introduce light sensitivity to NMDARs, we focused on a photopharmacological approach and developed a photochromic ligand (PCL), ATG (Azobenzene Triazole Glutamate), which is selective for NMDA receptors. ATG turned out to be inactive in its dark-adapted state (*trans*-conformation) and

converts into its active form (*cis*-conformation) upon irradiation with UV light (370 nm). Thus it is not excitotoxic when applied to neuronal networks in the dark, but becomes an effective agonist upon light activation, which is an advantage in comparison to our other glutamate receptor PCLs (ATA-3, 4-Gluazo). ATG can be actively *switched off* with blue light (420 nm) within a few milliseconds. In cortical neurons of wildtype mice (p=9-15) the electrophysiological properties of the active *cis*-ATG on the NMDA receptor are similar to the activation by NMDA itself. Because of its spectral properties ATG-photoswitching can be combined with calcium imaging, which frequently uses indicators excited around 480nm. Furthermore, the activation of ATG proved to be sufficient to trigger the phosphorylation of CREB in a light-dependent and spatially restricted (size of the illuminated area) fashion.

With ATG, we have developed a highly selective molecule to activate NMDA receptors, enabling the control and further investigation of neuronal activity with light. Since it is *cis*-active, it is not excitotoxic in the dark-adapted state and can easily be applied to the tissue of interest.

Replaying the "Tape of Life": Experimental interrogation of the path dependency of protein evolution

Bryan C. Dickinson
Harvard University, Department of Chemistry and Chemical Biology

Stephen Jay Gould famously hypothesized that if the "tape of life"— the long evolutionary trajectory that has resulted in present life on earth— were rewound and started again, completely different evolutionary outcomes would emerge due to the requisite stochasticity of evolution. In contrast, others point to evolutionary convergence, the acquisition of similar biological traits in unrelated lineages, as evidence that evolutionary outcomes are restricted and therefore reproducible and predictable. Therefore, the extent to which evolutionary outcomes are path-dependent— that is, whether evolution is a "state function"— remains unclear.
To answer this question, we used our recently developed phage-assisted continuous evolution (PACE) technology to construct a new evolutionary model system based off of evolving T7 RNA polymerase (T7 RNAP) to recognize different promoters. We continuously evolved populations of T7 RNAP through different "stepping stones" to acquire activity on either the T3 or SP6 promoters, resulting in divergent populations and two evolutionary "paths." We then continuously evolved each of the populations to recognize a final target promoter that is a combination of the T3 and SP6 promoters and more than 50% altered from the cognate T7 promoter. The populations survived more than 1800 rounds of evolution and resulted in variants with efficiencies comparable to the wild-type enzyme on each of the target promoters. We found that there are both path-dependent and path-independent mutations that confer fitness, and that both stochasticity within a path and the specific path taken can influence evolutionary outcomes, driven by varied epistatic interactions between mutations. This study shows that evolutionary history and trajectories can impact single protein evolutionary fate at both genotypic and phenotypic levels.

Evolutionary Engineering and Screening of Genetically Encoded FRET Calcium Indicators

Julia Litzlbauer
MPI of Neurobiology, Department of Cellular Dynamics

In neurons, each action potential is accompanied by a Ca^{2+} influx through voltage gated calcium channels, which can be quantified as a measure for neuronal activity. Genetically encoded calcium indicators (GECIs) have proven to be a valuable tool for monitoring this activity as they offer crucial advantages

over conventional calcium dyes, like targeting specific cell populations or intracellular compartments, simultaneous monitoring of a large number of cells, and chronic *in vivo* imaging (1).

Our group is focused on the design and development of FRET-based GECIs consisting of Troponin C, as a Ca^{2+} binding domain, fused between two fluorescent proteins. We have recently established directed evolution and primary and secondary screening techniques as tools for GECI improvement. Using these tools to supplement rational design, we were able to substantially improve our latest indicator, TN-XXL (2), incorporating the fluorescent proteins ECFP and cpCitrine, in terms of brightness, signal-to-noise ratio, low FRET in the Ca^{2+} free state, high FRET in the Ca^{2+} bound state, and kinetics. The same approach can also be used to create red shifted variants of our indicators, which will allow for deep tissue imaging, with reduced light scattering in tissue and better spectral separation.

Here, I will discuss our strategies for library design and screening and additionally present some of the most recent indicators.

Channelopathies of the retina

Martin Biel
LMU, Department of Pharmacy – Center for Drug Research

The retina is a neuronal network dedicated to converting visual stimuli (photons) into spatially and temporally controlled patterns of action potentials that are transmitted to the visual cortex. Ion channels play a fundamental role in both principal visual transduction and intraretinal information processing. Dysfunction of these proteins gives rise to various retinal diseases (retinal "channelopathies") that can eventually lead to blindness. In my lecture I will summarize our recent work on the role of two particular types of retinal ion channels, the cyclic nucleotide-gated (CNG) channels and the voltage-gated calcium channel (Cav1.4). CNG channels are present in photoreceptor outer segments where they play a fundamental role in transducing light-evoked changes of the cGMP concentration into electrical signals. Cav1.4 is required to trigger neurotransmitter release at the synapses of photoreceptors and bipolar cells. I will discuss the mechanisms that link dysfunction of these channels with specific types of blindness and retinal degeneration. Recently, our laboratory has developed viral vectors to rescue loss of retinal ion channels and restore vision in genetic mouse models. The impact of gene replacement on the therapy of human blinding eye diseases will be discussed.

SUNDAY 08 JULY TALKS

Molecular mechanisms of neurogenesis – comparing embryonic & adult neurogenesis & direct reprogramming

Magdalena Götz
LMU, Institute of Stem Cell Research, Helmholtz Center Munich and Institute of Physiology

Neurogenesis in the adult brain is restricted to few niches in the mammalian brain, prompting the question to which neurogenesis in these regions follows the mechanisms of their embryonic counter parts or occurs along largely different molecular rules. This will be discussed by comparing molecular mechanisms of neurogenesis in the embryonic telencephalon to the corresponding regions in adult mice. I will describe the concept of lineage priming which is a hallmark of adult but not embryonic neural stem cells. These data reveal the concept that adult neural stem cells are indeed globally more similar to mature glial cells while radial glial cells in the embryo rather resemble adult neuroblasts.

I will further describe our recent insights into the molecular basis of the neurogenic function of the transcription factor Pax6 which again show intriguing differences between embryonic and adult neurogenesis both in regard to the DNA binding domain involved in target gene regulation as well as in regard to interaction with an essential chromatin remodelling factor. These data highlight profound mechanistic differences in the regulation of neurogenesis in the adult and embryonic brain despite using the same molecular players. Finally I will compare these mechanisms of endogenous neurogenesis to recent insights into the molecular mechanisms underlying direct reprogramming into neurons, revealing yet again specific requirements, but also a common molecular logic.

How specific are receptor-type protein tyrosine phosphatases? The case of PTPRO and its role during neural development

Graziana Gatto
MPI of Neurobiology, Department of Molecular Neurobiology

During development neurons need to coordinate the activities of growth factors and guidance cues in order to reach their optimal growth rates and their correct synaptic targets. Among the prominent signaling pathways, tyrosine phosphorylation appears to be particularly important. To date, receptor tyrosine kinases (RTKs) have been extensively investigated, but much less is known about receptor tyrosine phosphatases (RPTPs) and their requirements during neural development. It is still unclear how RPTP substrate specificity is achieved: Is it regulated by the expression patterns of RTKs and RPTPs, or by their activation states? Furthermore, how well is their specificity conserved during evolution? In chick, PTP receptor type O (PTPRO) is required for motor axon outgrowth and Eph receptor-dependent retinotectal axon guidance. We have found that, in mice, PTPRO does not have the same *in vivo* functions, suggesting that chick and mouse PTPRO have different substrate specificities. Moreover, it has been published that $PTPRO^{-/-}$ mice show abnormal spinal pathfinding and decreased survival of specific trunk nociceptive (pain sensing) neurons. We found that during embryonic development of the mouse trigeminal ganglion, PTPRO is expressed in BDNF- and GDNF-sensitive mechanoceptive neurons, but not in NGF-sensitive nociceptive neurons. Consistently, primary cultures of trigeminal neurons from $PTPRO^{-/-}$ embryos were more responsive than $PTPRO^{+/+}$ neurons to BDNF and GDNF, but not NGF. *In vivo,* $PTPRO^{-/-}$ embryos showed increased arborization of the ophthalmic branch of the trigeminal nerve,

and defasciculation of the maxillary branch. In transfected cells, PTPRO directly dephosphorylates TrkB upon BDNF stimulation. These results indicate that PTPRO negatively controls BDNF/TrkB and GDNF/Ret signaling in trigeminal neurons. Loss of NGF-sensitive nociceptive neurons in PTPRO$^{-/-}$ mice may be non-cell autonomous due to the competitive growth advantage that BDNF- and GDNF-sensitive neurons have.

Direct lineage reprogramming of postmitotic callosal neurons into corticofugal neurons in vivo

Caroline Rouaux
Harvard University, Department of Stem Cell and Regenerative Biology

Once programmed to acquire a specific identity and function, cells rarely change *in vivo*. Neurons of the mammalian central nervous system (CNS) in particular are a classic example of a stable, terminally differentiated cell type. With the exception of the adult neurogenic niches, where a limited set of neuronal subtypes continue to be generated throughout life, CNS neurons are only born during embryonic and early postnatal development. Once generated, neurons become permanently postmitotic and do not change their identity for the life span of the organism. Here, we have investigated whether postmitotic excitatory neurons of the neocortex can be instructed to directly reprogram their identity postmitotically from one subtype into another *in vivo.* We show that embryonic and early postnatal callosal projection neurons (CPN) of layer II/III can be postmitotically lineage reprogrammed into layer V/VI corticofugal projection neurons (CFuPN) upon expression of the transcription factor *Fezf2*. Reprogrammed callosal neurons acquire molecular properties of corticofugal projection neurons and change their axonal connectivity from interhemispheric intracortical projections to corticofugal projections directed below the cortex. The data indicate that, at least during a defined window of postmitotic development, neurons can change their identity, acquiring critical features of alternate neuronal lineages.

Molecular determinants of corticospinal motor neuron segmental target specificity

Vibhu Sahni
Harvard University, Department of Stem Cell and Regenerative Biology

Corticospinal motor neurons (CSMN, and related cortico-brainstem motor neurons; together "CSMN") are located in layer V of the neocortex and make synaptic connections to motor output circuitry in the spinal cord and brainstem. CSMN axons form the corticospinal tract (CST), which is the major motor output pathway from the motor cortex, and critically controls voluntary movement. CSMN exhibit a great deal of anatomical heterogeneity: CSMN located rostro-laterally extend axons to proximal targets such as the pons and medulla (i.e. hindbrain) and the cervical cord (collectively "$CSMN_C$"), and those located caudo-medially extend their axons more distally to the lumbar spinal cord ("$CSMN_L$"). Molecular mechanisms underlying segmental specificity of this connectivity remain largely unknown.

Building on foundations of earlier studies in the lab, we are investigating molecular controls over development of CSMN segmental connectivity. We selectively isolated $CSMN_C$ and $CSMN_L$ at three critical time points of CST development, and identified differentially expressed genes between these two CSMN subpopulations during development. Using gain- and loss-of function analyses, we have identified molecular controls that function to direct CSMN axon extension to appropriate levels of the spinal cord (short axon extension by $CSMN_C$ and long axon extension by $CSMN_L$). In addition, we have also identified a complementary molecular control, which is expressed by $CSMN_C$ and non-cell-autonomously serves to limit $CSMN_L$ axonal collateral branching in the cervical spinal cord. Together, these controls constitute new, bi-directional mechanisms directing CSMN axonal targeting and collateral branching; and

lay the foundation for more in-depth studies of CSMN subtype-specification, as well as the development of precise corticospinal circuitry.

Amplification of neuron numbers in the telencephalon occurs by a heterogeneous progenitor population that includes a novel radial glia cell type

Gregor-Alexander Pilz
HMGU, Institute of Stem Cell Research

To investigate the mechanisms regulating expansion of neuron numbers during brain development we examined the mouse ventral telencephalon, which is characterized by a large subventricular zone (SVZ). Utilizing long-term imaging of progenitor cells labelled in embryonic brain slices (embryonic day 14) and immunohistochemistry, we discovered novel subapically dividing radial glia that contribute pivotally to the extensive progenitor amplification generating this expanded proliferative zone. This amplification of progenitor cells initiates already within the ventricular zone (VZ) and includes a series of intermediate progenitor (IP) cells with short cell cycle length and symmetric proliferative modes of cell division. In the mouse this is in pronounced contrast to the dorsal telencephalon where few IPs are generated dividing in a symmetric terminal mode. These mechanisms contribute to generate a larger neuronal output in the ventral compared to dorsal telencephalon in the mouse. In mammalian species with expanded neuron numbers in the dorsal telencephalon, namely a gyrified cerebral cortex, a similar progenitor heterogeneity including subapical radial glia are found in increasing numbers, implying key roles in ontogeny and phylogeny.

Wnt/β-catenin signaling in development and regeneration of mesodiencephalic dopaminergic neurons

Wolfgang Wurst
TU Munich, Institute of Developmental Genetics

Wnt/β-catenin signaling plays a crucial role in the generation of mesodiencephalic dopaminergic (mdDA) neurons, including the Substantia nigra pars compacta (SNc) and Ventral tegmental area (VTA) dopaminergic (DA) neurons. The preferential degeneration of the SNc DA neurons is a hallmark of Parkinson's Disease (PD), and the need of better therapies for this disease has fuelled basic research to understand the precise molecular mechanisms underlying SNc DA neuron generation and survival in recent years. However, the mechanisms underlying Wnt function in this context remain unknown.

We have recently uncovered a genetic cascade including the atypical secreted Wnt inhibitor Dickkopf 3 (Dkk3), the homeodomain transcription factors Lmx1a and Pitx3, and the secreted neurotrophin brain-derived neurotrophic factor (Bdnf), that is necessary and sufficient for the correct differentiation of a rostrolateral mdDA precursor subpopulation into SNc DA neurons and their survival in the developing mouse ventral midbrain. Interestingly, this feed-forward genetic cascade also appears to be active in the adult rodent ventral midbrain, where it is required for the neuroprotection of the SNc DA neurons against cytotoxic insults. Moreover, stimulation of this genetic cascade in cultured ventral midbrain progenitor cells or differentiating pluripotent stem cells selectively increases the differentiation and survival of mature mdDA neurons in vitro without affecting progenitor cell proliferation and precursor cell proportion, thus making it a good candidate for the improvement of regenerative and neuroprotective strategies in the treatment of PD.

MONDAY 26 JULY TALKS

Developing Covalent Kinase Inhibitors to Treat Cancer

Nathanael Gray
Harvard University, Department of Biological Chemistry and Molecular Pharmacology

There is an ever increasing demand for new protein kinase inhibitors both as potential therapeutics and as tools for dissecting complex signaling pathways. The vast majority of currently developed kinase inhibitors target the ATP-binding site and bind in a reversible fashion. An alternative mode of kinase inhibition involves developing compounds that form permanent covalent bonds with nucleophilic residues such as cysteines or lysines. A survey of the kinome reveals that there are over 150 kinases that possess a potentially accessible cysteine in or around the ATP-binding site. Despite this abundance of potential targets only a very limited of selective covalent kinase inhibitors are currently known. I will describe two distinct and general methods for efficiently developing novel covalent inhibitors with examples provided for T790M EGFR, FGFR and JNK.

Single-molecule studies of a ParB family chromosome segregation protein from *Bacillus subtilis*

Thomas G.W. Graham
Harvard University, Department of Systems Biology

ParAB systems play a role in chromosome segregation in a wide range of bacterial species. The DNA binding protein ParB (termed Spo0J in *Bacillus subtilis*) associates specifically with origin-proximal *parS* sites and also "spreads" in a poorly understood way by interacting nonspecifically with adjacent chromosomal DNA. Spo0J complexes in *B. subtilis* are required for early segregation of newly replicated origins and facilitate loading of the bacterial condensin homolog SMC. Using *in vitro* single-molecule imaging, we have studied the mechanism of Spo0J nucleoprotein complex formation by simultaneously observing Spo0J binding to DNA and motion of site-specific labels on the DNA chain. Our results suggest that Spo0J forms complexes by trapping long-distance loops between consensus *parS* sites and distal nonspecific segments of DNA. Detailed *in vitro* and *in vivo* analysis of mutants has allowed us to define the molecular determinants of DNA bridging by Spo0J.

Lin28-mediated control of let-7 microRNA expression by alternative TUTases Zcchc11 and Zcchc6

James Thornton
Harvard School of Public Health, Children's Hospital Boston

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression in numerous contexts including development and disease. The pluripotency factor Lin28 post-transcriptionally inhibits the tumor suppressor let-7 miRNA family in undifferentiated cells and many types of cancer. Lin28 recruits the 3' terminal uridyl transferase Zcchc11 (TUTase4), which adds a short uridine tail to the 3' end of let-7 miRNAs, signaling their degradation. Here we investigate the protein and RNA determinants for the interaction between let-7, Lin28, and Zcchc11. Biochemical dissection and reconstitution assays reveal the TUTase domains necessary and sufficient for Lin28-enhanced pre-let-7 uridylation. We also identify Zcchc6 (TUTase7) as an alternative TUTase that functions with Lin28 in vitro, and accordingly

we find Zcchc11 and Zcchc6 redundantly control let-7 biogenesis in embryonic stem cells. Overall our results provide insight into the mechanism of Lin28-mediated TUTase control of let-7 expression in development, stem cells, and cancer.

Synaptic Basis of Interaural Time Difference Coding in the Medial Superior Olive

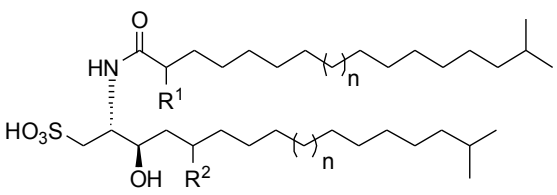
Michael H. Myoga
LMU, Department of Biology II

The manner in which mammals localize sound sources is crucial for their ability to navigate their environment and communicate with other individuals. The brainstem nucleus of the medial superior olive (MSO) encodes differences in the arrival time of sounds between the ears (interaural time difference or ITD), providing precise information about the location of sounds in the horizontal plane. ITD thresholds in humans can be as small as 10 μs, making the MSO the fastest known coincidence detection circuit in the brain. However, it is not understood how MSO neurons integrate their synaptic inputs to perform such fast and precise computations. In addition to phase-locked excitatory inputs from both ears, MSO neurons receive phase-locked glycinergic inhibition from the medial nucleus of the trapezoid body (MNTB). In the present study, we test the hypothesis that the precise timing of inhibition is important for coincidence detection in the MSO. We made patch-clamp recordings of MSO neurons in acute slices from adult Mongolian gerbils (*Meriones unguiculatus*) and investigated the interaction between EPSPs and IPSPs. We found that the peak of an EPSP is advanced and sharpened when it occurs during the rising phase of an IPSP and is delayed and broadened when it occurs during the falling phase of the IPSP. Experimental and modeling data further indicate peak-shifts during ongoing activity. Moreover, we found that inhibition controls the integration time window for which coincident EPSPs generate an action potential. These findings provide evidence that inhibition is important for coincidence detection and ITD tuning in the MSO, which has specific importance for sound localization processing in mammals and general importance for fast coincidence detection circuits in the brain.

Isolation and Synthesis of Novel Sulfonolipids – Small Molecules With Important Biological Impact

Christine Beemelmanns
Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology

Interactions between bacteria and their animal hosts play critical roles in animal development, metabolism, and evolution. However, little is known about the molecular mechanisms involved.[1] The gram-negative Bacteroidetes bacterium *Algoriphagus r*egulates an onset of development in one of the closest living relatives of animals, the choanoflagellate *Salpingoeca rosetta*.[2] Choanoflagellates are known to have both solitary and multicelled stages in their life histories,[3] and unraveling the mechanism of this simple single cell to multiple cell transition would provide an unparalleled insight into the origins of animal development. Only recently, we found that multicellular development in *S. rosetta* is induced by a chemical signal from the bacterium *A. machipongonensis*, and we were able to identify small diffusible molecule which are both necessary and sufficient to robustly induce this colony development.[4] The relative structure of the colony inducing sulfonolipids were analyzed by a combination of 1D and 2D NMR and the activity of the isolated compound was confirmed by using an established bioassay methodology.[4] Currently, we are synthesizing



the natural product as well as derivatives to determine the absolute configuration of the signaling molecule and to analyze the structure-activity relationship of this highly potent signaling molecule.

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HIV-infected T cells are migratory vehicles for viral dissemination

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After host entry through mucosal surfaces, HIV-1 disseminates to lymphoid tissues to establish a generalized infection of the immune system. The mechanisms by which this virus spreads among permissive target cells locally during early stages of transmission, and systemically during subsequent dissemination are not known. *In vitro* studies suggest that formation of virological synapses (VSs) during stable contacts between infected and uninfected T cells greatly increases the efficiency of viral transfer. It is unclear, however, if T cell contacts are sufficiently stable in vivo to allow for functional synapse formation under the conditions of perpetual cell motility in epithelial and lymphoid tissues. Here, using multiphoton intravital microscopy (MP-IVM), we examined the dynamic behavior of HIV-infected T cells in lymph nodes (LNs) of humanized mice. Unexpectedly, we found that most productively infected T cells migrated robustly, resulting in their even distribution throughout the LN cortex. A subset of infected cells formed multinucleated syncytia through HIV envelope (Env)-dependent cell fusion. Both dyssynchronous motility of syncytia as well as adhesion to $CD4^+$ LN cells led to the formation of long membrane tethers, increasing cell lengths to up to 10 times that of migrating uninfected T cells. Blocking the egress of migratory T cells from LNs into efferent lymph, and thus interrupting T cell recirculation, limited HIV dissemination and strongly reduced plasma viremia. Thus, we have found that HIV-infected T cells are motile, form syncytia, and establish tethering interactions that may facilitate cell-to-cell transmission through virological synapses. While their migration in LNs spreads infection locally, T cell recirculation through tissues is important for efficient systemic viral spread, suggesting new molecular targets to antagonize HIV infection.

Engineering Nucleic Acid-Based Fluorescent Probes for Multiplexed Super-Resolution Imaging

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DNA is now widely used as a programmable material for the construction of two- and three-dimensional nanostructures. Application of hierarchical assembly strategies as well as intramolecular folding as in “DNA origami” has resulted in structures with low assembly error densities. We currently explore the use of DNA origami in the context of biophysics, bionanotechnology and bioimaging. We recently developed

an assay based on transient binding of short fluorescently labeled imaging strands to their complements. It allows analyzing of binding and dissociation kinetics on the single molecule level. The method, termed DNA-PAINT, can also readily be used for super-resolution fluorescence imaging of DNA nanostructure with a resolution of up to 25 nm.

We here present the concept of a new kind of fluorescent molecule, a metafluorophore, a compact DNA nanostructure that displays fluorophore docking sites at prescribed locations with nanometer precision, and thus features precise control over color, brightness, and geometry based on DNA-PAINT. Metafluorophores have extended capabilities compared to conventional fluorophores as DNA nanotechnology allows us to precisely design and control the properties of these novel imaging probes. Using DNA origami as a scaffold, we demonstrate a first application of this concept by creating a nanoscopic barcode. For this purpose, color and shape of the metafluorophore can be precisely designed to create the first geometrically encoded super-resolution barcode that can be used to tag biomolecules of interest and thus distinguish between many different species.

We also describe a novel approach for true *in situ*, multiplexed super-resolution imaging based on the analysis of binding frequency to mRNA targets of interest. We envision this concept to be used to perform single-molecule, highly multiplexed super-resolution fluorescence in situ hybridization (FISH) experiments e.g. in E.coli.

Recombination-dependent repair of DNA crosslinks

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Fanconi anemia (FA) is a genetic disorder characterized by chromosomal instability and a predisposition to cancer. FA cells are extremely sensitive to DNA interstrand crosslinks (ICLs), which prevent separation of DNA strands. Using *Xenopus* egg extracts, we have established an *in vitro* system for studying the step-wise repair of a single ICL on a plasmid. Repair is initiated during replication when two forks run into the ICL. Next, incisions are made in one DNA strand, allowing the ICL to be "unhooked" and removed. However, the incisions also create a double-stranded break in the DNA. Ultimately, the DNA break is fixed by homologous recombination, which uses the intact sister chromatid as a template for error-free repair.

Super-SILAC allows Classification of Diffuse Large B-cell Lymphoma Subtypes by their Protein and Post-translational Modifications (PTMs) Expression Profiles

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Correct grouping of molecularly distinct tumor entities into clinical subtypes that can be treated in a specific manner remains one of the major challenges in cancer treatment. This is often reflected in a wide range of heterogeneity in terms of patient survival rates and response to therapy. Mass spectrometry (MS)-based proteomics has recently evolved to the stage where answering specific clinical questions is plausible. Global profiling of closely related tumors at the level of expressed proteins as well as post-translational modifications (PTMs) is an interesting avenue that we wanted to investigate its applicability using MS-based approaches.

We showed recently that high accuracy, quantitative proteomics can robustly segregate two histologically indistinguishable subtypes of diffuse large B-cell lymphoma (DLBCL), activated B-cell-like (ABC) and germinal-center B-cell-like (GCB) [1]. Our approach was based on generating a general lymphoma super-SILAC mix [2] and combining it with cell lines derived from five ABC-DLBCL and five GCB-DLBCL patients. Principal component analysis showed robust segregation of the subtypes where the main drivers of segregation included proteins known to be differentially expressed as well as novel candidates.

We also investigated the possibility of characterizing these tumor subtypes solely based on their pattern of post-translational modifications. We used the recently developed N-glyco FASP method for enrichment of cell surface proteins. We show for the first time the ability to characterize tumor subtypes based on their PTMs expression profiles. We also report results from quantification of the activated phosphoprotein signaling pathways of these two B-cell lymphomas.

Moving one step forward toward clinical settings where minimal sample amounts and measuring times are highly favorable, we investigated the feasibility of single-shot measurements of global proteomes in cell lines and patient samples with the aim of reaching the depth required for their segregation with high accuracy.

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The Personal Genome Project - Worldwide Open Access to Genomes Environments & Trait data

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The Project (personalgenomes.org) enables open observation and critique of a large cohort "test-driving" comprehensive participatory personalized medicine. Since 2004, we have helped push the cost of reading and writing DNA (and biological systems) down by a million-fold (5-fold faster exponential than Moore's law) and enabled fully open-access human Genome+Environment=Trait (GET) data, stem cells, and clinical community curation/interpretation tools (Evidence.PersonalGenomes.org). This involves inherited genomes plus day-to-day genomic variation -- cancers, microbes, allergens, vaccines, & subcellular-resolution epigenomics. We are also sequencing centenarians and long-lived mammals. We are developing human genome engineering technologies for personalized diagnostics as well as stem cell, synthetic organ, microbiome and immunome transplantation therapies.

POSTER ABSTRACTS

1. Smad4 and Trim33 synergistically regulate neural stem cells in the developing cortex

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Neural stem cells (NSCs) in different areas and at different time points during CNS development have to produce distinct types of cells in a precise manner and at accurate numbers. Accordingly the spatiotemporal control of proliferation and differentiation of NSCs is essential to produce a functional nervous system. We found different features of the TGFβ signaling pathway to be required in a particular brain area-specific manner to control the balance between NSC proliferation and differentiation. The TGFβ signaling cascade is initiated by TGFβ-ligand bound receptors triggering the phosphorylation and activation of R-Smads. Activated R-Smads bind Smad4, translocate the nucleus, and regulate target gene transcription. Trim33 is a molecule implicated in the regulation of the TGFβ signaling pathway activity by modulating Smad4 action through competing with Smad4 for interaction with receptor-activated Smad2/3. The complex Trim33-Smad2/3 then in turn controls the expression of specific target genes. However, depending on the context Trim33 can also work as an inhibitor of Smad4 function. In this study we revealed that canonical TGFβ signaling via Smad4 regulates the balance between proliferation and differentiation of NSCs in the entire developing midbrain. *Smad4* deletion leads to horizontal expansion of NSCs due to increased proliferation, decreased differentiation, and decreased cell cycle exit. In contrast, in the developing cortex, ablation of *Smad4* alone did not have any effect on proliferation and differentiation of NSCs. Strikingly, concomitant mutation of both *Smad4* and *Trim33* led to an increase of proliferative cells in the ventricular zone due to a decreased cell cycle exit, revealing synergistic function of Smad4 and Trim33 on a genetic level. Furthermore, we show that in *Smad4-Trim33* double mutant embryos cortical precursor cells generate an excess of deep layer neurons concurrent with a delayed and reduced production of upper layer neurons and in addition fail to undergo the neurogenic to gliogenic switch at the right developmental stage. Thus, our data discloses that in different regions of the developing CNS different aspects of the TGFβ signaling pathway are required to ensure proper development.

2. Neuron-type-specific signals for reward and punishment in the ventral tegmental area

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Dopamine plays a key role in motivation and reward. Dopaminergic neurons in the ventral tegmental area (VTA) signal the discrepancy between expected and actual rewards (i.e., reward prediction error, RPE), but how they compute such signals is unknown. Anatomical and physiological evidence supports the idea that dopamine neuron firing can be modulated by GABAergic neurons, which comprise about 30-40% of all VTA neurons. The role of these GABAergic neurons in regulating dopaminergic neuron activity has been studied extensively in the context of addiction, but their role in normal reward processing has yet to be determined. We recorded the activity of VTA neurons while mice associated different odour cues with appetitive and aversive outcomes. We found three types of neurons based on responses to odours and outcomes: approximately half of the neurons (Type I, 52%) showed phasic excitation after reward-predicting odours and rewards in a manner consistent with RPE coding. The other half of neurons showed persistent activity during the delay between odour and outcome, that was

modulated positively (Type II, 31%) or negatively (Type III, 17%) by the value of outcomes. While the activity of Type I neurons was sensitive to actual outcomes (i.e., when the reward was delivered as expected vs. unexpectedly omitted), the activity of Types II and III neurons was determined predominantly by reward-predicting odours. We "tagged" dopaminergic and GABAergic neurons with the light-sensitive protein channelrhodopsin-2 (ChR2) and identified them based on their responses to optical stimulation while recording. All identified dopaminergic neurons were of Type I, and all GABAergic neurons were of Type II. These results show that VTA GABAergic neurons signal reward expectation, a key variable for calculating RPEs.

3. Impact of interleukin-22 in human lung cancer

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Introduction: Interleukin-22 (IL-22) is an interleukin-10-related cytokine with unique functions on interleukin-22-receptor-1 (IL-22-R1) expressing epithelial cells. Recently, it has been suggested that IL-22 may be an autocrine factor in lung cancer. However, the prevalence of this cytokine and its role in the promotion of human lung cancer are not known.
Methods: Expression of IL-22 in primary lung cancer tissue was analyzed by immunohistochemistry. IL-22 serum levels were measured by ELISA. Expression of the IL-22-R1 was addressed by Western blot and quantitative PCR. Analysis of downstream signaling was performed by Western blot. Cell vitality and impact on apoptosis was assessed by cell titer blue and annexin V-propidium iodide staining, respectively.
Results: We first screened two cohorts of 205 and 2145 lung cancer samples (on a tissue microarray) for IL-22 expression. IL-22 was detected most frequently in small cell (n = 50) and large cell lung cancer (n = 303) with 58 % and 46 % respectively. IL-22 expression did not correlate with survival time in any of these subtypes. 123 sera of lung cancer patients were analyzed for IL-22 concentrations. Among the subtypes analyzed large cell lung cancer patients had the highest mean serum level (548 pg/ml, n = 4). Next, we addressed why, despite the expression of IL-22 as a putative protumoral factor, the course of the disease seems unaltered. We analyzed the effects of IL-22 in five human lung cancer cell lines (A549, HCC827, H1339, H187 and LOU-NH91). IL-22-R1 was expressed in all analyzed cell lines but the expression level differed between the cell lines. High levels of IL-22-R1 were associated with a high cellular response rate to IL-22 exposure. IL-22 induced proliferation of these lung cancer cell lines. We found no increased resistance to chemotherapy by IL-22 treatment. In contrast, when cells were continuously exposed to cisplatin until they grew drug-resistant, we found a striking upregulation of the IL-22-R1 both on protein and mRNA level. IL-22-stimulated cisplatin-resistant cells exhibited higher proliferation rates than the non-resistant controls.
Conclusions: Our data give no evidence for IL-22 expression in tumor tissue as a prognostic factor in resectable lung cancer at the time of diagnosis. In contrast, our results indicate that in chemotherapy-

resistant tumor cells, upregulation of IL-22-R1 and of IL-22-responsiveness may contribute to more aggressive behavior of the disease.

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4. Acivicin as a probe for ABPP

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Nature provides us with a huge number of scaffolds, containing electrophiles that served as inspiration for drug design. The fermentation product of *Streptomyces sviceus* Acivicin contains a chloro-dihydro-isoxazol as the electrophile moiety, that is unique among natural products. It is considered as a glutamine antimetabolite and inhibits mainly L-glutamine amidotransferases. Acivicin showed antitumor activity and was already subject to several clinical trials, but due to side effects went never higher than phase 2. Despite this, Acivicin is still an interesting compound for research and there are still questions regarding its mechanism. To confirm already established targets and to find new targets, we applied a chemical proteomics technique called activity-based protein profiling (ABPP). Furthermore a small library of probes based on the chloro-dihydroisoxazole was designed and applied to living cells to elucidate their targets.

6. Signaling networks controlling multiple phases of axonal morphogenesis

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During development, neurons undergo a series of morphogenetic changes in order to form functional neural circuits. They include polarization, migration, process outgrowth and branching and synapse formation. We demonstrated previously that the signaling pathway defined by LKB1 and SAD-A and –B Ser/Thr kinases is essential for early axon-dendrite polarization in forebrain neurons. We asked whether these regulators are required to execute later phases of axonal differentiation. We have found that the LKB1-SAD pathway is dispensable for polarization of many other neuronal types, but that SAD kinases play a number of additional LKB1-independent roles in neuronal development. First, SAD kinases act within sensory neurons to regulate the formation of terminal axon branches in the central nervous system. Our data reveal that SAD kinases are critical signaling intermediates that transduce both long- and short-term signals from neurotrophic cues in the periphery to regulate patterns of connectivity within the CNS. At later times, SADs also act presynaptically to promote structural and functional maturation of diverse classes of nerve terminals. We have thus identified essential multifunctional regulators of discrete phases of neuronal morphogenesis in vivo.

8. Effects of sensory experience on the development and maintenance of a motor program underlying a complex motor sequence

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Many complex sensorimotor behaviors, such as speech, emerge from the interaction between innate developmental programs (nature) and sensory experience (nurture). How neural circuits underlying such behaviors are shaped by these interactions is not well understood. The zebra finch, a songbird, provides a good model for exploring these questions. Their songs are highly species-specific, yet sensory experience is essential for proper development and maintenance of song. In the absence of a tutor early in life, zebra finches develop impoverished ('isolate') songs with species-typical elements; without proper auditory feedback later in life, learned vocal patterns degrade. Here we explore the effect of sensory experience on the development and maintenance of the motor program underlying zebra finch song by recording from projections neurons in RA, a motor cortex analogue, in birds undergoing one of two manipulations. In one group birds are raised in isolation thus depriving them of tutor song exposure; in the other group, birds are surgically deafened after song has been learned to deprive them of future auditory feedback. By comparing the RA motor program in these groups of birds with age matched controls, we show that sensory experience profoundly affects how the RA motor program develops and is maintained.
In isolate birds, the maturation of the RA motor program, as observed in normal birds (Ölveczky et al. 2011), was significantly slowed and never reached the level of normal adults, suggesting that learning-related synaptic reorganization within the motor circuit is, to a large extent, a function of the bird having access to a tutor template. In deafened birds changes in the song brought on by sensory feedback deprivation were not accompanied by obvious changes in the statistics of the underlying RA firing patterns. While our results suggest that RA may be the locus of tutor-dependent motor learning, they also suggest that different mechanisms may underlie feedback deprivation-induced song degradation. We are currently investigating what, if any, changes occur in the upstream premotor nucleus HVC during these perturbations.

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Ölveczky, Otchy, Goldberg, Aronov and Fee (2011), J. Neurophys. doi:10.1152/jn.00018.2011

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Birdsong, motor control, sensorimotor learning

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9. OpCon: Automated High-Throughput Operant Conditioning System

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Many human behaviors, like speaking, typing, and dancing, consist of complex sequences of movements in a particular order and often with precise timing. Despite the ubiquity of movement sequences in our behavioral repertoire and the ease with which we acquire new ones, very little is known about their neural basis and correlates. Training model organisms, like the rodent, to perform precise and complex motor behaviors to study their neural basis is very laborious. As such, we have developed a highly flexible, scalable and cost-effective technology to automatically (without human supervision) train large numbers of animals simultaneously in a variety of behavioral tasks including the production of sequences of movements in response to visual and auditory cues.

The system consists of several layers of hardware and software. An inexpensive desktop computer controls one or many behavior boxes containing various sensors (cameras, joysticks, levers) and actuators/outputs (speakers, LEDs, valves). The computer runs software that executes a declaratively specified behavioral task abstracting hardware details and continuously records behavioral data including precise high resolution timestamps of events that can then be correlated with neural data. A suite of software built on top of this basic framework, allows specifying the complete training protocol, with its various stages, the criteria for progression from each stage to the next, and the times and durations of training sessions, via a graphical user interface allowing the entire several-week training process to run automatically. Furthermore, the detailed performance of each rat, along with live and saved video, can be remotely viewed over the internet and, if necessary, appropriate adjustments to the protocol be made.

The implementation of this system in our laboratory consists of rats housed permanently in custom designed behavior boxes that are outfitted with joysticks, LEDs, a speaker, an infra-red camera and a water port with an infra-red lick sensor connected to a solenoid valve. This system has made training a large cohort of animals in a variety of movement sequence tasks nearly effortless. Furthermore, with the availability of a large number of automated training setups, discovering the best training/shaping procedure is much, much simpler.

11. Photosensitizing Family A G-Protein Coupled Receptors

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The combination of a seven-helix transmembrane protein with a photo-isomerizable chromophor is the basis of visual perception in animals (i.e. rhodopsin).

Using synthetic organic chemistry, we are developing new photo-sensitive molecules that allow applying this combination to G-protein coupled receptors (GPCRs), which are not inherently sensitive to light.
While nature is using the covalently attached polyene retinal, we make use of soluble small molecules that are equipped with an azobenzene light-switch, which – in analogy to retinal – undergoes *cis-trans*-isomerization upon exposure to light of distinct wavelengths.

Our work addresses structurally well investigated family A GPCRs including the dopamine D2 receptor. Preliminary data demonstrate light-induced changes in both affinity and efficacy of light-switchable agonists, paving the way for photosensitizing a wide range of family A GPCRs using photoswitchable ligands.

12. Population imaging of odor representation by granule cells in the mouse olfactory bulb

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The first sensory stages of the brain play a critical role by formatting the relevant environmental information in an efficient manner that facilitates easy and flexible extraction by downstream areas. Computations in these early stages, for example the main olfactory bulb, are facilitated by a diverse range of inhibitory interneurons. Granule cells (GCs) represent a major class of interneurons in the olfactory bulb, and are more numerous than principal neurons, the mitral/tufted cells (MCs). GCs receive excitatory inputs from MCs and make reciprocal as well as lateral inhibitory synapses on MCs. In addition to sensory inputs from MCs, GCs also receive abundant excitatory input from cortical feedback. To elucidate the role of GCs in odor processing, we imaged the activity of hundreds of GCs simultaneously using genetically encoded calcium indicators and multiphoton laser scanning microscopy in vivo. We found that any particular odor activates only a small fraction of GC somata, but odor responses were more readily observed in individual dendritic spines, the sites of excitatory synaptic inputs. Increasing concentration of odorants led to stronger responses in the somata of active GCs, but does not necessarily recruit additional GCs. Interestingly, while the total number of responding synapses seems to increase with stimulus strength, responses in individual local dendritic regions can be largely concentration independent. Additionally, juxtacellular recordings show that the majority of granule cells respond with only very few spikes to either odor or optogenetic stimulation. Our experiments suggest that suprathreshold responses are sparse in GCs although local activation may be more prevalent. Cortical feedback may play a crucial role in biasing GCs to become more active.