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HARVARD UNIVERSITY

LMU-Harvard Young Scientists' Forum

From Molecules to Organisms VII
Munich, June 28 – July 2, 2015

The LMU-Harvard Young Scientists' Forum (YSF) seeks to unite PhD students and Postdoctoral fellows from the Harvard University and the Ludwig-Maximilians-Universität (LMU Munich) 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.

Conference Agenda

Arrival	Individually arranged
19:00 – open	**Welcome dinner** (Spatenhaus, Kleine Opernstube, 1st floor, Residenzstraße 12, 80333 München)

Monday, June 29

CAS, Seestraße 13, 80802 München

09:00 – 09:30	**Welcome address** (Stefan Lauterbach; Head LMU International Office)
09:30 – 10:30	**Lecture 1** – **Jovica Ninkovic**: "Cellular and molecular changes in adult NSCs allowing successful regeneration in the adult zebrafish brain" (Intro: Stefan Lichtenthaler)
	Coffee break (catered)
11:00 – 13:00	**Session 1** – "Stem cell research and neural regeneration" **Grade/ Klim/ McAvoy/ Pusch** (Chair: Stefan Lichtenthaler)
	Lunch break (catered)
14:30 – 16:30	**Session 2** – "Neural development" **Colombo/ Druckenbrod/ Harris/ Poulopoulos** (Chair: Tobias Rose)
	Coffee break (catered)
17:00 – 18:00	**Lecture 2** – **Florian Engert**: "A sensory motor circuit for binocular motion integration in larval zebrafish" (Intro: Tobias Rose)
18:00 – open	**Conference BBQ** (CAS, garden)

Tuesday, June 30

CAS, Seestraße 13, 80802 München

09:00 – 10:00	**Lecture 3** – **Amar Sahay**: "Local circuits and neural pathways linking adult hippocampal neurogenesis with mood" (Intro: Stylianos Michalakis)
	Coffee break (catered)
10:30 – 12:30	**Session 3** – "Neural coding" **Goltstein/ Haesemeyer/ Mauss/ Odstrcil** (Chair: Martin Biel)
	Lunch break (catered) & YSF faculty meeting (oval office)

14:30 – 16:30	**Session 4** – "Neural circuits" **Hou/ Lodato/ Pecka/ Rose** (Chair: Benedikt Grothe)
	Coffee break (catered)
17:00 – 18:00	**Lecture 4** – **Leanne Godinho**: "Investigating the cellular and molecular mechanisms of interneuron genesis in the vertebrate retina" (Intro: Benedikt Grothe)
18:00 – open	At free disposal (student representative activities)

Wednesday, July 1

LMU Biocenter Martinsried, Großhaderner Straße 2, 82152 Martinsried

08:15 – 09:00	Pre-arranged transfer hotel – **Biocenter**
09:00 – 10:00	**Lecture 5** – **Wilhelm Haas**: "Probing the cancer proteome using multiplexed quantitative proteomics technology" (Intro: Axel Imhof)
	Coffee break (catered; seminar room D00.013)
10:30 – 12:30	**Session 5** – "Proteomics and cell organisation" **Peleg/ Sonnett/ Stadlmeier/ Wühr** (Chair: Axel Imhof)
	Lunch break (catered) & YSF poster session (foyer at D00.003)
	Arenz/ Coneva/ Damijonaitis/ Engerer/ Haile/ Hüll/ Jäpel-Schael/ Künzel/ Lapek/ Millman/ Pilz/ Resnik/ Rhee/ Stange-Marten
14:30 – 16:00	**Session 6** – "Physiology and Behavior" **deBekker/ Haile/ Pilz** (Chair: Karin Meissner)
16:00 – 17:00	**Lecture 6** – **Axel Imhof**: "Chromatin proteomics – decoding the epigenome" (Intro: Karin Meissner)
17:00	*Closing remarks*
17:15	Pre-arranged transfer Biocenter – hotel/ individually: beergarden

Thursday, July 2

Hotel Carlton Astoria, Fürstenstraße 12, 80333 München

09:00 – 19:00	Pick-up, excursion to **Castle Neuschwanstein**

Friday, July 3

Departure	Individually arranged

Participants*

Harvard University Delegation

- **Kenneth Blum**, Executive Director, Harvard Center for Brain Science
- **Noah Druckenbrod**, Postdoctoral Fellow, Harvard Medical School, Department of Neurobiology, Laboratory of Lisa Goodrich
- **Florian Engert**, Professor, Department of Molecular and Cellular Biology
- **Wilhelm Haas**, Professor, Harvard Medical School, Massachusetts General Hospital
- **Martin Haesemeyer**, Postdoctoral Fellow, Department of Molecular and Cellular Biology, Laboratory of Alex Schier
- **James Harris**, PhD Student, Department of Stem Cell and Regenerative Biology, Laboratory of Paola Arlotta
- **Helen Hou**, PhD Student, Harvard Medical School Department of Neurobiology, Laboratory of Bernardo Sabatini
- **Joe Klim**, Postdoctoral Fellow, Department of Stem Cell and Regenerative Biology, Laboratory of Kevin Eggan
- **John Lapek**, Postdoctoral Fellow, Harvard Medical School, Massachusetts General Hospital Cancer Center, Laboratory of Wilhelm Haas
- **Simona Lodato**, Postdoctoral Fellow, Department of Stem Cell and Regenerative Biology, Laboratory of Paola Arlotta
- **Kathleen McAvoy**, Postdoctoral Fellow, Harvard Medical School, Massachusetts General Hospital, Laboratory of Amar Sahay
- **Dan Millman**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Venkatesh Murthy
- **Iris Odstrcil**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Florian Engert
- **Alexandros Poulopoulos**, Postdoctoral Fellow, Department of Stem Cell and Regenerative Biology, Laboratory of Jeffrey Macklis
- **Jennifer Resnik**, Postdoctoral Fellow, Harvard Medical School, Department of Otology and Laryngology, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Laboratory of Daniel Polley
- **Julie Rhee**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of David Cox
- **Amar Sahay**, Professor, Harvard Medical School, Massachusetts General Hospital
- **Matt Sonnett**, PhD Student, Harvard Medical School, Department of Cell Biology, Department of Systems Biology, Laboratory of Steven Gygi
- **Martin Wühr**, Postdoctoral Fellow, Harvard Medical School, Department of Cell Biology, Department of Systems Biology, Laboratory of Steven Gygi

Harvard University Nominating Faculty

- **Paola Arlotta**, Assoc. Professor, Department of Stem Cell and Regenerative Biology
- **David Cox**, Ass. Professor, Department of Molecular and Cellular Biology
- **Kevin Eggan**, Professor, Department of Stem Cell and Regenerative Biology
- **Lisa Goodrich**, Professor, Harvard Medical School, Department of Neurobiology
- **Steven Gygi**, Professor, Harvard Medical School, Department of Cell Biology
- **Jeffrey Macklis**, Professor, Department of Stem Cell and Regenerative Biology
- **Venkatesh Murthy**, Professor, Department of Molecular and Cellular Biology
- **Daniel Polley**, Assoc. Professor, Harvard Medical School, Department of Otology and Laryngology, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary
- **Bernardo Sabattini**, Professor, Harvard Medical School, Department of Neurobiology
- **Alex Schier,** Professor, Department of Molecular and Cellular Biology

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) Delegation

- **Alexander Arenz**, Postdoctoral Fellow, MPIN, Department Circuits – Computation – Models, Laboratory of Alexander Borst
- **Oliver Baron**, Managing Director, LMU, Center for Integrated Protein Science Munich (CIPSM)
- **Oliver Behrend**, Managing Director, LMU, Munich Center for Neurosciences (MCN)
- **Martin Biel**, Professor, LMU, Department of Pharmacy, CIPSM
- **Lena Bouman**, Academic Coordinator (Natural Sciences and Medicine), Center for Advanced Studies (CAS[LMU])
- **Alessio Colombo**, Postdoctoral Fellow, TUM, German Center for Neurodegenerative Diseases, Laboratory of Stefan Lichtenthaler
- **Cvetalina Coneva**, PhD Student, MPIN, Department Synapses – Circuits – Plasticity, Laboratory of Tobias Bonhoeffer

- **Charissa de Bekker**, Postdoctoral Fellow, LMU, Institute of Medical Psychology, Laboratory of Martha Merrow
- **Arunas Damijonaitis**, PhD Student, LMU, Department of Chemistry, Laboratory of Dirk Trauner
- **Susanne Dietrich**, Project Manager, LMU International Office
- **Peter Engerer**, PhD Student, TUM, Institute of Neuronal Cell Biology, Laboratory of Thomas Misgeld
- **Leanne Godinho**, PI, TUM, Institute of Neuronal Cell Biology
- **Pieter Goltstein**, Postdoctoral Fellow, MPIN, Department Synapses – Circuits – Plasticity, Laboratory of Mark Hübener
- **Sofia Grade**, Postdoctoral Fellow, LMU, HMGU, Institute of Stem Cell Research, Laboratory of Magdalena Götz
- **Benedikt Grothe**, Professor, LMU, Division of Neurobiology, Department Biology II
- **Anja Haile**, PhD Student, LMU, Institute of Medical Psychology, Laboratory of Karin Meissner
- **Katharina Hüll**, PhD Student, LMU, Department of Chemistry, Laboratory of Dirk Trauner
- **Axel Imhof**, Professor, LMU, Adolf Butenandt Institute, CIPSM
- **Juliane Jäpel-Schael**, PhD Student, MPIN, Department Synapses – Circuits – Plasticity, Laboratory of Tobias Bonhoeffer
- **Andrea Künzel**, PhD Student, LMU, Nucleic Acid Chemistry, CIPSM, Laboratory of Thomas Carell
- **Stefan Lauterbach**, Head of LMU International Office
- **Stefan Lichtenthaler**, Professor, TUM, German Center for Neurodegenerative Diseases
- **Alex Mauss**, Postdoctoral Fellow, MPIN, Department Circuits – Computation – Models, Laboratory of Alexander Borst
- **Karin Meissner**, PI, LMU, Institute of Medical Psychology
- **Stylianos Michalakis**, PI, LMU Department of Pharmacy, CIPSM
- **Jovica Ninkovic**, PI, LMU, HMGU Institute of Stem Cell Research, Laboratory of Magdalena Götz
- **Michael Pecka**, Postdoctoral Fellow, LMU, Division of Neurobiology, Department Biology II, Laboratory of Benedikt Grothe
- **Sahaf Peleg**, Postdoctoral Fellow, LMU, Physiological Chemistry, CIPSM, Laboratory of Andreas Ladurner
- **Luisa Klaus Pilz**, PhD Student, LMU, Institute of Medical Psychology, Laboratory of Toll Roenneberg
- **Melanie Pusch**, PhD Student, LMU, HMGU Institute of Stem Cell Research, Laboratory of Magdalena Götz
- **Tobias Rose**, PI, MPIN, Department Synapses – Circuits – Plasticity, Laboratory of Tobias Bonhoeffer
- **Michael Stadlmeier**, PhD Student, LMU, Nucleic Acid Chemistry, CIPSM, Laboratory of Thomas Carell
- **Annette Stange-Marten**, Postdoctoral Fellow, LMU, Division of Neurobiology, Department Biology II, Laboratory of Benedikt Grothe
- **Dirk Trauner,** Professor, LMU, Department of Chemistry, Chemical Genetics and Chemical Biology, CIPSM
- **Christian Wahl-Schott**, Professor, LMU Department of Pharmacy, CIPSM

LMU Munich Nominating Faculty

- **Tobias Bonhoeffer**, Professor, MPIN, Department Synapses – Circuits – Plasticity
- **Alexander Borst**, Professor, MPIN, Department Circuits – Computation – Models
- **Thomas Carell**, Professor, LMU, Nucleic Acid Chemistry, CIPSM
- **Magdalena Götz**, Professor, LMU, HMGU Institute of Stem Cell Research
- **Mark Hübener**, Professor, MPIN, Department Synapses – Circuits – Plasticity
- **Andreas Ladurner**, Professor, LMU, Physiological Chemistry, CIPSM
- **Martha Merrow**, Professor, LMU, Institute of Medical Psychology
- **Thomas Misgeld**, Professor, TUM, Institute of Neuronal Cell Biology
- **Till Roenneberg**, Professor, LMU, Institute of Medical Psychology

Abstracts of lectures and posters

Probing the neural circuit underlying the detection of visual motion in the fly

Alexander Arenz
MPI of Neurobiology, Department Circuits – Computation – Models

The detection of visual motion, both the movement of objects in a visual scene and of the visual scene itself, helps animals to spot prey, predators or mates and to navigate in their environment. Yet visual motion is not directly detected by photoreceptors in the retina, but has to be computed from the luminance changes across at least 2 different points in space and time. Algorithmic models based on the correlation of the activity of one photoreceptor with the temporally delayed (low-pass filtered) signal of a neighboring photoreceptor describe both the behaviour and the electrical activity of direction-selective neurons of the fly very well. However, how these computations are implemented on a cellular basis to give rise to direction-selective responses in T4 and T5 neurons in the optical lobes of Drosophila is unknown. We manipulate synaptic inputs and dynamics of these elementary motion detectors in order to understand how visual motion is computed.

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DR6 shedding negatively regulates myelination in PNS

Alessio-Vittorio Colombo
TUM, German Center for Neurodegenerative Diseases

Death receptor 6 (DR6), also known as TNFRSF21, is a type I trasmembrane protein belonging the Tumor Necrosis Factor receptor superfamily (TNFRSF). Even if the main receptor ligand is not known yet, DR6 has been already described as a key regulator in cell differentiation and to play an important role during physiological development and pathological conditions in the central nervous system. In particular DR6 has been proposed as a key player in the myelination process occuring in the central nervous system (CNS) negatively regulating oligodendrocyte survival and maturation. Within the TNFRSF, some members like p75 neurotrophin receptor (p75NTR) have been shown to undergo a regulated intramembrane proteolysis (RIP) process. RIP is an elegant way for cells to transduce signals or simply to quickly remove proteins from the cell surface. It is a tightly regulated mechanism fundamental for key biological processes like embryonic development, regulaton of immune response and nervous system function. Because of its importance, its misregulation is often associated with pathological conditions like cancer or neurodegenerative disorders. We investigated the role of DR6 in myelination in the peripheral nervous system (PNS), showing that it is a negative regulator for myelination not only for oligodendrocyte but also for schwann cells (SC). Furthermore, we demonstrated for the first time that DR6 undergoes RIP under physiological conditions, thus releasing biologically active proteolytic fragments affecting PNS myelination.

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Activity-dependence and target specificity of synapse formation during long-term potentiation

Cvetalina Coneva, Tobias Bonhoeffer and Tobias Rose
MPI of Neurobiology, Department Synapses – Circuits – Plasticity

Long-term potentiation (LTP) of synaptic connections results in the formation and stabilization of new dendritic spines *in vitro*. Similarly, experience-dependent plasticity *in vivo* is associated with changes in the number and stability of spines. However, to date, the contribution of excitatory synaptogenesis to the strengthening of synaptic connections remains elusive. Do new spines form functional synapses with the inputs stimulated during LTP induction and thereby follow Hebbian co-activation rules, or do they connect with random partners? Furthermore, at which time-point are *de novo* spines functionally integrated into the network?
We developed an all-optical approach to stably and exclusively stimulate a defined channelrhodopsin-2 (ChR2)-transduced subset of CA3 cell axons in mature hippocampal slice culture over extended periods of time (up to 24h). We continuously monitor synaptic activation and synaptic structure of CA1 dendrites using two-photon imaging. To control the dendritic location where LTP and associated spinogenesis are allowed to take place, we globally block Na^+-dependent action potential firing and directly evoke neurotransmitter release by local light-evoked depolarization of ChR2-expressing presynaptic boutons (in TTX, 4-AP). We induce robust optical LTP specifically at this location by combining optogenetic activation with chemical pairing (in low $[Mg^{2+}]_o$, high $[Ca^{2+}]_o$, forskolin, and rolipram). Taking advantage of the NMDA-receptor mediated Ca^{2+} influx during synaptic activation we assess the formation of functional synapses using the genetically encoded Ca^{2+} indicator GCaMP6s.
We find that optical LTP leads to the generation of new spines, decreases the stability of preexisting spines and increases the stability of new spines. Under optical LTP conditions, a fraction of new spines responded to optical presynaptic stimulation within hours after formation. However, the occurrence of the first synaptic Ca^{2+} response in *de novo* spines varied considerably, ranging from 1.1 to 25 h. Most new spines became responsive within 4 h (2.2 ± 0.9 h, mean ± S.D., n = 16 out of 20), whereas the remainder showed their first response only on the second experimental day (19.2 ± 3.7 h). Importantly, new spines generated under optical LTP are more likely to build functional synapses with light-activated, ChR2 expressing axons than spontaneously formed spines (new responsive spines under optical LTP: 64 ± 4 %; control 1: 0%; control 2: 13 ± 4 %; control 3: 11 ± 4 %). Furthermore, new spines that are responsive to optical presynaptic stimulation are less prone to be eliminated overnight than new spines that fail to respond (% spine overnight survival; 81 ± 3 % new responsive spines; 58 ± 4 % of new unresponsive spines).

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Cholinergic Photopharmacology – Controlling nicotinic and muscarinic Acetylcholine Receptors with photoswitchable molecules

Arunas Damijonaitis, Johannes Broichhagen, Laura Laprell, Jatin Nagpal, Matthias Schönberger, Tatsuja Urushima, Tobias Bruegmann, Philipp Sasse, Martin Sumser, Alexander Gottschalk, Dirk Trauner
LMU, Department of Chemistry

Nicotinic and muscarinic acetylcholine receptors (n/mAChRs) play a prominent role in shaping excitatory postsynaptic potentials and intracellular signaling pathways. To study these systems, a vast pharmacological toolset has been developed over the last decades. However, the possibility to reversibly control the receptor activation selectively and with high spatiotemporal precision has been lacking. To address this issue, two novel photochromic ligands (PCLs) have been generated: **AzoCholine** to target nicotinic AChRs and **AzoCarbachol** to target muscarinic AChRs. Here, we show that, **AzoCholine** specifically targets neuronal nAChRs, allowing light control of cholinergic activity in hippocampal mouse brain slices and triggering of behavior in the nematode *C.elegans*. On the other hand, **AzoCarbachol** applied in a whole heart preparation of the mouse (Langendorff) modulates beat frequency in a light-dependent fashion. Therefore, these molecules can be used to optically control behaviour and cardiac rhythm and can be envisioned to enable further elucidation of AChR function *in vivo*.

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Photopharmacology

Photochromic Ligand PCL

hv_1 / hv_2

effect

receptor, ligand, photoswitch,
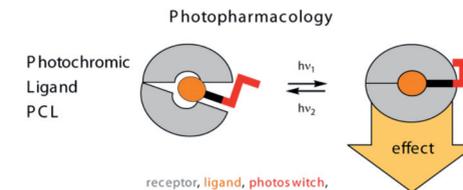
Manipulation of animal behavior: How can a parasite control its host's brain?

Charissa de Bekker
LMU, Institute of Medical Psychology

Many parasites have evolved the ability to manipulate the behavior of their hosts to advance transmission. These manipulations range from slightly changed existing behaviors to the establishment of completely novel ones that are not part of the regular repertoire. The stereotypical biting behavior right before death in Carpenter ants infected with the fungus *Ophiocordyceps unilateralis* (the so-called "zombie ants") is and example of the latter. I use this parasite-host interaction as a model system to ask the question how a microbe can control an animal's brain to precisely change the behavioral output.

By combining behavioral ecology techniques with molecular biology techniques and several types of –"omics" datasets I am taking the first steps towards unraveling the mechanisms underlying parasitic behavioral manipulation. Infection studies together with metabolomics on ex vivo fungus-ant brain interactions demonstrated the species-specificity of parasitic manipulation. Moreover, these studies led to the discovery of the first fungal candidate compounds in this system that might be involved in changing host behavior. This study was followed by a mixed transcriptomics approach investigating gene expression of both parasite and host during manipulated biting. Among the up-regulated fungal genes during manipulation were those that putatively encode for proteins with reported effects on behavioral outputs, proteins involved in neuropathologies and proteins involved in the biosynthesis of secondary metabolites such as alkaloids. We also found that the fungal parasite might be regulating immune- and neuronal stress responses in the ant host during manipulated biting, as well as impairing its chemosensory communication and causing apoptosis.

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Cochlear wiring specificity is achieved by sequential segregation of neuronal processes before birth

Noah Druckenbrod
Harvard, Department of Neurobiology

A hallmark of the nervous system is the presence of precise patterns of connections between different types of neurons. Although many possible mechanisms have been described, we still know little about how developing neurons find each other and form synapses in predictable numbers and locations. We addressed this question in the developing cochlea, where Type I and Type II spiral ganglion neurons (SGN) navigate together to the sensory epithelium and then diverge to contact inner or outer hair cells, respectively. By analyzing the morphology and dynamic behaviors of individual fibers and their branches, we found that even before birth, SGN processes follow stereotyped paths that predict their final organization in the mature cochlea. Our results suggest that Type I SGN processes are steered towards their targets by repulsive cues that first position the terminals and then corral them to this region during a postnatal stage of exuberant branching and refinement.

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Uncoupling of mitosis and differentiation allows for fast and synchronous CNS development *in vivo*

Peter Engerer
TUM, Institute of Neuronal Cell Biology

The prevailing view of neuronal development is that specific ontogenetic events occur in a defined sequence. Thus, following cell-cycle exit, newly generated neurons are believed to migrate to specific locations and differentiate, acquiring molecular and morphological features that permit their integration into synaptic circuits. How these events are coordinated to accommodate the generation of a rapidly developing nervous system is not well understood. Using the zebrafish retina as a model for *in vivo* CNS development, we show that mitosis and neuronal differentiation are largely independent of each other. Rather than dividing at a stereotypic point in their developmental trajectory, we find that vsx1+ progenitors of retinal bipolar interneurons can undergo mitosis at different stages of differentiation. For example, late-dividing vsx1+ progenitors already target their neuronal processes to synaptic neuropil, reposition their soma to their final stratum of residence prior to mitosis and show gene expression dynamics similar to the post-mitotic bipolar cells that surround them. Intriguingly, the differentiation of post-mitotic and progenitor cells towards mature bipolar cells appears to be locally regulated rather than being time-locked to mitosis. We propose that uncoupling of mitosis and differentiation allows for accelerated neuronal development and synchronizing neuronal differentiation within a local population. Our findings are compatible with a reinterpretation of previous observations from neuronal development in mammals, and hence reveal a new neuro-developmental strategy that might be operating in a wide range of species and brain structures.

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A sensory motor circuit for binocular motion integration in larval zebrafish

Florian Engert
Harvard, Department of Molecular and Cellular Biology

Zebrafish process whole field visual motion, extract the net direction of such stimuli and use this information to guide their swimming behavior to match the direction and speed of these external cues. This innate behavior, called the optomotor reflex (OMR) is ubiquitous in the animal kingdom and presumably serves to stabilize an animal's position in the world when it is being moved by external forces.
Here we use a closed loop behavioral assay in freely swimming fish that allows specific and independent stimulation of the two eyes – with coherent as well as conflicting motion signals. We can then answer questions of how the two eyes interact to combine, suppress and filter the various permutations of motion stimuli.
We subsequently use whole brain imaging in tethered larvae to identify the complete neural circuitry underlying these various sensory motor transformations. Specifically we provide a working model of the complete circuit, that quantitatively captures the complete behavioral output as well as the response characteristics of the majority of the active neurons identified by independent cluster analysis.
This rate based computational model makes very specific predictions about connectivity and synaptic polarity of the functionally identified neurons, is easy to test and falsify and serves as an ideal platform and hypothesis generator for a whole range of future experiment.

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Investigating the cellular and molecular mechanisms of interneuron genesis in the vertebrate retina

Leanne Godinho
TUM, Institute of Neuronal Cell Biology

The central nervous system is composed of a multitude of neuronal cell-types, each of which sub-serves unique functions. How this cellular diversity is generated *in vivo* is not completely understood. Using the zebrafish retina as a model for studying CNS development *in vivo*, we have been investigating how bipolar cells, excitatory interneurons located in the inner nuclear layer, are generated. In line with previous work, we find that bipolar cells are generated by the terminal divisions of visual system homeobox 1 (vsx1) – expressing progenitors that are committed to their production. Intriguingly, our time-lapse observations reveal that bipolar cells are generated by two modes of terminal divisions – symmetric divisions, yielding two bipolar cells or asymmetric divisions where the bipolar cell sibling is an amacrine cell, an inhibitory interneuron. We have been exploring the molecular mechanisms underlying these diverging fates and find the Notch receptor and the transcription factor ptf1a to be key players. Our work suggests that Notch and ptf1a act in concert within a limited time-window following mitotic divisions to instruct the amacrine cell fate in one daughter cell. In the absence of such signaling two bipolar cells are generated. Further gain-of-function experiments reveal that Notch can impose a surprising plasticity in new-born neurons of the vsx1 lineage, permitting their adoption of other cell fates.

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Visual category learning and its neural correlate in mouse V1

Pieter Goltstein, Sandra Reinert, Tobias Bonhoeffer, Mark Hübener
MPI of Neurobiology, Department Synapses – Circuits – Plasticity

Everything we know is stored in networks of associative memories. The ability to assign these into discrete functional groups is a fundamental aspect of behavior. Learned categories can be represented in tuning of single neurons as well as population coding, but are ultimately stored in the underlying pattern of synaptic connectivity. To investigate the underlying cellular and synaptic mechanisms, we developed a mouse model for visual category learning.
We constructed a two-dimensional stimulus space consisting of gratings that varied continuously in orientation and spatial frequency, and applied a diagonal category boundary to distinguish two categories (21 stimuli each) based on specific parameter combinations. Mice (n=8) were trained to discriminate stimuli from the rewarded and the non-rewarded category in a touch-screen equipped operant chamber. Training started with only a single stimulus from each category, both having a large distance from the category boundary. All mice discriminated these stimuli well (>80% correct, 6 days). Further stimuli with decreasing distance to the category boundary were added in a stepwise fashion. The very first stimuli of each added set were discriminated at >70% correct, indicating that generalization occurs within categories.
After successful learning, we performed two-photon microscopy in layer 2/3 of the primary visual cortex, approximately 2 months after cells had been transduced with a viral construct expressing a calcium indicator (AAV2.1.hSyn.mRuby2.GCaMP6f). In each mouse, we recorded calcium activity of 600 to 800 cells in response to moving gratings (24 directions, 11 spatial frequencies). Many cells were selective for orientation and spatial frequency, with preferred spatial frequency being much higher in the awake mice than under anesthesia. Some cells in awake mice showed spatial-directional tuning curves resembling learned categories, but the same cells did not show this under anesthesia. As a next step, we will employ this paradigm to search for neurons and brain regions representing learned categories, ultimately allowing us to study the synaptic changes underlying category learning.

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Transplanted neurons wire into damaged brain circuits: from connections to function

Sofia Grade[1,2], Susanne Falkner[3], Leda Dimou[1,2], Klaus Conzelmann[4], Mark Hübener[3], Magdalena Götz[1,2]
1 LMU, Biomedical Center
2 HMGU, Institute of Stem Cell Research
3 MPI of Neurobiology
4 LMU, Max von Pettenkofer Institute and Gene Center

The capacity of the adult mammalian brain to restitute neurons upon neuronal loss is extremely limited. Transplantation studies aiming at cell replacement in animal models of neurological disease or injury have shown host-graft synapse formation and extension of efferents from the grafted cells to proper anatomical targets through the adult brain (Fricker-Gates et al., 2002; Gaillard et al., 2009). However, it remains largely unexplored whether new afferent synapses are adequate and thus able to convey genuine input information. To tackle this question, we induced cell death of upper layer callosal projection neurons in the primary visual cortex (V1) of adult mice and investigated whether young neurons transplanted after lesion may integrate properly or aberrantly into the native circuitry, a critical question for functional reconstruction. We demonstrate here that a great majority of transplanted cells has the appropriate upper layer neuron identity and survive for several months. Using the monosynaptic rabies virus-based tracing approach (Wickersham et al., 2007), previously adapted in our group to target new neurons (Deshpande et al., 2013), we dissected the whole brain host-to-graft circuitry. Local neuronal populations within the visual cortex connect massively with transplanted neurons, but also other sensory and associative areas including the somatosensory cortex, motor cortex, auditory cortex, retrosplenial cortex and the posterior parietal association cortex. Moreover, synaptic input from distant areas including the thalamic lateral geniculate nucleus – the primary relay center for the visual information coming from the optic nerve – and the contralateral V1 was found. A comparative analysis with the endogenous developmentally-generated connectivity suggests that the newly formed circuits strikingly resemble the normal afferents of V1 neurons in the naïve brain. In addition, using two-photon calcium imaging we demonstrate that transplanted neurons become visually responsive and show orientation and/or direction selectivity. Altogether, our results indicate that new neurons may properly integrate and function in an injured neocortex.

Deshpande, A. et al. Retrograde monosynaptic tracing reveals the temporal evolution of inputs onto new neurons in the adult dentate gyrus and olfactory bulb. Proc Natl Acad Sci U S A 110: E1152-61 (2013).
Fricker-Gates, R. A. et al. Late-stage immature neocortical neurons reconstruct inter-hemispheric connections and form synaptic contacts with increased efficiency in adult mouse cortex undergoing targeted neurodegeneration. J Neurosci 22: 4045-56 (2002).
Gaillard, A. et al. Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral transplants. Neurobiol Dis 35: 477-88 (2009).
Wickersham, I. R. et al. Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron 53: 639-647 (2007).

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Probing the cancer proteome using multiplexed quantitative proteomics technology

Wilhelm Haas
Harvard Medical School, Massachusetts General Hospital

Mass spectrometry-based proteomics is currently undergoing a major technological shift as multiplexed quantitative strategies are closing a historical performance gap between proteomics and genomics platforms both in sample throughput and sensitivity. The proteome is a crucial link between genotype and phenotype and access to the information of changes at the proteome level between healthy and diseased states has high potential to allow us understanding the molecular mechanisms underlying a disease and may inform ways to interrupt these mechanisms when treating patients. We are using state-of-the-art multiplexed proteomics technologies to study multiple questions in cancer research and I will present our general strategies to address these question as well as results from specific projects.

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Temporal receptive fields of heat perception in larval zebrafish

Martin Haesemeyer, Drew N. Robson, Jennifer M.B. Li, Alexander Schier, Florian Engert
Harvard, Department of Molecular and Cellular Biology

Avoiding temperatures outside the physiological range is critical for animal survival. Therefore many animals employ strategies to navigate temperature gradients but, especially in vertebrates, it is currently unknown how temperature dynamics are transformed into behavioral output. In order to understand how the nervous system generates meaningful behavior in response to temperature changes it is crucial to understand these sensorimotor transformations. In the present study we set out to characterize the temporal receptive fields of heat sensation that drive behavioral decisions in larval zebrafish. Using an infrared laser we challenged freely swimming larval zebrafish with „white-noise" heat stimuli and built models that relate external sensory information as well as internal state to behavioral output. These models revealed that larval zebrafish integrate temperature information over a time-window of 400ms immediately preceding a swim-bout gated by a refractory period. Our results suggest that larval zebrafish compute both an integral and a derivative across heat in time to guide their next movement. Our models put important constraints on the type of computations that occur in the nervous system in order to process somatosensory temperature information to guide behavioral decisions.

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Neurophysiological correlates of the placebo effect in nausea

Anja Haile[1], M Watts[1,2], S Aichner[1], Verena Hoffmann[1], F Stahlberg[1], Karin Meissner[1]
1 LMU, Institute of Medical Psychology
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Background: Nausea, in the context of motion sickness, is associated with specific changes in low frequencies (0.5-4 Hz) above central cortical areas in the electroencephalogram (EEG) (Hu et al., 1999). Interestingly, several studies suggest that nausea can be modulated by placebo interventions (Quinn & Colagiuri, 2014), but little is known about the underlying cognitive mechanisms. The goal of the present study was to compare behavioral and electroencephalography (EEG) measures of nausea with and without placebo intervention in healthy controls.
Methods: In total, 100 subjects were exposed to a nauseating stimulus (i.e., alternating black and white stripes projected onto a semicircular screen in front of the participants) for 20 minutes on two separate days. On day one, all subjects watched the nauseating stimulus without intervention. On day two, subjects were either randomized to sham acupuncture point stimulation (placebo group) or to no treatment (control group). To evaluate the effects of the placebo manipulation, severity of nausea was assessed on a numeric rating scale from 0 to 10 and EEG signals were collected before and during the nauseating stimulus.
Results: Preliminary results are based on 45 subjects. On day one, both groups showed significant nausea and a higher net increase in EEG power in the low frequency Delta band (0.5-4Hz) at electrodes C3 and C4 in the nauseated condition compared to the measures at rest. Severity of nausea correlated positively with the rise in delta power. On day two, the sham acupuncture manipulation in the placebo group significantly reduced nausea ratings as well as the increase in delta waves at C3 and C4 in comparison to the control group. The behavioral placebo effect on nausea ratings correlated positively with the electrophysiological placebo effect on delta waves.
Discussion: Sham acupuncture point stimulation significantly reduced nausea and low frequencies in the EEG at C3 and C4. The present data emphasize the effectiveness of sham acupuncture for inducing a placebo effect on nausea and allow first insight into underlying central mechanisms. In particular, we found a partial reversal of nausea-specific changes above central cortical areas in the EEG.

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Exploring the role of extrachromosomal circular DNAs in the development of cortical projection neurons

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The DNA code, written in an organism's genome, is its fundamental blueprint for life. It is believed that the sequence of DNA is largely identical in most cells within an individual organism. However, recent evidence suggests that neurons of the cerebral cortex exhibit variations, not only in their DNA content, but also in the form of this DNA. In particular, extrachromosomal circular DNAs (eccDNAs) have been detected in early embryonic brain tissue and are believed to be the products of chromosomal excisions. Furthermore, eccDNAs can form as a result of DNA damage that accumulates over the long life of neurons in the brain and this accumulated DNA damage is thought to be a central driver of aging and cortical neurodegenerative diseases. However, the biological significance of these eccDNAs and their corresponding chromosomal alterations is unknown. Here we develop methodologies for sequencing eccDNAs from small populations of FACS purified cells of the cerebral cortex. Using these new techniques, we plan to characterize eccDNA populations in specific cortical projection neuron subtypes during development and neurodegenerative disease.

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To pee or not to pee: Dissecting the neural circuit for bladder control

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All animals integrate information about their environment, internal state, and previous experience to guide motor output and determine behavioral choices. The neural mechanisms underlying the context-dependent selection of behaviors are poorly understood, largely due to the complexities of neural circuits. We tackle this problem by examining a system where simple cues trigger a binary decision to execute rapid movements: the neural control of micturition (urine release from the bladder) for territorial marking. Adult male lab mice scatter urine in their environment to attract mates and suppress micturition to evade predators. This innate behavior depends on olfactory cues in the environment: the frequency and location of micturition changes if the mouse senses predators, potential mates and conspecific competitors. Although the local control from spinal cord to bladder is well understood, we know little about how olfactory information is relayed and processed in the brain to dictate bladder movement.

The pontine micturition center (PMC), also known as Barrington's nucleus, is a brainstem nucleus through which different information from the brain has to funnel through in order to command the bladder. Cats with bilateral lesion of the PMC cannot empty the bladder, whereas electrical stimulation in the PMC in rats leads to micturition. Anatomical and functional evidence suggests that the PMC could be a bottleneck nucleus, a site that integrates various inputs from the brain and sends a commanding output to the bladder. Therefore, in order to understand how the brain controls the bladder, we use PMC as an entry point to probe its inputs, local circuitry and outputs. To understand the neural circuit underlying micturition control, we use electrophysiological, as well as neuroanatomical, optogenetic, pharmacogenetic and behavioral approaches to study if, and how information flows to and undergoes transformation PMC. We test the hypothesis that the PMC acts as a master switch for micturition, integrating various neural inputs to send an on/off command to the bladder.

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Photopharmacology targeting the Adenosine A1 Receptor

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G-Protein coupled receptors (GPCRs) are important transmembrane proteins and target of up to 40% of marketed drugs. The adenosine A1 receptor, a class A GPCR, is found in the brain, especially the cerebral cortex and the striatum. Activation of the adenosine A1 receptor has an inhibitory effect to the cell by inhibiting adenylyl cyclase and activating G protein coupled inwardly rectifying potassium (GIRK) channels. We have developed three photochromic agonists for the adenosine A1 receptor. Photophysical properties of all molecules, including a red shifted derivative, have been investigated using NMR and UV-Vis spectroscopy. Furthermore, pharmacological photoswitching has been demonstrated using patch clamp electrophysiology in HEK cells. Our novel photoswitchable ligands for adenosine A1 receptors enable reversible light-mediated control of their target proteins. In future, they might be used for orthogonal receptor activation with high spatiotemporal precision in complex systems.

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Chromatin proteomics – decoding the epigenome

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Constitutive heterochromatin is typically defined by high levels of DNA methylation and H3 lysine 9 trimethylation (H3K9Me3), whereas facultative heterochromatin displays DNA hypomethylation and high H3 lysine 27 trimethylation (H3K27Me3). The two chromatin types generally do not coexist at the same loci, suggesting mutual exclusivity. During development or in cancer, pericentromeric regions can adopt either epigenetic state, but the switching mechanism is unknown. We used a quantitative locus specific purification method to characterize changes in pericentromeric chromatin-associated proteins in mouse embryonic stem cells deficient for either the methyltransferases required for DNA methylation or H3K9Me3. DNA methylation controls heterochromatin architecture and inhibits Polycomb recruitment. To analyze the inheritance of epigenetic information we also combined nascent chromatin capture and SILAC labeling to track histone modifications and histone variants during replication and across the cell cycle. We show that old histones maintain methylation marks upon recycling onto newly replicated DNA offering a blueprint for chromatin restoration. Methylation of new histones is slow, step-wise and continues into the next cell cycle. Indeed, H3K9 and H3K27 tri-methylation requires passage through mitosis and extents beyond one cell cycle. Therefore, methylation state increases with histone age and exit from the cell cycle is accompanied with global increase in histone methylation. This work reveals that DNA replication has a global long-lasting impact on chromatin state and provides a foundation to understand how epigenetic states are propagated.

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Experience-dependent structural and functional plasticity of eye-specific thalamocortical axons

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A classic example of experience-dependent plasticity is the shift in ocular dominance (OD) after monocular deprivation (MD). It is well established that long-term MD is accompanied by the retraction of axonal projections to visual cortex originating from dorsal lateral geniculate nucleus (dLGN). However, these structural changes occur long after the onset of functional OD plasticity in mice. It is unclear, whether the rapid changes in cortical responsiveness to the deprived and non-deprived eye might partly be mirrored by eye-specific fine-scale presynaptic rearrangements along thalamocortical (TC) axons.
In order to follow the changes in responsiveness and synapse-scale structure of TC afferents, we established long-term chronic *in vivo* two-photon Ca^{2+} imaging of the same axons and presynaptic boutons in layer 1 of adult mouse visual cortex. For structural and functional axonal imaging, we conditionally expressed the genetically encoded calcium indicator GCaMP6m in LGN neurons of partially thalamus-specific Scnn1a-Tg3-Cre mice using AAV-mediated transduction.
We repeatedly measured the eye-specific Ca^{2+}-responses of TC afferents to visual stimulation both before and after MD. In adult mice, the OD of the same identified axons imaged over weeks is largely stable under baseline conditions. To our surprise, preliminary results suggest prominently decreased deprived eye responses of TC afferents after MD which would argue against an exclusively cortical locus of OD plasticity. We are currently assessing whether these functional changes are accompanied by eye-specific structural changes to evaluate if indeed early changes in TC connectivity may be a driving force of OD plasticity.

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Transcriptional and proteomic profiling of human pluripotent stem cell-derived motor neurons: Implications for familial amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative disease characterized by the selective loss of upper and lower motor neurons. The identification of genetic triggers (e.g. mutations in */C9ORF72, SOD1/, /FUS/*, and */TARDBP)/* has informed our biological understanding of this devastating disease, but we still do not know how mutations in these genes cause selective motor neuron degeneration. Global, large-scale molecular studies on disease-relevant cell types is an attractive option to investigate the pathology of ALS, but the inaccessibility of human motor neurons combined with our inability to expand them in culture like cancer cells remains a barrier to these types of studies. Opportunely, human pluripotent stem cells can be directed to efficiently differentiate into substantial quantities of motor neurons. Here, we combined pluripotent stem cell technologies with both RNA sequencing and mass spectrometry-based proteomics to map alterations to mRNA and protein levels in motor neurons expressing mutant SOD1. Specifically, we introduced the severe */SOD1 A4V/* mutation into a stem cell line that reports for GFP under the control of the motor neuron-specific promoter for */HB9/*. This approach enabled us to study the effects of mutant SOD1 in purified populations of motor neurons using multiple metrics over time. These investigations have afforded an unprecedented glimpse at the biochemical make-up of human stem cell-derived motor neurons and how they change in culture. Moreover, our results revealed subtle yet reproducible differences in gene and protein expression between motor neurons with and without the */SOD1 A4V/* mutation. Interestingly, several of the altered proteins regulate aspects of neuronal excitability, which our group previously described a hyper-excitability phenotype induced by mutant SOD1 in motor neurons. In conclusion, our global profiling efforts offer a greater understanding of stem cell-derived neurons and provide possible links between mutant proteins and molecular pathology. Perhaps their greatest value, however, is in identifying new therapeutic targets for inter-vention into the disease course of ALS.

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Tête-à-Tet – Elucidating the interactome of the tet enzymes

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Currently in the focus of ongoing research are the ten eleven translocation (Tet) 1-3 proteins, which belong to the family of DNA hydroxylases[1].Tet enzymes are capable of changing the DANN methylation state, which is important for epigenetic regulation. They can generate the only recently discovered modified bases 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine by successive oxidation of 5-methylcytosine[2,3,4]. However, it is unknown how the Tet proteins are regulated in this process. To identify the proteins interacting with the Tet enzymes, we conducted modified immunoprecipitations using GFP-Tet fusion constructs and analyzed the samples via mass spectrometry and label-free quantification. The thus obtained results indicate that the Tet enzymes interact mostly with proteins involved in epigenetic regulation and transcription. In future experiments the interaction network of the Tet enzymes will be further elucidated by using MS-cleavable crosslinking reagents in combination with mass spectrometry.

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Functional network prediction in breast cancer derived from MS3-based multiplexed quantitative proteomics

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Historically, a lack of sample throughput in mass spectrometry-based proteomics, compared to genomics tools, has resulted in a lack of proteomic analysis for large sample sets. In this study, we show that the use of multiplexed proteomics using TMT-10 reagents coupled with synchronous precursor selection on an Orbitrap Fusion, allows us to analyze a large panel of breast cancer cell lines in a high throughput manner. At an analysis rate of 4.5 hours per quantitative proteome, this puts quantitative proteomics technology on same time line as genomic tools for handling large sample sets. Quantitative proteomic data generated at this pace adds an important dimension to the characterization of cancer cell lines. Protein from 42 breast cancer cell lines, representing basal, luminal, claudin-low, ERBB2+, triple negative and non-malignant subtypes, were analyzed. We generated quantitative proteomic profiles from just 50 µg of protein per cell line. This was performed in biological duplicate for each cell line. Quantitative data was obtained for 10,535 proteins overall, with an average of 9,115 proteins quantified per cell line. There was an overlap of 6,911 proteins quantified across all cell lines. All results were filtered to a peptide and protein false-discovery rate of less than 1 %. Comparisons with RNAseq data will be presented, along with comparison of co-expression analysis to define functional networks from each dataset.

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Molecular mechanisms of local microcircuit assembly in the neocortex

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The activity and function of the mammalian cerebral cortex rely on the precise assembly of specialized neuronal circuits involving an extraordinary diversity of excitatory projection neurons and inhibitory interneurons. Although they have different developmental origins, these two classes of neurons ultimately co-reside in the cortex, where they assemble into balanced local microcircuitry. The developmental events governing the proper interaction between excitatory projection neurons and inhibitory interneurons are poorly understood, in particular the cellular and molecular events that direct interneurons to position precisely within specific cortical layers. We recently reported that different subtypes of projection neurons uniquely and differentially determine the laminar distribution of cortical interneurons. We found that in Fezf2-/- cortex, the specific absence of subcerebral projection neurons and their replacement by commissural projection neurons causes abnormal lamination of inter-neurons and altered overall GABAergic inhibition in the mutant cerebral cortex. In addition, experimentally-induced ectopic corticofugal or callosal neurons selectively recruit distinct subtypes of cortical interneurons, based on the identity of the projection neuron. These data demonstrate that, in the cerebral cortex, individual populations of projection neurons cell-extrinsically control the laminar fate of interneurons and the assembly of local inhibitory circuitry (Lodato et al., Neuron, 2011). Here, we investigated the molecular mechanisms that mediate the precise pairing between distinct subtypes of projection neurons and inter-neurons in the early stages of radial migration of cortical interneurons, a process that is fundamental for the assembly of specialized cortical circuits. To address this question, we FACS-purified and transcriptionally profiled pairs of projection neuron and interneuron subtypes which preferentially reside in the same cortical layers, over multiple developmental time points. Using custom bioinformatic analysis, we have identified potential candidates that are functionally relevant for the lamination of interneurons within the cortex, and therefore crucial for the assembly of specialized cortical circuits. These molecules have potential roles in the etiology of neurological disorders where excitation and inhibition balanced is altered, such as autism and schizophrenia.

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Local inhibitory interneurons conveying optic flow-field selectivity in the fly

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When navigating in their environment, animals use visual motion cues as feedback signals that are elicited by their own motion. Such signals are provided by wide-field neurons sampling motion directions at multiple image points. Each one of these neurons responds selectively to a specific optic flow-field representing the spatial distribution of motion vectors on the retina as occurring during a particular maneuver. Here, we describe the discovery of a group of local, inhibitory interneurons in the fruit fly *Drosophila* that play a decisive role in this context. Using anatomy, molecular characterization, genetic activity manipulation and physiological recordings, we demonstrate that these interneurons convey direction-selective inhibition to wide-field neurons with opposite preferred direction. Blocking their activity, we find that wide-field neurons now respond unselectively to a variety of different flow-fields. We conclude that these circuit elements eliminate unspecific responses and, thus, ensure selectivity for particular flow-fields in downstream wide-field neurons.

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Modulating lineage homeostasis to rejuvenate aging memory circuits

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Neural stem cells in the dentate gyrus (DG) generate dentate granule neurons throughout life, a process exquisitely sensitive to the environment. Adult-born dentate granule neurons contribute to encoding functions, such as pattern separation, important for minimizing interference during storage of episodic memories. These observations suggest that adult hippocampal neurogenesis represents an adaptive mechanism of encoding by which generation and integration of new neurons is governed by environmental demands on hippocampal circuitry to maintain memory precision. However, the underlying mechanisms by which mature dentate granule neurons sense and transduce changes in activity to dictate lineage homeostasis are poorly understood. Here, we interrogated the impact of decreasing synaptic inputs onto mature dentate granule neurons on their competition for perforant path inputs with adult-born dentate granule neurons and also on neural stem cell activation. Using a novel genetic system by which we reversibly eliminate a subset of dendritic spines on mature dentate granule neurons, we found that adult-born dentate granule neuronal integration and activation of neural stem cells are bidirectionally sensitive to these alterations. We have harnessed this strategy to determine how rejuvenating the DG with expanded cohorts of adult-born dentate granule neurons impacts encoding and memory precision in adulthood and in aging. We have found that targeting adult hippocampal neurogenesis is sufficient to improve behavioral measures of memory precision. Furthermore, increasing adult neurogenesis leads to decreased overlap between context-dependent neuronal ensembles, suggesting that global remapping in the dentate gyrus may contribute to memory precision in adulthood and aging.

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Sensory and reward codes in olfactory cortex

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Quickly learning whether novel objects encountered in the environment should be sought or avoided can have life-or-death consequences. We have developed a behavioral task that requires mice to learn the valence (i.e. positive or negative association) of each odor among a panel of novel odors. Interestingly, mice learn to respond appropriately based on the assigned valence after experiencing only 5-10 one-second trials with each odor, given any arbitrary panel of odors and odor-valence associations. Once learned, mice correctly determine the valence of odors within a single (~100ms) sniff. To better understand the neural mechanisms underlying this rapid and flexible sensorimotor learning and decision making, we investigated the coding of odors versus valence at two key stages of the olfactory system. In particular, we recorded single units during behavior in two areas of the olfactory system that are near the top of the sensory processing hierarchy and have projections to cognitive and motor brain regions, namely the posterior piriform cortex and the olfactory tubercle. We find a transformation from an implicit to an explicit code for valence between these two brain areas.

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Cellular and molecular changes in adult NSCs allowing successful regeneration in the adult zebrafish brain

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In the adult zebrafish brain, adult neural stem cells (aNSCs) exist not only in a more wide-spread manner as compared to mammals, but also react to injury by regenerating neurons. Indeed, after stab wound injury the cellular architecture is restored, including additional neurogenesis and recruitment of new neurons to the injury site. As aNSCs are positioned close to the brain surface, the behavior of aNSCs in the intact brain and during regeneration of the injured brain can be examined non-invasively by live *in vivo* imaging.

We used repetitive imaging to follow single aNSCs in the intact and injured adult zebrafish telencephalon *in vivo* and found that neurons are generated by direct conversion of aNSCs into post-mitotic neurons, by asymmetric divisions or via intermediate progenitors amplifying the neuronal output. We also observed an imbalance of asymmetric and symmetric self-renewing divisions, depleting aNSCs over time. After brain injury, neuronal progenitors are recruited to the injury site and generate additional neurons. Our data suggest that several changes in neurogenesis contribute to the response to injury: fewer stem cells remain quiescent compared to the intact brain and a pathway is added by which symmetric NSC division generates 2 inter-mediate progenitors. This new type of division observed only after injury gives rise to a larger neuronal progeny, but rapidly depletes the aNSC pool.

The analysis of transcriptional changes in the aNSCs and their progeny revealed activation of the aryl-hydrocarbon receptor (AhR) signaling in a novel subset of aNSCs, characterized by the absence of PSA-NCAM expression in response to injury. Our data suggest that the activation of the AhR signaling is crucial for the change of the type of aNSC division and recruitment of aNSCs to the injury site, as observed also by live imaging. Comparing this pathway to a model of mammalian brain injury in mice, we discovered that the activation of AhR signaling in reactive astrocytes after stab wound injury in the cerebral cortex enhances their de-differentiation and gain of the aNSC characteristics, as measured in the neurosphere assay. Taken together, our data imply conserved pathways in radial glia and reactive astrocyte reaction in zebrafish and mice and suggest that the increase in neurogenesis after injury comes at the expense of depletion of aNSCs, perhaps drawing on quiescent NSCs. Therefore, the maintenance of aNSCs is a key factor for long-term regeneration.

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"Efferent" modulation in the mechanosensory lateral line of the larval zebrafish

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Fish and amphibians possess a mechanosensory lateral line system that enables them to sense water motion relative to their bodies. This information can be used to identify moving objects in their vicinity or water currents and therefore contributes to a variety of behaviors including schooling, prey capture, predator avoidance and rheotaxis. To accurately interpret mechanosensory input, animals must distinguish between externally generated (exafferent) vs. self-generated (reafferent) sensory inputs. To understand the neuronal circuits that underlie such stimulus discernment, we are studying the lateral line system of larval zebrafish as a model circuit. Using standard labeling techniques, we have traced circuit connectivity, finding both ascending and descending connections to the mechanosensors themselves. Additionally, functional imaging of circuit activity during locomotion has unraveled that a subset of descending neurons is activated during motion and inhibits the primary sensory stream. This is suggestive of an efference copy generation system, whereby motor areas inform sensory processing areas about impending movements such that the expected self-generated stimulation can be nullified or compensated for via subtraction.

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Efficient coding of binaural localization cues by monaural input adaptation

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Information about the location of high-frequency azimuthal sound sources is computed by neurons of the lateral superior olive (LSO) by detecting direction-specific differences in the intensity level at the two ears (interaural level differences, ILDs) that are generated by the head shadow. Specifically, LSO neurons integrate excitatory inputs from the ipsilateral side with inhibitory inputs from the contralateral side to gauge the relative sound levels at the two ears. However, it is known that under natural acoustic conditions the monaural inputs upstream to the LSO continually adapt in their dynamic range to match the intensity distribution of the concurrent stimulus statistics. Consequently, for large ILDs, the inputs to the LSO at the left and right ear will be adapted to drastically different intensity levels. Yet, it is unknown how the binaural computation and the coding of ILDs in LSO neurons is affected by the divergent input adaptations. We performed *in vivo* extracellular single-cell recordings in anesthetized Gerbils and used computer modelling to investigate the coding of ILDs in the LSO under different monaural adaptation levels. Broadband stimuli with similar bimodal intensity statistics but divergent absolute sound levels were presented to the two ears to mimic natural binaural acoustic conditions. We find that the divergent intensity-dependent monaural adaptation between ipsi- and contralateral inputs account for drastic dynamic range shifts in LSO neurons. Remarkably, these shifts resulted in near-optimal separability of nearby ILD in the range of ILDs that were most likely to occur. We conclude that sound intensity-dependent shifts of LSO inputs allow for the efficient coding and separation of sound source locations by the LSO under a wide range of natural conditions.

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Attenuation of age-associated increases in acetylation promotes *Drosophila* longevity

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Old age is associated with a progressive decline of mitochondrial function and changes in nuclear chromatin. However, little is known about how metabolic activity and epigenetic modifications change during midlife. I will present how cellular metabolism and protein acetylation change during the onset of aging in Drosophila. Midlife flies alter metabolic activity, increased oxygen and acetyl-CoA levels and display altered protein and histone acetylation levels. Targeting and lowering the activity of the acetyl-CoA synthesizing enzyme ATP citrate lyase (ATPCL) or of the histone H4K12 specific lysine acetyltransferase Chameau promote longevity. These findings reveal how increases in protein acetylation during midlife regulate lifespan and evoke alternative strategies to delay aging

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Enlightening the effects of artificial light on biological rhythms

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5 Visiting Professor at UFRGS/CAPES

Quilombos are settlements originally founded far away from civilisation by African slaves who were forced to work in Brazilian plantations (the Quilombolas). The remaining Quilombos have a wide range of electrification-status, from people that have no electricity or were only recently connected to the grid to communities living in modern conditions. Artificial light has profoundly changed human life in the last century: it has become quite common to be awake or work at night since people have become independent in their activities of natural daylight. Quilombolas are, therefore, a unique opportunity to study the historical transition in rest-activity behaviour from the pre-electricity era until today.

We are investigating rest-activity- and sleep-patterns in the Quilombolas' daily context, with wrist actimetry and the Munich ChronoType Questionnaire. By comparing people with different levels and histories of light exposure, we aim to unveil how human sleep changed with the possibilities brought by artificial light.

The data collected so far refer mostly to the Quilombolas living away from the cities. Preliminary analyses suggest that, similar to other rural residents, Quilombolas show earlier sleep patterns than the general population, as expected for people who rely more on natural light and work mostly outdoors. They are also less dependent on alarm clocks: less than a third report to use them on workdays, while in the industrialised world alarm-clock usage can exceed 80%. Similar to findings in a variety of populations, chronotype is age-dependent also in the Quilombos. Young people are later chronotypes and they also experience the highest levels of social jetlag.

Quilombos represent a contrast to the industrialised urban societies that work and are connected in a 24/7 mode. Studying this Brazilian population may help us to understand the impact of the modern lifestyles on health.

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The subcellular transcriptomes and proteomes that execute brain wiring

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The most striking feature of brain structure is arguably the complexity of wiring schemes with which neurons connect. The developmental mechanisms capable of forming such complex connections can be viewed as emergent from the interplay between multitudes of molecular and cell biological phenomena, spanning chromatin dynamics, gene expression, signaling, cytoskeletal rearrangements, and cell surface interactions, each of which is the subject of intense focused research. Aiming to understand how the interplay between these processes leads to the development of specific brain circuits, we established a new approach that can extract systems-level molecular information from native neuronal projections in the mouse brain. Combining *in vivo* labeling of neuron subtypes, biochemical fractionation, and subcellular flow cytometry with quantitative proteomics and transcriptomics, we were able to obtain comparative datasets of the subcellular RNA and protein networks that execute the formation of specific projections in the developing brain. We compare the native growth cone proteomes of distinct projection classes to identify the molecular effectors of specific circuit elements. We also examine the local transcriptomes and proteomes of growth cones compared to those of their parent cell bodies, and uncover hundreds of previously unidentified locally enriched coding and non-coding RNA species. These data exemplify the capacity of this new approach to be applied to various neuronal systems and mouse lines in order to reveal the underlying molecular signatures of distinct circuits, and for molecular phenotyping in models of neurological disease. It additionally provides unique paired datasets to address fundamental cell biology of projection neurons, regarding the extent and relationship of local translation, and protein trafficking mechanisms in the development, homeostasis, degeneration, and regeneration of brain circuits.

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Choroid plexus secreted microRNAs maintain neural stem cells by regulating neurogenic fate determinants

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Neurogenesis in the mammalian brain persists throughout life in specific regions, the subependymal zone (SEZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus, embedded in otherwise gliogenic environment. A genome-wide transcriptome analysis of prospectively isolated neural stem cells (NSCs) and their progeny revealed the mRNA and no protein for known, potent neurogenic factors already in the NSCs, but not in the bona fide astrocytes without the neurogenic potential residing in the diencephalon. These data, therefore, suggest a neurogenic priming of NSCs in the adult SEZ allowing them to generate neurons in otherwise gliogenic environment.
We identified two microRNAs involved the neurogenic priming and the maintenance of NSCs in their niche by regulating neurogenic transcription factors posttranscriptionally. Overexpression of both microRNAs in vitro lead to a reduction of neurons produced by NSCs, while inhibition *in vivo* lead to premature differentiation and a loss of quiescent NSCs. Furthermore we could identify the choroid plexus (CP) of the lateral ventricle as a source of one microRNA that would be transported via the cerebrospinal fluid (CSF) to the SEZ and would be taken up by the NSCs to regulate neurogenic priming. Taken together, our results suggest a novel mechanism to maintain the NSCs in their specific niche via secreted microRNAs that regulate neurogenic fate determinants in the NSCs.

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Homeostatic gain control gradually restores neural and perceptual sensitivity to sound following profound auditory nerve degeneration

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Whether as a consequence of acute trauma or normal aging, spiral ganglion neurons that form the auditory nerve will degenerate, decreasing the bandwidth of information that can be transmitted from the cochlea to the brain. Individuals with this type of hearing loss have difficulty processing temporal patterns that convey meaning in speech, while their hearing thresholds and basic sound detection remains relatively normal. The mechanisms linking neuropathy in the peripheral auditory system to genetics, aging and noise exposure have been studied extensively. However, the strategies employed by the brain to compensate for the diminished input remain unclear. Here, we propose that homeostatic plasticity enables higher levels of the central nervous system to partially compensate for a degraded input, thereby reinstating neural encoding and perceptual awareness of basic sound features.
We tested this hypothesis by measuring behavior and recording from units in the central nucleus of the inferior colliculus and primary auditory cortex of awake mice after ~95% of Type-I auditory nerve fibers were selectively eliminated. Thirty days after auditory nerve degeneration physiological and behavioral measures of cochlear and brainstem function indicated near-complete deafness. However, cortical unit responses and psychometric tone detection functions were nearly normal. We found that tone burst sensitivity had progressively recovered while temporal encoding of broadband chirp trains remained abnormal. Our data suggest that central nervous system's compensatory plasticity supports the recovery of rudimentary sound features that can be encoded by spike rate (tone detection), though complex sound features that are encoded by precise spike timing (e.g., pulse train synchronization) do not recover to the same degree. Our ongoing studies examine the dynamic interplay between interneurons and excitatory neurons that enables homeostatic adjustment in the face of extreme afferent loss.

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A rodent model of high-level vision

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The brain's visual system translates ambiguous and rapidly-changing patterns of light falling on the retina into a coherent representation of the external world that can be used to guide behavior. The hierarchical organization of sensory cortex is thought to play a key role in its ability to extract and learn about latent structure from visual inputs. However, the nature of transformations that occur from one level of the hierarchy to the next remain poorly understood.

Though rodents have not traditionally been used as models for high-level visual behaviors, such as object recognition, recent evidence suggests that rats possess surprisingly sophisticated visual abilities. Building on these findings, we have undertaken a systematic electrophysiological investigation of object-evoked responses in rat extrastriate cortex, finding that rat visual cortex exhibits signatures of hierarchical progression across areas similar to those observed in the primate visual hierarchy. Critically, we observe increasing visual object selectivity and tolerance to identity-preserving transformations as one progresses from early visual cortex (i.e. V1) to higher-level, extrastriate areas, such as areas LL and LI. To better understand how these higher visual areas represent visual information at the population level, we have begun characterizing object-evoked responses using wide-field and two-photon imaging. We aim to leverage the experimental advantages of this new model for high-level vision to ask previously intractable questions about the transformation of visual information across cortical populations, and how learning and experience shape this process.

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Individual neurons in visual cortex retain a memory of their inputs after monocular deprivation

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Sensory experience shapes structure and function of neuronal circuits during development and adulthood. However, how mature circuits achieve a stable representation of sensory input while maintaining the capacity to adapt to changes in the environment is unresolved. The classic model of experience-dependent cortical plasticity is monocular deprivation (MD), where temporary closure of one eye evokes a prominent shift of eye-specific responses in the visual cortex towards the non-deprived eye. In adult mice, re-opening the deprived eye reverts the shift in ocular dominance (OD) on the population level, even though both, the initial shift and recovery are accompanied by prominent structural and functional synaptic rearrangements. It is unclear if single neurons recover their initial tuning, or whether the original population OD is reestablished by compensatory changes in different cells, which would imply that plasticity acts on the population mean rather than single cell level. We follow the activity of the same excitatory layer 2/3 neurons in primary visual cortex of adult mice over months during multiple episodes of OD plasticity (MD, binocular recovery, and repeated MD) using ratiometric two-photon Ca^{2+} imaging (AAV1/2-mRuby2-P2A-GCaMP6s). We find that the change in population OD after MD is driven by an increase in open eye and a prominent decrease in deprived eye responsiveness. Surprisingly, most cells not only return to their pre-deprivation OD after recovery, but also the pre-MD microcircuit structure appears to have been faithfully restored as suggested by the analysis of pair-wise neuronal response correlations. When challenged with a second MD episode, cells that showed plasticity during the first MD largely also changed their OD for a second time, further emphasizing circuit stability after experience-dependent plasticity. Therefore, both receptive field properties and the underlying microcircuits are remarkably resilient to massive perturbations of sensory input, but individual neurons nevertheless maintain their capacity for experience-dependent changes.

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Local circuits and neural pathways linking adult hippocampal neurogenesis with mood

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The generation of adaptive fear responses to ambiguous threats in the environment is critically dependent on how contextual information is encoded. Inefficient encoding of ambiguous threats results in inappropriate retrieval of aversive memories and activation of fear circuits to produce heightened avoidance behavior, overgeneralization of fear, hyper vigilance and arousal, symptoms that characterize anxiety disorders such as post-traumatic stress disorder. Recent studies by us and others has found that adult-born neurons generated from neural stem cells in the hippocampus play a critical role in discrimination of ambiguous threats and modulating generalization of fear. One neural mechanism by which this is accomplished is pattern separation, a process by which interference between similar memories is minimized. However, despite these advances, the local circuit mechanisms by which adult-born neurons modulate pattern separation are poorly understood. Furthermore, how these encoding operations are linked with circuits subserving stress and fear responses are not known. I will discuss ongoing studies addressing these fundamental questions and how we have harnessed insights to re-engineer connectivity of memory circuits in adulthood and aging.

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A quantitative description of the metaphase spindle

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The metaphase spindle is composed of chromosomes, microtubules, and an unknown number of proteins. Spindle microtubules are very dynamic with an average lifetime of 17 seconds making faithful isolation of spindles extremely challenging. Using Xenopus cell free extracts we isolated metaphase spindles in less than 15 seconds without dilution or drug addition. We measured the composition of these spindles with quantitative multiplexed proteomics to precisely quantify each protein's enrichment on the spindle and determine the absolute number of molecules of each protein bound to DNA and microtubules. Additionally, we systematically measured the turnover of many spindle proteins by adding X. tropicalis extract to X. laevis spindles and following the exchange dynamics with multiplexed proteomics. Taken together, these measurements can quantitatively define the composition and turnover of the metaphase spindle for the first time.

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Distinct myelination patterns adjust conduction velocity and transmission delay of action potentials in auditory brainstem neurons in frequency-specific manner

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The timing of inputs in microcircuits plays a crucial role in information processing. The relative arrival time of inputs is largely determined by the relative conduction velocity of action potentials along individual axons, which is effectively shaped by the axon diameter and the internode length. Traditionally, it has been thought that axon diameter and internode length are uniformly correlated with each other. However, recent data from our lab has revealed that the ratio of internode length and axon diameter deviates significantly in globular bushy cell (GBC) axons tuned to low- and high frequencies. Nevertheless, the effect of this morphological specialization on the actual conduction velocity and the functional relevance remain obscure.

Here, we investigated the structure-function relationship of axon morphology, conduction velocity and synaptic delay in a frequency-specific manner. To this end, we measured the action potential conduction velocity in GBC axons *in vivo* by electrically stimulating GBC somata and simultaneously recording synaptic volleys extracellularly in the target region of GBCs, the medial nucleus of the trapezoid body (MNTB).

We find that the action potential propagation latency correlated with the characteristic frequency of the cell, as predicted by the morphological data. In particular, action potential conduction velocity was higher in low frequency GBC axons than in high frequency ones. Measuring the synaptic delay between the presynaptic GBC volley and the postsynaptic MNTB spike revealed that activity-dependent changes in synaptic delay increased as a function of axonal action potential propagation latency. Together, these findings suggest a close link between axonal morphology and the tuning of conduction velocity and synaptic transmission for frequency-specific processing of auditory stimuli.

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Proteomic clues to cell organization

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The composition of the nucleoplasm determines the behavior of key processes such as transcription, yet there is still no reliable and quantitative resource of nuclear proteins. Furthermore, it is still unclear how the distinct nuclear and cytoplasmic compositions are maintained. To describe the nuclear proteome quantitatively, we hand isolated the large nuclei of frog oocytes and measured the nucleocytoplasmic partitioning of ~9000 proteins by mass spectrometry. Most proteins localize entirely to either nucleus or cytoplasm, only ~17% partition equally. Native size but not polypeptide-molecular-weight is predictive of localization: partitioned proteins exhibit native size larger than 100 kDa. To evaluate the role of nuclear export in maintaining localization, we inhibited Exportin 1. This resulted in the expected relocation of proteins towards the nucleus, but only 3% of the proteome was affected. Thus, complex assembly and passive retention, not continuous active transport, is the dominant mechanism for the maintenance of distinct nuclear and cytoplasmic compositions.

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