LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

HARVARD UNIVERSITY

LMU–Harvard Young Scientists' Forum

From Molecules to Organisms IX

Munich, July 23 – July 27, 2017

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

- **LMU-Harvard Young Scientists' Forum at the Center for Advanced Studies (CAS[LMU]) and the LMU Biocenter:** From Molecules to Organisms, July 23 – July 27, 2017
- **Under the auspices of** Prof. Dr. Hans van Ess, Vice President for International Affairs, LMU
- **Program Management:** Dr. Anna Jakubowska (LMU International Office)
- **Participating academic units:** Center for Integrated Protein Science Munich (CIPSM), Munich Center for Neurosciences (MCN), Graduate School for Systemic Neurosciences (GSN)
- **Academic Management:** Prof. Dr. Oliver Behrend (MCN/GSN), Prof. Dr. Benedikt Grothe
- **Institutional Responsibility:** LMU International Office, Center for Advanced Studies (CAS[LMU]), LMU Biocenter

Conference Agenda

Sunday, July 23

	CAS, Seestraße 13, 80802 Munich
18:30 – 19:00	**Welcome reception** (Benedikt Grothe; Lena Bouman)
19:00 – open	**Lecture 1** – **Alexander Borst:** "How does direction selectivity emerge in fly motion detecting cells"
	– followed by snacks/drinks

Monday, July 24

	LMU Biocenter, Grosshadernerstr. 2, 82152 Martinsried, D00.003
08:15 – 09:00	Pre-arranged transfer hotel – Biocenter
09:15 – 09:30	**Welcome address** (Stefan Lauterbach; Head LMU International Office)
09:30 – 10:30	**Lecture 2** – **Nadine Gogolla:** "From circuits to behavior in the mouse insular cortex" (Intro: Steffen Katzner)
	Coffee break (catered; foyer D00.003)
11:00 – 13:00	**Session 1** – "Modulation of Neural Processing and Behaviour" **Vogt/Sayin/Odstrcil/Wotjak** (Chair: Steffen Katzner)
	Lunch break (catered; foyer)
14:30 – 16:30	**Session 2** – "Sensory Circuits" **Guggiana-Nilo/Aimable Naumann/Weiler/Myoga** (Chair: Anton Sirota)
	Coffee break (catered; foyer)
17:00 – 18:00	**Lecture 3** – **Benjamin de Bivort:** "Light-dependent modulation of individual behavior reveals converging streams of sensory information in the central brain" (Intro: Anton Sirota)
18:00 – open	*Neuro summer BBQ* (on lawn behind the LMU Biocenter)

Tuesday, July 25

	LMU Biocenter Martinsried, D00.003
08:15 – 09:00	Pre-arranged transfer hotel – Biocenter
09:00 – 10:00	**Lecture 4** – **Luk Vandenberghe:** "Gene therapy for neurosensory disorders: engineering delivery" (Intro: Stylianos Michalakis)
	Coffee break (catered; foyer)
10:30-12:00	**Session 3** – "Neuropathologies" **Schön/Franzmeier/Wells** (Chair: Stylianos Michalakis)
	Lunch break (catered; foyer) & YSF faculty meeting (faculty club)

14:00 – 15:30	**Session 4** – "Modulation of Neural Acivity" **Kapoor/Granger/Hammelmann** (Chair: Hans Straka)
	Coffee break (catered; foyer)
16:00 – 17:00	**Lecture 5** – **Laura Busse:** "A causal test of V1's role in visual awareness" (Intro: Hans Straka)
17:00 – open	At free disposal (student representative activities)

Wednesday, July 26

	LMU Biocenter Martinsried, D00.003
08:15 – 09:00	Pre-arranged transfer hotel – Biocenter
09:00 – 10:00	**Lecture 6** – **Zhigang He:** "From axon regeneration to functional recovery" (Intro: Florence Bareyre)
	Coffee break (catered; foyer)
10:30 – 12:30	**Session 5** – "Neurodevelopment and Regeneration" **Itoh/del Toro Ruiz/Camargo-Ortega/Kölsch** (Chair: Martin Kerschensteiner)
	Lunch break (catered) & YSF poster session (foyer)
	Asgharsharghi/Franzmeier/Genewsky/Hatch/Jacobi/Leonhardt/ Njavro/Sarin/Smith/Weiler/Weili/Wells/Yonar
14:30 – 16:00	**Session 6** – "Memory and Motor Systems" **Karalis/Yonar/Mearns** (Chair: Christian Leibold)
16:00 – 17:00	**Lecture 7** – **Anton Sirota:** "Network and behavioral dynamics of sensory integration in the rodent hippocampal system" (Intro: Christian Leibold)
17:00	**Closing remarks**
17:15	Pre-arranged transfer Biocenter – hotel / individually: beergarden

Thursday, July 27

	Hotel Carlton Astoria, Fürstenstr. 12, 80333 Munich
09:00 – 19:00	Pick-up, excursion to Castle Neuschwanstein

Friday, July 28

Departure, individually arranged

Participants*

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

Harvard University Delegation

- **Eva Aimable Naumann**, Postdoctoral Fellow, Department of Molcular and Cellular Biology, Laboratory of Florian Engert
- **Benjamin de Bivort**, Professor, Harvard Center for Brain Science, Department of Molecular and Cellular Biology
- **Adam Granger,** Postdoctoral Fellow, Harvard Medical School, Department of Neurobiology, Laboratory of Bernardo Sabatini
- **Drago Guggiana-Nilo**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Florian Engert
- **John Hatch**, PhD Student, Department of Stem Cell and Regenerative Biology, Laboratory of Jeffrey Macklis
- **Zhigang He**, Professor, Harvard Medical School, F.M. Kirby Neurobiology Center, Boston Children's Hospital
- **Yasuhiro Itoh**, Postdoctoral Fellow, Department of Stem Cell and Regenerative Biology, Laboratory of Jeffrey Macklis
- **Anne Jacobi**, Postdoctoral Fellow, Harvard Medical School, Boston Children's Hospital, Department of Neurology, Laboratory of Zhigang He
- **Vikrant Kapoor**, Postdoctoral Fellow, Harvard Center for Brain Science, Department of Molecular and Cellular Biology, Laboratory of Venkatesh Murthy
- **Iris Odstrcil**, PhD Student, Department of Molcular and Cellular Biology, Laboratory of Florian Engert
- **Sumeet Sarin**, Postdoctoral Fellow, Harvard Center for Brain Science, Department of Molecular and Cellular Biology, Laboratory of Joshua Sanes
- **Matthew Smith**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Benjamin de Bivort
- **Luk H. Vandenberghe**, PI, Department of Ophthalmology, Director of Grousbeck Gene Therapy Center, Ocular Genomics Institute
- **Katrin Vogt**, Postdoctoral Fellow, Harvard Center for Brain Science, Department of Physics, Laboratory of Aravinthan Samuel
- **Michael Wells**, Postdoctoral Fellow, Department of Molecular and Cellular Biology, Laboratory of Kevin Eggan
- **Abdullah Yonar**, PhD Student, Department of Molecular and Cellular Biology, Laboratory of Sharad Ramanathan

Harvard University Nominating Faculty

- **Kenneth Blum**, Executive Director, Harvard Center for Brain Science
- **Benjamin de Bivort**, Professor, Harvard Center for Brain Science, Department of Molecular and Cellular Biology
- **Kevin Eggan**, Professor, Harvard Center for Brain Science, Department of Stem Cell and Regenerative Biology
- **Florian Engert**, Professor, Harvard Center for Brain Science, Department of Molecular and Cellular Biology
- **Zhigang He**, Professor, Harvard Medical School, Boston Children's Hospital
- **Jeffrey Macklis**, Professor, Harvard Center for Brain Science, Department of Stem Cell and Regenerative Biology
- **Venkatesh Murthy**, Professor, Harvard Center for Brain Science, Department of Molecular and Cellular Biology ,
- **Sharad Ramanathan**, Professor, Harvard Center for Brain Science, Department of Molecular and Cellular Biology
- **Bernardo Sabatini**, Professor, Harvard Medical School, Department of Neurobiology
- **Aravinthan Samuel**, Professor, Harvard Center for Brain Science, Department of Physics
- **Joshua Sanes**, Professor, Harvard Center for Brain Science, 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)
Max Planck Institute of Psychiatry (MPIPsy)
Technische Universität München (TUM)
Delegation

- **Amir Asgharsharghi**, PhD Student, HMGU, Laboratory of Hernán López-Schier
- **Herwig Baier**, Professor, MPIN, Department Genes – Circuits – Behavior
- **Florence Bayreyre**, PI, LMU, Institute of Clinical Neuroimmunology
- **Oliver Behrend**, Managing Director, LMU, Munich Center for Neurosciences (MCN)
- **Martin Biel**, Professor, LMU, Department of Pharmacy, Center for Integrated Protein Science Munich (CIPSM)
- **Tobias Bonhoeffer**, Professor, MPIN, Department Synapses – Circuits – Plasticity
- **Alexander Borst**, Professor, MPIN, Department Circuits – Computation – Models

- **Lena Bouman**, Academic Coordinator (Natural Sciences and Medicine), LMU, Center for Advanced Studies (CASLMU)
- **Laura Busse**, Professor, LMU, Department Biology II, Division of Neurobiology
- **Germán Carmargo Ortega**, PhD Student, HMGU, Institute of Stem Cell Research; LMU, Department of Physiological Genomics, Laboratory of Magdalena Götz
- **Daniel del Toro Ruiz**, Postdoctoral Fellow, MPIN, Department Molecules - Signaling - Development, Laboratory of Rüdiger Klein
- **Michael Ewers**, Professor, LMU, Institute for Stroke and Dementia Research (ISD)
- **Nikolai Franzmeier**, PhD Student, LMU, Institute for Stroke and Dementia Research (ISD), Laboratory of Michael Ewers
- **Andreas Genewsky**, PhD Student, MPIPsy, Department Stress Neurobiology and Neurogenetics, Laboratory of Carsten Wotjak
- **Nadine Gogolla**, PI, MPIN, Research Group Circuits for Emotion
- **Magdalena Götz**, Professor, LMU, Department of Physiological Genomics, HMGU
- **Benedikt Grothe**, Professor, LMU, Department Biology II, Munich Center for Neurosciences (MCN), Graduate School of Systemic Neurosciences (GSN)
- **Ilona Grunwald Kadow**, Professor, TUM, Munich Center for Neurosciences (MCN)
- **Verena Hammelmann**, Postdoctoral Fellow, LMU, Department of Pharmacy, Center for Drug Research, Laboratory of Martin Biel
- **Anna Jakubowska**, Project Manager, LMU International Office
- **Nikolas Karalis**, PhD Student, LMU Department Biology II, Laboratory of Anton Sirota
- **Steffen Katzner**, PI, LMU, Department Biology II, Division of Neurobiology
- **Martin Kerschensteiner**, Professor, LMU, Institute of Clinical Neuroimmunology
- **Yvonne Kölsch**, PhD Student, MPIN, Department Genes – Circuits – Behavior Laboratory of Herwig Baier
- **Stefan Lauterbach**, Head of LMU International Office
- **Christian Leibold**, Professor, LMU, Department Biology II, Computational Neuroscience
- **Aljoscha Leonhardt**, PhD Student, MPIN, Department of Circuits – Computation – Models, Laboratory of Alexander Borst
- **Stefan Lichtenthaler**, Professor, TUM, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE)
- **Hernán López-Schier**, PI, HMGU
- **Duncan Mearns**, PhD Student, MPIN, Department Genes – Circuits – Behavior, Laboratory of Herwig Baier
- **Stylianos Michalakis**, PI, LMU, Department of Pharmacy, Center for Integrated Protein Science Munich (CIPSM)
- **Mike Myoga**, Postdoctoral Fellow, LMU, Department Biology II, Laboratory of Benedikt Grothe
- **Jasenka Njavro**, PhD Student, German Center for Neurodegenerative Diseases, Laboratory of Stefan Lichtenthaler
- **Sercan Sayin**, PhD Student, TUM, School of Life Sciences, Laboratory of Ilona Grunwald Kadow

- **Christian Schön**, Postdoctoral Fellow, LMU, Department of Pharmacy, Laboratory of Stylianos Michalakis
- **Anton Sirota**, Professor, LMU, Department Biology II, Bernstein Center for Computational Neuroscience (BCCN)
- **Hans Straka**, Professor, LMU, Department Biology II, Division of Neurobiology
- **Simon Weiler**, PhD Student, MPIN, Department Synapses – Circuits – Plasticity, Laboratory of Tobias Bonhoeffer
- **Tian Weili**, PhD Student, HMGU, Laboratory of Hernán López-Schier
- **Carsten Wotjak**, PI, MPIPsy, Department Stress Neurobiology and Neurogenetics

LMU Munich Nominating Faculty

- **Herwig Baier**, Professor, MPIN, Department Genes – Circuits – Behavior
- **Oliver Baron**, Managing Director, LMU, Center for Integrated Protein Science Munich (CIPSM)
- **Martin Biel**, Professor, LMU, Department of Pharmacy, Center for Integrated Protein Science Munich (CIPSM)
- **Tobias Bonhoeffer**, Professor, MPIN, Department Synapses – Circuits – Plasticity
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- **Michael Ewers**, Professor, LMU, Institute for Stroke and Dementia Research (ISD)
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- **Ilona Grunwald Kadow**, Professor, TUM, Munich Center for Neurosciences (MCN)
- **Rüdiger Klein**, Professor, MPIN, Department: Molecules – Signaling – Development
- **Stefan Lichtenthaler**, Professor, TUM, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE)
- **Hernán López-Schier**, PI, HMGU, Research Unit Sensory Biology and Organogenesis
- **Stylianos Michalakis**, PI, LMU, Department of Pharmacy, Center for Integrated Protein Science Munich (CIPSM)
- **Anton Sirota**, Professor, LMU, Department Biology II, Cognition and Neural Plasticity
- **Carsten Wotjak**, PI, MPIPsy, Department Stress Neurobiology and Neurogenetics

Notes ***

Abstracts of lecturers and posters

Brain-scale neural circuits for visual motion processing in zebrafish

Eva Aimable Naumann
Harvard Department of Molecular and Cellular Biology

The larval zebrafish presents an exciting opportunity to investigate the neural basis of vertebrate behavior at the brain scale. However, it has been particularly difficult to distill neural circuits from whole-brain measurements of neural activity. By combining detailed psychophysics, anatomy, cellular resolution whole-brain imaging, and circuit perturbations, we establish critical links between brain- and circuit-level descriptions of the zebrafish optomotor response. Specifically, we find diverse neural response types distributed across multiple brain regions and show that to transform visual motion into action, these regions sequentially integrate eye- and direction-specific sensory streams, refine representations via interhemispheric inhibition, and demix locomotor instructions into distinct motor modules. Ultimately, we develop a quantitative whole-brain model that explains the behavior and reduces the space of possible synaptic connections into a few critical dimensions of functional connectivity among identified neural response types. More generally, our methodology illustrates a flexible paradigm for studying diverse brain-scale computations related to individuality, learning, and motivational states. DR6 shedding negatively regulates myelination in PNS

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Rheotaxis behavior in larval zebrafish

Amir Asgharsharghi[1,3], Gema Valera[1], Jean-Pierre Baudoin[2] and Hernán López-Schier[1,2]
[1] **HMGU Research Unit Sensory Biology & Organogenesis**
[2] **CRG-PRBB, Laboratory of Sensory Cell Biology, Barcelona**
[3] **TUM School of Life Sciences**

For animals that can be displaced by currents, visual cues are the most salient sensory percepts to evaluate self-motion relative to an external reference frame. Yet, in the absence of visual information fishes can orient to the direction of water currents via an innate behavior called rheotaxis. Here we use behavioral data from controlled environmental modifications to show that larval zebrafish exploit mechanical inhomogeneities across the horizontal plane to determine water-flow direction. We use a predictive model to guide experimental manipulations, which reveal that orientation to flow is sensitive to unilateral ablations of lateral-line inputs. Remarkably, regenerating lateralis axons always re-establish synapses with hair cells of the original polarity but frequently fail to re-innervate their original receptor, highlighting pressure to preserve directional tuning as opposed to fine-grained topographic mapping. Accordingly, somatotopic rewiring does not disrupt rheotaxis. Our findings indicate that rheotaxis involves at least three processes: vectorial fractionation of mechanical signals, sensory updating by recurrent yaw rotations, and integration of bilateral sensory information.

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How does direction selectivity emerge in fly motion detecting cells?

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

In the fly visual system, the local direction of motion is computed in two parallel ON and OFF pathways. Within each pathway, a retinotopic array of four direction-selective T4 (ON) and T5 (OFF) cells represents the four Cartesian components of local motion vectors. Since none of the presynaptic neurons as identified by EM analysis turned out to be directionally selective, direction selectivity emerges within T4 and T5 cells. Now the question is: How?
Preferred direction enhancement and null direction suppression have been discussed as two alternative algorithms that both lead to a direction-selective output. Surprisingly, we found both mechanisms at work in T4 cells implemented in different areas within their receptive field. Algorithmically, this can be realized by a three-input model where the output of a multiplicative interaction is divided by a third, spatially displaced input. We recently characterized the spatio-temporal receptive field properties of all T4 and T5 input neurons. Mapping specific cell-types to specific input lines of the three-input detector allowed us to construct T4 and T5 cell models with a degree of direction selectivity and temporal tuning properties that closely match the ones of their natural counterparts. At present, we test these models by blocking individual cell types while recording the responses of T4 and T5 cells.

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A causal test of V1's role in visual awareness

Laura Busse
LMU Department Biology II, Division of Neurobiology

The primary visual cortex (V1) is the main source of visual input to downstream cortical areas; it is a long-standing question, however, whether V1 activity is critical for conscious visual perception. Previous studies have exploited visual illusions that render stimuli invisible, such as binocular rivalry or backward masking, but have provided conflicting results. Moreover, in these paradigms, the role of a candidate area in visual perception was often based on correlations between its neural activity and subjective reports of visibility. Mere correlations, however, may reflect spurious relationships, and ultimate answers about an area's contribution to perception will require methods demonstrating causality. Here we provide a causal test of V1's role in perception by porting the backward masking paradigm to the mouse model, where we combine behavior, electrophysiology, and optogenetic manipulations of neural activity.
We first demonstrate that the behavioral signatures of visual backward masking known from humans and non-human primates are also present in the mouse. We then characterize a pro-longed response component of V1 neurons, which is indeed correlated with the mouse's report of visibility. Despite this correlation, we find that this prolonged V1 response component is not causally linked to perception, because temporally precise suppression of it leaves behavioral performance fully intact. We conclude that V1 functions as an input source of visual information to later areas, but is not part of the circuitry critical for visual perception.

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The novel centrosomal protein Akna, a regulator of microtubule organizing activity, controls neurogenesis

Germán Camargo Ortega[1,2*], Pia A Johansson[1,2*], Sven Falk[1,2], Stanislav Vinopal[3], Kalina Draganova[1,2], Kaviya Chinnappa[1,8], Anna Gavranovic[1], Juliane Merl-Pham[4], Arie Geerlof[5], Regina Feederle[6,7], Camino De Juan Romero[8], Stefanie M. Hauck[4], Victor Borrell[8], Frank Bradke[3], Michaela Wilsch-Bräuninger[9], Wieland Huttner[9] and Magdalena Götz[1,2,7]

*** equal contribution**

[1] HMGU Institute of Stem Cell Research

[2] LMU Biomedical Center, Physiological Genomics

[3] German Center for Neurodegenerative Diseases

[4] HMGU Research Unit Protein Science

[5] HMGU Protein Expression and Purification Facility, Institute of Structural Biology

[6] HMGU Core Facility Monoclonal Antibody Development

[7] SYNERGY Excellence Cluster of Systems Neurology, Biomedical Center

[8] Instituto de Neurociencias, Universidad Miguel Hernández

[9] MPI of Molecular Cell Biology and Genetics

Understanding mechanisms regulating neural stem cell (NSC) homeostasis and their fate commitment is fundamental for their efficient manipulation and future usage in regenerative medicine. To contribute to this goal, our laboratory interrogates novel neurogenic molecular pathways by identifying common regulators in neural stem cells of the developing and adult brain (Pinto et al., 2008, Mol Cell Neurosci; Beckervordersandforth et al., 2010, Cell Stem Cell). These studies have proved successful for the identification of new factors essential for embryonic and adult neurogenesis (Pinto et al., 2009, Nat Neurosci; Stahl et al., 2013, Cell) and key in direct neuronal reprogramming (Masserdotti et al., 2015, Cell Stem Cell). Here we show the functional and molecular analysis of another novel factor common to embryonic and adult neurogenesis, called Akna. We demonstrate that Akna, previously annotated as AT-hook transcription factor, is rather a centrosomal protein located predominantly at the subdistal appendages regulating microtubule nucleation and anchoring. Akna expression is higher in differentiating NSCs and their progeny, the basal progenitors, than self-renewing NSCs and mature neurons. Accordingly, in vivo knock-down and overexpression experiments combined with live imaging demonstrate that reducing Akna abolishes delamination, while its elevation results in very fast delamination and differentiation. Akna overexpression also retains cells at the place of highest expression, in the subventricular zone (SVZ), showing that it is essential for young neurons leaving the SVZ to migrate into the neuronal layers. Delamination of RGCs (the NSCs) from their apical anchoring to become basal RGCs (bRGCs) is crucial for the generation of the extended outer SVZ, a unique germinal layer present in brains with expanded cerebral cortex size and spatially correlated with the stereotypic formation of cortical folds (Martínez-Martinez et al., 2016, Nat Commun; Taverna et al., 2014, Annu Rev Cell Dev Biol). Accordingly, we found highest Akna mRNA levels in the VZ of ferret at the critical time window for RGC delamination into bRGC and OSVZ formation. In support of Akna being relevant for human cerebral cortex expansion and folding, human Akna protein is also localized at the centrosome and Akna mRNA is expressed in the human fetal forebrain in a modular pattern along the VZ, similar to other genes involved in gyrification (de Juan Romero et al., 2015, EMBO J). At a functional level, Akna overexpression in human cerebral organoids mediated delamination of NSCs. Thus, the central role of Akna in NSC biology and cortical development seems to be conserved between mouse, ferret and human. We identified a novel centrosomal protein with heterogeneous expression in stem cell subtypes mediating the epithelial-mesenchymal transition (EMT)-like process of NSC delamination and differentiation in neurogenesis.

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Light-dependent modulation of individual behavior reveals converging streams of sensory information in the central brain

Benjamin de Bivort
Harvard Department of Molecular and Cellular Biology

Variability across populations of animals is often considered to be biological or experimental noise. However, it is clear that individuals display considerable idiosyncrasies in behavior, beyond what would be expected to be generated simply by sampling error. Individual Drosophila, locomoting in simple Y-shaped mazes display individual biases in their preference to turn in one direction or the other as they pass through the center point. Populations of flies display a unimodal distribution of individual locomotor biases, centered around choosing randomly, that are greatly over-dispersed compared to the expected distributions driven purely by sampling error. These biases are generally stable over the short and long time scales and could be generated by individual genetics, environmental history or stochastic developmental noise. However, I have recently found individual biases to be context dependent. When these flies are exposed to whole field illumination with white light, their locomotor turning bias can dramatically shift. These changes in bias can be easily reversed by turning the illumination off. The fly can toggle between these light-dependent and light-independent locomotor biases continuously for any experimental time. From initial perturbation studies with a collection of Gal4 and split-Gal4 driver lines, these transitions are modulated by the microcircuitry of the protocerebral bridge and are mediated by the early visual circuits. The light-dependent differences in behavioral bias appear to be present in distinct streams of information into the protocerebral bridge. Connections from the ellipsoid body convey visual information imparting the light-bias, while connections from the posterior slope likely convey self-motion information imparting the dark-bias. This hints at general principles by which context-dependent behavior may be governed at the level of individual animals.

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Regulation of cerebral cortex folding by controlling neuronal migration via FLRT adhesion molecules

Daniel del Toro Ruiz[1], Tobias Ruff[1], Erik Cederfjäll[1], Ana Villalba[2], Gönül Seyit-Bremer[1], Víctor Borrell[2] and Rüdiger Klein[1]
[1] **MPI of Neurobiology, Department Molecules – Signaling – Development**
[2] **Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas & Universidad Miguel Hernández, Sant Joan d'Alacant, Spain**

The folding of the mammalian cerebral cortex into sulci and gyri is thought to be favored by the amplification of basal progenitor cells and their tangential migration. Here we provide a molecular mechanism for the role of migration in this process by showing that changes in intercellular adhesion of migrating cortical neurons result in cortical folding. Mice with d eletions of FLRT1 and FLRT3 adhesion molecules develop macroscopic sulci with preserved layered organization and radial glial morphology. Cortex folding in these mutants does not require progenitor cell amplification, but is dependent on changes in neuron migration. Analyses and simulations suggest that sulci formation in the absence of FLRT1/3 results from reduced intercellular adhesion, increased neuron migration and clustering in the cortical plate. Notably, FLRT1/3 expression is low in the human cortex and in future sulcus areas of ferrets, suggesting that intercellular adhesion is a key regulator of cortical folding across species.

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Towards a neural understanding of cognitive reserve in Alzheimer's disease

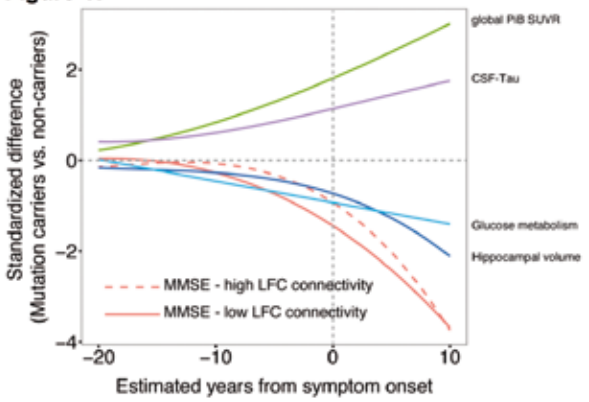
Nicolai Franzmeier
LMU Institute for Stroke and Dementia Research

Alzheimer's disease (AD) is characterized by progressive neurodegeneration and ensuing cognitive impairment. The susceptibility of cognition to the impact of brain pathology, however, can vary substantially between subjects (Jack et al., 2013). Post-mortem brain studies reported the surprising finding that subjects who fulfilled the neuropathological criteria of AD based on amyloid and tau pathology were ante-mortem cognitively normal (Katzman et al., 1989). The theory of reserve proposes that such a discrepancy between the level of pathology and cognition is not due to incomplete characterization of the pathology, but due to varying levels of resilience against the impact of pathology on cognition. An important question is which brain changes may underlie such reserve capacity. If we knew the brain mechanism that underlies reserve, therapeutic interventions to target and enhance such protective brain mechanisms could be developed to slow down or halt the development of AD dementia.

Epidemiological studies revealed that specific intellectual and life-style factors such as greater education, IQ or occupational attainment, are associated with higher reserve. Such protective factors have been associated with a variety of functional brain changes in AD (Barulli & Stern, 2013). Yet, no consistent pattern of functional brain changes has been identified previously. We recently developed a novel hypothesis-driven approach to identify functional brain mechanisms underlying reserve. We stipulated that any putative brain mechanism must be 1) related to higher levels of protective factors (e.g. education) that promote reserve, and 2) associated with attenuated effects of AD pathology on cognition. We focused on functional hubs in the brain, i.e. brain regions that are connected to many other brain regions and are thus of high importance for ensuring efficient information flow in the brain (Buckner et al., 2009). In particular, we focused on a hub in the left frontal cortex (LFC; Brodmann area 6/44), that was previously shown to be highly associated with mental capacity, cognitive flexibility and to be relatively spared in AD (Buckner et al., 2009; Cole, Yarkoni, Repovs, Anticevic, & Braver, 2012). In a resting-state functional MRI study in subjects with mild AD, we showed that the global connectivity of the LFC hub was associated with 1) protective factors including greater years of education or higher IQ and 2) milder impact of parietal FDG-PET hypometabolism, i.e. a major pathological hallmark of AD, on memory performance (Franzmeier et al., 2017). We recently replicated these findings based on memory-task fMRI assessed LFC connectivity in an independent sample of subjects with mild cognitive impairment (MCI) (Franzmeier et al., in press). These findings provide cross-validated support for LFC connectivity as a functional substrate of reserve at the stage of mild AD.

However, is higher LFC connectivity is associated with higher reserve already decades before the onset of dementia and thus could significantly delay the onset of dementia? In order to address this question, we studied LFC connectivity in subjects with autosomal dominant AD (ADAD), in whom the age of dementia onset is genetically determined, hence the current disease stage, even if years away from dementia onset, can be well estimated based on a subject's age. In collaboration with the multicenter Dominant Inherited Alzheimer's Network (DIAN) in the USA and Europe (Bateman et al., 2012), we obtained cross-sectional resting-state fMRI and cognitive data (MMSE, delayed memory recall) in ADAD (74 mutation carriers and 55 non-mutation controls). Polynomial regression analysis showed that the trajectory of cognitive decrease against disease stage was pushed back by several years in individuals with high global LFC connectivity, suggesting that in ADAD subjects with higher LFC connectivity a decline in cognition was delayed during AD progression years before the onset of dementia (Figure 1) (Franzmeier et al., submitted). We could confirm this result pattern in a sample of subjects with sporadic AD (i.e. 75 amyloid positive participants), where greater levels of global LFC connectivity were associated with attenuated decreases in global cognition (as measured by MMSE) and delayed recall. Together, these results suggest that global LFC connectivity contributes to reserve, i.e. a delay in the development of cognitive deficits over the course of AD. In future studies, we will aim to test the therapeutic effect of enhancing LFC connectivity by non-invasive transcranial magnetic resonance imaging or cognitive training to enhance functional reserve mechanism in AD.

Figure 1:



Cognitive and biomarker changes as a function of AD progression. MMSE (a measure of global cognitive ability) is plotted separately for individuals with high vs. low global LFC-connectivity. We plotted the standardized difference between mutation carriers and non-carriers against the estimated years from symptom onset based on the polynomial regression models. The plot suggests that high global LFC-connectivity is associated with a delay of cognitive decline towards a later timepoint with more progressed levels of AD pathology (from Franzmeier et al, submitted).

References:

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Midbrain structures control the expression of extremes in anxiety

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Background: Animal models and testing situations for the study of anxiety-like behavior often utilize a mouse's innate avoidance of brightly illuminated areas. One classical example of such a task is the elevated plus-maze (EPM). The selective breeding for extreme behavior on the EPM, resulted in two mouse lines namely high-anxiety behaving (HAB) and low-anxiety behaving (LAB) mice. We were interested whether the extremes in trait-anxiety in these animals are accompanied by altered innate fear responses. Therefore we developed two novel, multi-sensory behavioral tasks, which assay repeated, innate escape behavior towards an impeding threatening stimulus.
Results: Using these two novel tests, we could demonstrate (a) that HAB animals exhibit exaggerated fear responses, which are reflected by a strong but maladaptive escape behavior and an extreme disposition to vocalize, and (b) that LAB animals show a profound deficit in escaping impeding threats. We further discovered that LAB animals suffer from complete retinal blindness, which however, can only in part explain their fear response deficiency. Using manganese-enhanced magnetic resonance imaging (MEMRI) we have further identified an increased activation of the periaqueductal gray (PAG) in HAB mice, as well as a decreased activation of the superior collicullus (SC) in LAB mice, two important midbrain areas mediating fear responses. In order to test whether these two brain areas are involved in the generation of both extreme phenotypes, we applied either local injections of the potent GABAA-agonist muscimol within the PAG of HAB animals, or the pharmacogenetic activation using the *designer receptor exclusively activated by designer drugs* (DREADD) hM3Dq in the SC of LAB. Thereby we could successfully normalize the extreme behavior of both strains.
Conclusion: In this study we have demonstrated the applicability of tow novel ethiologically inspired innate fear assays (Robocat & IndyMaze). Further we have shown that midbrain structures govern the expression of anxiety-like behavior in two strains of mice, which were selectively bred for extremes in trait-anxiety. These findings emphasize the importance of primordial midbrain '*emotion*' centers, in the generation of affect, suggesting a role which is beyond mere behavioral output.

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From circuits to behavior in the mouse insular cortex

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Our behavior is governed by basic emotions and internal states. The insular cortex is an intricate part of a wider neuronal network orchestrating emotions and feelings into complex decisions. Despite compiling evidence from human imaging studies implicating the insular cortex in emotion regulation, the neuronal circuit mechanisms underlying its role in mediating affective behavior are currently unknown.
In my talk, I will introduce some ongoing work in the lab aimed at exploring how functional microcircuits within the insular cortex are embedded in a wider network of prefrontal, sensory and limbic brain structures. I will give insights into the approaches we are taking combining modern viral tracing tools in conjunction with projection- and cell-type specific optogenetics as well as functional in vivo imaging in behaving animals to dissect neuronal circuits implicated in emotion processing from their precise structure to function.

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Cortical disinhibition by cholinergic interneurons

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Acetylcholine is a major neuromodulator in the brain, important for maintaining alertness, directing attention, promoting learning, and detecting salient sensory cues. The net effect of acetylcholine transmission on downstream neurons is excitation, indicating that acetylcholine increases cortical activity. However, we have recently demonstrated that all cholinergic neurons have the potential to package and release the inhibitory neurotransmitter GABA, fundamentally altering our view of how acetylcholine-releasing neurons influence cortical circuits. In particular, very little is known about how a local population of cortical interneurons that express choline acetyltransferase (ChAT), a marker for cholinergic identity, influences downstream cortical neurons. We find that these interneurons are a subset of previously described class of interneurons marked by expression of vasoactive intestinal peptide (VIP), and that activation of these neurons results in GABA release onto all other classes of inhibitory cortical interneurons, providing a powerful disinhibitory signal to the cortex. Though we have yet to identify a definitive physiological effect of acetylcholine release, we have confirmed that all of the necessary cellular machinery to produce and release acetylcholine is expressed by these neurons and present in their presynaptic terminals. We are continuing our search for a physiological role for this putative acetylcholine release, and hypothesize that it acts in concert with disinhibition provided by GABA to promote cortical processing.

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Colors underwater: a study on color vision circuitry in the larval zebrafish

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Color vision is a highly adaptive and widespread visual modality. In its broadest definition, any visual system capable of extracting information from the wavelength of light is using color vision. There is a large variety of visual systems complying with this definition in nature, spanning the range from basic wavelength recognition in Daphnia to the use of color categorization in humans. Despite this ubiquity, there is a scarcity of studies that probe large populations of neurons from a color vision perspective, especially when it is the circuitry following the cones in the eye that truly determines the capabilities of a visual system in terms of color. The larval zebrafish, Danio rerio, is an attractive vertebrate model organism for studying color vision circuitry, given its tetra-chromatic retina combined with its suitability for functional calcium imaging of cell populations. In this study, we showed the larva a battery of colored visual stimuli, and recorded neural responses either at the interface between the Retinal Ganglion Cells (RGCs) coming from the eye, or their targets in the Optic Tectum (OT) of the fish, two of the most relevant visual areas in this organism. We find differential responses to a wide range of colors and spatiotemporal patterns. Overall, the whole system was found to be more sensitive to lower wavelengths, and the inferred connection patterns seem to be stereotypic. In terms of neural encoding, the circuit has the capability of discerning between responses at the single terminal level. Additionally, we used a machine learning approach to probe the population responses, and we show that color can also be decoded from these ensembles of responses. In summary, we find evidence that supports a functional color vision system in the larval zebrafish, both in terms of spectral sensitivity and neural encoding, and we establish its value as a model organism for color vision research.

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Cyclic AMP-dependent modulation of HCN2 controls thalamocortical activity

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels pass a current, termed Ih, that is found throughout the nervous system. The basic properties of these channels have been investigated in quite some detail. By contrast, much less is known about the physiological implications of the regulation of Ih by the cAMP system. Using a HCN2 mouse model deficient for cAMP binding (HCN2 EA) we examined this aspect of HCN channel modulation in different thalamic nuclei. We found that loss of cAMP modulation has profound effects on the firing modes of thalamocortical (TC) neurons in the ventrobasal (VB) and the dorsolateral geniculate nucleus (dLGN). Specifically, TC neurons expressing the HCN2 EA channel were profoundly impaired with regard to the transition from burst to tonic firing. On the whole animal level the changes in thalamic firing led to the generation of spike-and-wave discharges (SWDs) in the EEG and caused specific learning deficits in a visual discrimination task. Local knockdown of HCN2 in the VB region also induced SWDs supporting a key role of HCN2 in the control of network activity in this particular thalamic nucleus. Taken together, our data indicate that cAMP-dependent modulation of HCN2 is crucial for normal thalamic information processing.

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Subcellular localization of growth cone molecular machinery in subtype-specific cortical circuit development

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During development, growth cones (GCs) of diverse cortical or other projection neuron (PN) subtypes navigate complex extracellular environments to reach distant, subtype-specific targets. These axon-terminal structures must respond to substrate-bound and diffusible signals in a subtype- and stage/context-specific fashion to construct specific functional circuitry. Recent studies strongly indicate that subcellular localization of specific molecular machinery to GCs might underlie the precise behaviors of these structures during circuit formation. While great progress has been made toward identifying diffusible and substrate-bound signals that guide axon growth, it is becoming increasingly clear that intracellular, local growth cone biology underlies the distinct responses of specific neuronal subtypes at specific stages in specific contexts. Molecular determinants of these critical processes remain largely unstudied with respect to distinct neuronal subtypes under physiological conditions. Our laboratory has recently developed an innovative approach that enables high-throughput proteomic and transcriptomic investigation of GCs from fluorescently labeled cortical PN. This strategy combines molecular, genetic, and biochemical methods to isolate pure, subtype-specific populations of GCs directly from the developing brain. We demonstrate that the approach yields GCs with intact membranes that are amenable to fluorescence-activated small particle sorting and are free from significant contamination from somatodendritic components. Further, parallel isolation of labeled GCs and their anatomically distant parent somata has identified hundreds of transcripts specifically enriched in the GCs of developing callosal PN. Because most current knowledge of growth cone biology was identified in vitro, often with heterogeneous populations, access to subtype-specific growth cones in their native environment during normal development will substantially elucidate molecular bases of cortical and other neural circuit formation.

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From axon regeneration to functional recovery

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Injured axons do not regenerate spontaneously, accounting permanent functional deficits after injury. In analyzing the underlying mechanisms for such regeneration failure, we identified several pathways, such as PTEN/mTOR and SOCS3/STAT3, as critical regulators of axon regeneration from retinal ganglion cells (RGCs) after optic nerve injury. Our further studies also suggested that these pathways are critical for promoting axon regeneration from corticospinal neurons from spinal cord injury. However, we showed that regenerating axons induced by such treatments are able to reform functional synapses, but fail to be myelinated. In a traumatic optic tract injury model, we found that administration of voltage-gated potassium channel blockers restores conduction and results in increased visual acuity. Thus, our results revealed that in addition to axon regeneration, myelination is an additional limiting step towards functional recovery. In addition, we have also developed more translatable strategies to mimic the effects of genetic manipulations. Together, our studies demonstrated the feasibility of achieving functional recovery by enhancing both the regeneration of injured axons and conduction of regenerated axons.

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Molecular controls over corticospinal motor neuron axonal branching at specific spinal segments

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Corticospinal motor neurons (CSMN, and related cortico-brainstem neurons; together "CSMN") are located in layer V of the neocortex, and make synaptic connections with circuitry in the spinal cord and brainstem. CSMN axons form the corticospinal tract (CST), the major motor output pathway from the cerebral cortex essential for voluntary motor control. CSMN are also clinically important. CSMN degeneration in amyotrophic lateral sclerosis (ALS), along with degeneration of spinal motor neurons, causes spasticity and paralysis. In humans, damage to the CST in spinal cord injury is the principal cause of loss of voluntary motor control. Previous studies in our lab have identified combinatorial molecular controls over the specification and differentiation of CSMN.
CSMN themselves exhibit striking anatomical and functional diversity: Some CSMN extend axons to innervate cervical spinal cord targets and control forelimb movement, while others extend far more caudally to innervate lumbar segments and control hindlimb movement.
The underlying molecular basis for this diversity is strongly suggested by the stereotypic organization of these populations in the sensorimotor cortex, which is largely conserved from rodents to primates, and by their precise topographic pattern of connectivity in the spinal gray matter. Our investigation has identified a set of candidate molecular controls over development of this diversity and specificity of CSMN segmental connectivity. We selectively isolated bulbar-cervical- and thoracic-lumbar-projecting CSMN (CSMNBC and CSMNTL, respectively) at three critical time points of CST development, and identified differentially expressed genes between these two subpopulations of CSMN during development. Using gain- and loss-of-function analyses, we identified a secreted proteoglycan, Lumican, expressed specifically by CSMNBC that non-cell-autonomously limits CSMNTL axonal collateral branching in the cervical spinal cord. These results represent a novel mode of control over circuit connectivity, via non-cell-autonomous regulation of CSMNTL axonal branching by CSMNBC, thus over development of segmentally and functionally specific corticospinal circuitry.

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Identification of candidates promoting axon regeneration after injury

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Many retinal ganglion cells (RCGs) die following axotomy (optic nerve crush) and only some of the survivors can be induced to regenerate axons by interventions such as down-regulation of PTEN. The mouse retina contains about >40 different RGC types, and a recent analysis of several of these types showed that they differ dramatically in their ability to survive and regenerate (Duan et al., Neuron, 2015). This result suggests that comparing patterns of gene expression in susceptible and resilient populations could provide a strategy for finding factors that could be harnessed clinically to promote survival or regeneration. To test this idea, we are initiating the following studies: 1) Using high-throughput single cell RNAseq (scRNAse), we are classifying RGC types; the current estimate is ~55 type. 2) Using immunohistochemistry and scRNAseq, we are assessing the fraction of each RGC type that survives at least two weeks following axotomy. 3) Using scRNAseq at 6-48 hours after axotomy, we are identifying gene expression patterns that correlate with, and could underlie, resilience. 4) Using novel retrograde labeling methods, we are marking cells that regenerate following interventions such as PTEN loss, so we can catalogue their types and analyze their gene expression patterns.

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Diverse effects of serotonergic modulation on olfactory behavior in mice

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The serotonergic system modulates a wide range of perceptual and cognitive functions. It affects a wide variety of learned behaviors such as decision-making and reward prediction, as well as innate behaviors (e.g., novelty exploration). One critical limitation of existing research has been the use of system-wide non-specific manipulation of serotonergic populations. Since the serotonergic neurons project broadly in the vertebrate brain, broad manipulations could lead to confounding results. In this study, we used virally-targeted, chemogenetic inhibition (Gi-DREADDs) in mice to test the effects of serotonergic modulation on different olfactory behaviors by either selectively blocking activity of serotonergic projections to the olfactory bulb or by system wide inhibition of serotonergic modulation. We found that targeted block of serotonergic projections to the olfactory bulb (with local CNO injections) during olfactory figure-ground segregation task impaired the animals' ability to detect single components embedded in an odor mixture. This impairment is highly correlated with the complexity the task. The performance of mice is unaffected at lower difficulties (i.e, low number of components in background mixture ~4) while decreasing rapidly with increasing difficulty (~ number of components in the background mixture). In addition, impairment was highly dependent upon the identity of target odors. We found similar impairment in animal's performance during a two-alternate force choice task in presence of variable background mixtures, where targeted block of serotonergic projections to the bulb resulted in odor identity and task complexity dependent impairment of performance. In contrast, system wide inhibition of serotonergic modulation with systemic injection of CNO resulted in qualitatively distinct and more severe behavior deficits. The systemic block of raphe projections resulted in nonspecific impairment that was neither dependent upon the complexity of the task nor on the identity of target odors to be detected.
To further compare the targeted versus system-wide effects of serotonergic modulation, we tested mice in an open field arena. We found that the systemic manipulations resulted in increased mobility, decreased patience and impaired animals' ability to correctly identify attractive and repulsive olfactory stimuli. In comparison, targeted manipulation of serotonergic projections had no effects on these tasks.
Together, these results point to a specific role of serotonergic projections to the olfactory bulb in complex, but not simple, olfactory behaviors.

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Respiratory entrainment of memory circuits

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Decades of research have identified neural oscillations as a mechanistic substrate for the formation of cell assemblies and the coordination of information transfer between remote brain regions. During exploratory behavior, the hippocampus and the prefrontal cortex are organized by theta oscillations, known to support memory encoding and retrieval, while during sleep the same structures are dominated by slow oscillations that are believed to underlie the consolidation of recent experiences.
Although most known neural oscillations are generated by intra-cerebral pacemakers and circuits, here we focused our attention to breathing, the most fundamental and ubiquitous rhythmic activity in life. We report respiratory entrainment of limbic circuits, including the prefrontal cortex and hippocampus, two structures critically involved in memory consolidation and retrieval.
Using a combination of extracellular recordings using high-density silicone probes, calcium imaging, photometry, pharmacological and optogenetic manipulations in mice, we identify that a rhythmic oscillation (2-6 Hz and termed respiratory θ rhythm) entrains neuronal activity across structures. We characterize the translaminar and transregional profile of the respiratory entrainment of the prefrontal cortex and hippocampus and demonstrate a causal role of re-afferent respiratory inputs in synchronizing neuronal activity and network dynamics between these structures in a variety of behavioral scenarios in the awake and sleep state. Prefrontal 4Hz oscillations, recently identified as a physiological signature of fear memory in mice, are a manifestation of the differential cortical entrainment by the respiratory θ rhythm during behavior.
Our results highlight respiration, a persistent rhythmic input to the brain, as a novel oscillatory mechanism mediating inter-regional synchronization of limbic memory circuits and contributing to the formation and expression of neuronal ensembles.

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Role of Slit-Robo signaling in the formation of retinotectal layers

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Retinal ganglion cells (RGCs) are the sole output of the eye and transmit visual information for further processing in the brain. Their main innervation site is the optic tectum, a highly layered structure in the midbrain. In the zebrafish tectum, RGC axons terminate in four innervation domains from superficial to deep: SO, SFGS, SGC and SAC/SPV. Our laboratory has recently shown that Slit signaling through Robo receptors expressed by RGCs is essential for the layer-specific targeting of RGC axons within the tectum (Xiao et al., *Cell* 2011). Slit1a, provided by tectal cells, appears to act as a classical axon guidance molecule steering axons into the correct tectal layers. However, the *slit1a* gene is also expressed by a subpopulation of RGCs, suggesting that retina-derived Slit1a shapes axon trajectories. We have set out to disentangle the relative contributions of the two Slit1a sources in the formation of visual pathways. First, we investigated the retinotectal projection of a novel *slit1a* loss-of-function mutant. Whereas, in wildtype larvae, each RGC axon forms a flat terminal arbor, restricted to a single layer, this planar morphology is severely disrupted in *slit1a* mutants. SO- and SFGS-terminating RGC axons branch aberrantly and frequently cross between layers. Even more dramatically, the deep-projecting subset of RGCs fails to innervate the SGC and SAC/SPV layers altogether. Next, we generated a *slit1a:Gal4* BAC transgenic line allowing us to morphologically classify slit1a-expressing RGCs. Intersectional labeling revealed that the majority of this heterogeneous population terminates in SGC and SAC/SPV, which are the layers most affected by *slit1a* loss of function. Together, these findings prompt the hypothesis that Slit1a may cell-autonomously help to guide RGC axons to the deep tectal layers. Future experiments will employ tissue-specific rescue and knockout experiments to explore which developmental aspects of the retinotectal projection depend on RGC-derived versus tectum-derived Slit1a.

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Neurogenetic manipulation of visuomotor circuitry in freely flying Drosophila

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Locomotion in complex environments poses a severe challenge to sensory systems controlling an animal's course. For flies, visual estimation of self-motion is thought to play a critical role in stabilizing trajectories during walking or flight. Robust sensory feedback seems particularly important when considering the rapid velocities at which flies maneuver through varied and adverse surroundings. The extent, however, to which flies make use of optic flow remains unclear, especially in comparison with proprioceptive feedback mediated by halteres. Recently, a circuit-level account of how Drosophila detects motion has come within reach. The vast majority of these discoveries was made in either resting or head-fixed tethered animals. Here, we probe the function of optomotor circuitry under real-world conditions: in freely behaving Drosophila. Our set-up allows us to track multiple flies online while presenting high-resolution visual stimuli. We express opto- and thermogenetic tools in precisely circumscribed groups of neurons within the optic lobe, silencing and activating cells during episodes of untethered locomotion in two or three spatial dimensions. Emphasis rests on the role of local motion detector arrays T4 and T5 for active navigation. The two cell types process moving bright and dark edges, respectively, and provide the sole direction-selective input to the intricate course control networks of the lobula plate. Our previous work on tethered walking flies has demonstrated that these elements are required for correcting artificially imposed course deviations. Here, we show path stability to be strongly diminished in freely walking flies rendered motion-blind through inactivation of T4 and T5, even without external perturbation. We extend these findings to three-dimensional trajectories, focusing on differential effects between walking and flying. Our approach thus represents a critical step toward quantifying and understanding the contribution of optomotor circuitry to natural navigation in the fly.

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Kinematics of the prey capture strike in zebrafish larvae

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Zebrafish larvae rely on their visual system to hunt single-celled protists such as paramecia. This complex innate behaviour requires the detection, localisation, pursuit and capture of prey in three-dimensional space. Pursuit involves eye convergence, low amplitude swims and turns that bring prey within striking distance of the fish. Our lab has recently described the stimuli that drive these movements and the underlying visual circuits. However, the movements of the fish during the final stage of hunting – which we call the prey capture strike – are poorly characterised. Consequently, without the ability to reliably identify the prey capture strike in freely swimming or restrained larvae, the specific visual cue that drives this phase of the behaviour and the underlying neural circuits have remained elusive. As a first step towards understanding the neural circuits for prey capture, we have developed a behavioural assay that allows entire hunting sequences of freely swimming larvae to be studied simultaneously from above and from the side using high-speed videography. For the first time, we combine automated tracking of the tail and eyes of the fish with jaw and cranial kinematics to produce a multi-dimensional representation of the behaviour. Our results suggest that a stereotyped jaw movement associated with prey ingestion represents a unique kinematic signature of the prey capture strike, whereas tail movements appear to be more variable and may be subject to modulation by prey location. Our assay will allow us to objectively identify different phases of prey pursuit and capture. By combining this behavioural analysis with genetic and 2-photon ablations we will be able to test the role of specific neuronal populations in hunting behaviour. In the future, we will use the kinematic signature of the prey capture strike we have identified to develop a semi-restrained preparation suitable for psychophysics and imaging experiments that will elucidate the visual and motor circuits underlying prey consumption.

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Imaging representations of natural stimuli in auditory cortex

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Primary auditory cortex is thought to be particularly sensitive to higher order statistics of sounds compared to lower brain areas, making it ideally suited to the processing of natural sounds. However, the manner in which cortical circuits process such complex sounds, particular at the level of ensemble activity, is not well understood. We performed in vivo 2-photon calcium imaging of cortical neurons in anaesthetised mice. We indeed found subpopulations of neurons that responded selectively to natural sounds that could not simply be explained by their frequency tuning. Our ongoing work is investigating the functional connectivity of these neuronal subpopulations and the mechanisms that underlie their specialised tuning properties. These findings provide new insights into the functional specialisations in auditory cortex for processing behaviourally-relevant sounds.

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BACE1 activity modulates the cell surface proteome of neurons

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Proteolysis is a mechanism to control the levels and functions of cell surface membrane proteins. One of the contributing proteases is the b-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), which cleaves off the ectodomain of its mostly single-span transmembrane protein substrates. BACE1 is a central drug target in Alzheimer's disease (AD), but has multiple substrates besides APP which possess different functions in neurobiology. This raises the concern that therapeutic BACE1 inhibition may result in mechanism-based side-effects due to reduced cleavage of its substrates and their subsequent accumulation on the cell surface. Yet, the consequences of BACE1 inhibition on the neuronal cell surface proteome are only partly known. Therefore, primary neurons were treated with a BACE inhibitor, and cell surface proteins were labeled using click chemistry-mediated biotinylation. Label-free proteomics identified over 30 membrane proteins to be enriched at the neuronal surface. This included both BACE1 substrates and surprisingly also membrane proteins which are unlikely to be BACE1 substrates, such as tetraspanins. A small subset of BACE1 substrates, APLP1, SEZ6/L, CHL1 and contactin-2, was strongly enriched – 2.5 to 7-fold – at the neuronal surface upon BACE1 inhibition. Other known substrates, including L1, were mildly enriched – less than 2-fold. Several proteins were further validated by immunoblots and in BACE1-deficient mouse brains. This revealed that increased surface levels were accompanied by increased cellular levels. Taken together, this study demonstrates that BACE1 cleavage is a mechanism to control the surface proteome of neurons, regulating the abundance of a subset of its substrates, but also indirectly altering the amount of many other membrane proteins.

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Efferent modulation in the mechanosensory lateral line of the larval zebrafish

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Animals need to identify the source of sensory stimuli to guide appropriate responses. However, sensory systems can be activated both by events occurring in an animal's environment, as well as self-stimulation generated by an animal's own movement. This poses a fundamental challenge given that sensory receptors are intrinsically unable to discriminate between external and self-generated inputs; they can merely report their presence. In addition to informational ambiguity, movement also generates sensitivity challenges. By strongly driving sensory activity, movement may mask external inputs that occur concurrently or desensitize primary afferents leaving the animal unresponsive to stimuli following motion.
Aquatic organisms are particularly confronted with this challenge. Fish and amphibians guide multiple behaviors by using the lateral line, a collection of mechanosensory organs distributed along their body that sense watercurrents or displacements caused by other animals or objects. However, fluid drag during locomotion also strongly activates this sensory modality. Nevertheless, fish are able to filter out this confounding contribution and respond appropriately to external stimuli.
To understand the neuronal circuits that underlie such stimulus discernment, we study the lateral line system of larval zebrafish as a model circuit. Using standard labeling techniques, we have found two distinct nuclei providing direct descending inputs to mechanosensory organs: a dopaminergic hypothalamic nucleus and a cholinergic hindbrain nucleus.

Using calcium imaging in a head-embedded preparation, we have observed that both descending nuclei are activated during locomotive events such as swims and escapes, while the primary sensory stream gets inhibited. Interestingly, pharmacological manipulations and laser ablations have shown that inhibition is largely mediated by descending cholinergic neurons. 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. Current efforts are directed towards elucidating the precise relationship between inhibition strength and expected sensory feedback. Changes in the coding capabilities of sensory neurons during locomotion would have profound implications on behavior. For example: all-off inhibition would render the animals insensitive to external cues during motion, while inhibitory signals that scale with expected-feedback would still allow for stimulus detection during motion.

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Combining RNA-Seq and somatic CRISPR mutagenesis to study mouse neural development *in vivo*

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Forward genetic screens have been invaluable for identifying genes that regulate synaptogenesis in invertebrates, but few such screens have been performed in mammals owing to the expense and difficulty of germ-line mutagenesis. Here, we propose an alternative approach: purification of synaptic partners at key developmental stages; transcriptional profiling (RNA-seq) to identify candidate mediators of their interactions; and somatic CRISPR/Cas9-based mutagenesis to assess their roles in vivo. We applied this strategy to mouse outer retina, in which axons of rod and cone photoreceptors form synapses on dendrites of rod bipolar and cone bipolar interneurons, respectively, in a thin neuropil called the outer plexiform layer (OPL). We purified and profiled these four cell types, identifying hundreds of genes encoding cell surface and secreted proteins that were differentially expressed among them as synapses are forming. We then generated guide RNAs to inactive a set of them, and introduced them into outer retina by electroporation, along with Cas9 and a fluorescent marker to identify transduced cells. Using this strategy, we found that inactivation of Wnt5a and 5b, which are selectively expressed by rod bipolars, leads to formation of a supernumerary OPL. To investigate Wnt5's mechanism of action, we used cell type-specific promotors to inactivate 8 candidate signal transduction components. We found that Wnt5 acts on rods through a non-canonical pathway using Ryk, Fzd4 and Fzd5 as receptors. Neither the initial screen nor the investigation of signaling mechanism could have been performed in a timely manner using germ-line constitutive and conditional mutagenesis. These methods can be employed to study circuit development in many part of the developing mouse brain.

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A subset of octopaminergic neurons asserts impulse control on odor tracking in food-deprived flies

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The expression of a behavior or an impulse has to be tightly controlled in a context-dependent manner. A hungry animal must spend energy in food-seeking despite its high cost. On the other hand, a fed animal will prioritize safety and suppress exposure or energy expenditure required for food seeking. Therefore, the internal state of an animal and its drive or ability to express or suppress a behavior are tightly linked. Olfactory behavior in the fly represents a good model for studying the neuronal mechanisms underlying the interaction of internal state and behavioral control. Using a combination of high-resolution behavioral analysis and genetics, we uncovered a neural circuit element that integrates feeding state and controls the animal's impulse to follow the odor of a putative food source. First, we show that repetitive odor exposure does not lead to adaptation, but instead increases running bouts in cue direction. The length and speed of these food-seeking behaviors strongly correlate with hunger state. Second, a small cluster of neurons releasing the insect analog of norepinephrine, octopamine, the VPM4, integrates feeding state and suppresses food seeking in hungry flies. Furthermore, we find that a specific output neuron of the mushroom body, the inhibitory MVP2 neuron, is required for the expression of food seeking behavior in hungry animals. Preliminary whole cell recordings suggest that feeding state regulates the neuron's activity and thereby the impulse and motivation of the animal to seek food. We propose that octopaminergic neurons such as VPM4 control the expression of food seeking behavior through regulating the activity of MVP2 mushroom body output neurons. Given that norepinephrine is an important regulator of impulse and motivation in many animal species including humans, the identified circuit element provides a potential mechanism for the integration of motivational drive and control of impulsive behaviors.

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A central role of two-pore channels type 2 in the pathogenesis of age-related macular degeneration

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Age-related macular degeneration (AMD) is a leading cause for loss of visual acuity in the industrial countries. About 10-15 % of AMD patients suffer from the exudative form of the disease which is characterized by abnormal, leaky blood vessels invading the retina from the choroid. This process, called choroidal neovascularization (CNV), leads to loss of macula integrity and visual acuity. In this project, we focused on the role of two-pore channels (TPCs) in CNV formation. TPCs are expressed in the membrane of the endolysosomal system and have been linked to the formation of blood vessels and the migration and proliferation of cancer cells. In order to model the wet form of AMD and to induce CNV formation, laser photocoagulation was applied to the retina of wild type and TPC2 knockout (KO) mice. After laser treatment, TPC2 KO animals developed significantly smaller or less CNV lesions in comparison to wild type mice. The pharmacological inhibition of TPC2 channels resulted in a similar reduction of CNV formation. In addition, laser-treated TPC2 KO animals showed decreased expression levels of various factors important for neoangiogenesis. In conclusion, we identified TPC2 as an important modulator of CNV and thereby a novel therapeutic target for wet AMD.

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Network and behavioral dynamics of sensory integration in the rodent hippocampal system

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What are the systems-level mechanisms allowing for formation of memories? The conceptual picture that emerges is that the representation of a novel object or event is incorporated into representation of the spatio-temporal context in the hippocampus, a structure critical for memory. The accepted broad mechanistic framework for this process is that perceived information about the world is transferred from multimodal neocortical areas to the hippocampal region where it is
actively encoded. Both transfer of information to and encoding in the hippocampus relies on active sampling of the external sensory inputs and internal network dynamics. However the quantitative link between diverse exploratory behaviour that rodents use to actively sample external sensory inputs during learning, oscillatory network dynamics that controls information flow and hippocampal population code for space and memory is not established. We use marker-based high-resolution tracking of rat biological motion to quantitatively and objectively segment and classify its exploratory behaviour. We combine behaviour analysis with multichannel extracellular recording of populations of neurons and oscillatory dynamics in entorhino-hippocampal circuits. In the talk I will present our recent advances in these project along several directions. First, I will show how high-resolution tracking gives rise to quantification of known behaviours and discovery of new behavioural motifs. Second, how population activity of hippocampal neurons is changing dynamically with exploratory state. Third, I give an overview of the oscillatory synchronization dynamics across entorhinal-hippocampal circuits. Taken together the constraints imposed by spontaneous exploratory behaviour and network dynamics on activity of hippocampal neurons give rise to a novel temporal framework for the analysis of the mechanisms of memory encoding.

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Individual-to-individual differences in classical conditioning within *Drosophila melanogaster*

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Performance in learning tasks varies across individuals. While it is understood that many factors contribute to variability in learning, the comprehensive study necessary to determine the weight of environment, genetics, and neural physiology on variability in learning is challenging and has not been conducted. We can begin to answer this question by taking advantage of the characterized neural circuitry, rich behavior, and extensive molecular genetics tools of *Drosophila melanogaster*. Genetically identical individuals display variability in their physiology, morphology and behaviors even when reared in identical environments. To evaluate variation in learning we have engineered an automated classical conditioning odor assay. By expressing light-sensitive ion channels (Chrimson) in bitter taste receptor neurons, we can deliver a consistent unconditioned stimulus to a population of flies. We have observed individual-to-individual variation in the magnitude of behavioral change after training, and individual-to-individual variation in the duration of the memory. Interestingly, we have found evidence of a generality in learning odor associations, which suggests that within an isogenic population there are flies that learn associations faster and some flies that learn associations slower. We are currently working to determine if this generality will correlate to other learning modalities, such as visual learning. Preliminary evidence suggests there is generality to learning across modalities. Using real-time PCR we are determining if there are genes that predict a fly's ability to learn, these genes include: *chrimson*, dop1R1, *rutabaga*, and *dunce*. Our work suggests that *Drosophila melanogaster* could be an attractive model organism for studying individuality in learning and could further the understanding of the molecular mechanisms behind variability in learning.

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Gene therapy for neurosensory disorders: engineering delivery

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Under study for over four decades, therapeutic gene transfer is now demonstrating safety and efficacy in clinical studies. Some of the more impactful studies have been in ophthalmology, in particular targeting retinal disorders and diseases of visual decline. The eye is a compartmentalized organ allowing limited doses and local delivery while benefiting from relative immune privilege. The genetics of retinal disease, and our understanding of pathophysiology of many retinal diseases, have progressed substantially. Lastly, improvements on vector delivery technology have allowed, for select retinal cell targets, to successfully translate preclinical proof-of-concept studies to human trials. Limitations however remain, curtailing the potential of gene therapy toward broad application. Here, we describe our work to unlock novel strategies for retinal gene therapy by translating programs to the clinic for forms of inherited retinal degeneration, studying surgical delivery routes, and optimizing vector design. In light of the translational success in ophthalmology, it is remarkable how gene therapy for hearing and balance disorders has not progressed as far. Here, we discuss the hurdles for cochlear gene therapy on the path to clinical reality and our research on viral vector design to surmount some of these challenges.

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Modulation of olfactory processing in the *Drosophila* larva

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Detecting and processing sensory cues allows us to select essential information from the environment and subsequently to enact purposeful behaviors. Appropriate decisions can however only be made when this sensory information is processed together with our internal and external state (such as hunger or exposure to toxic substances). Many studies have focused on how sensory input is perceived on the receptor level and how it influences motor output, while the impact of internal physiological state and current environmental factors on sensory processing is less studied. In case of odor detection, the decision to approach or avoid an odor source must be appropriate to the body's requirements for food and the possibility of exposure to harmful stimuli. For *Drosophila* larvae, a wide array of genetic tools and a detailed map of the underlying neural circuit are available. Thus, it is especially suited to investigate modulation of olfactory processing.
We found that after starvation, a change of the internal state, larvae change their preference towards monomolecular odors. Previously, detailed EM reconstruction revealed that there are several types of local inhibitory and neuromodulatory neurons innervating the antennal lobes of larvae. Hence, modulation of olfactory information might already take place at that early stage of sensory processing. By manipulating relevant cell types during behavioral preference tests and by recording neural activity in intact larvae, we will dissect the circuit underlying starvation modulation of olfactory processing in the *Drosophila* larva.

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Integrated circuit analysis of layer 2/3 pyramidal cells in mouse visual cortex

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Neocortical pyramidal cells (PCs) display functional specializations defined by their connectivity as well as their molecular, anatomical and electrophysiological properties. PCs in layers 5 (L5) and 6 (L6) have been classified into different subtypes based on their in vivo functional response properties and corresponding connectivity patterns as well as genetic and electrophysiological characteristics (Velez-Fort et al. Neuron 2014, Kim et al. Neuron 2015). For layer 2/3 (L2/3) PCs no unique genetic markers corresponding to individual functional subtypes have been discovered so far (e.g. Zariwala et al. Front Syst Neurosci 2010). However, L2/3 PC have been shown to project functionally target-specific to higher visual areas (Glickfeld et al. Nat Neurosci 2013).
Here, we ask whether L2/3 PCs differ in their connectivity patterns in mouse primary visual cortex (V1) and whether this is related to differences in L2/3 PC stimulus preferences. To address this question, we characterize the excitatory and inhibitory cortical inputs of the same L2/3 PCs using laser-scanning photostimulation (LSPS) by UV glutamate uncaging in brain slices. The majority of L2/3 pyramidal cells receive strong excitatory input from layer 4 (L4) (77/106 PCs, exc. L4 input >50% of total input) and strong local inhibitory input from L2/3 (67/106 PCs, inh. L2/3 input >50% of total input) and varying degrees of additional input from the other layers (e.g. 19/106 PCs, exc. L5 input >25% of total input). Furthermore, the spatial overlap between excitatory and inhibitory synaptic input within a given L2/3 PC varies, e.g. L5 excitatory input is mostly unbalanced by L5 inhibitory input.
In order to explore the functional implications of the different input patterns we developed an in vivo / in vitro approach: First, we characterize the visual response properties (orientation/direction selectivity, temporal/spatial preferences, ocular dominance and spontaneous activity) of individual neurons expressing genetically encoded calcium indicators (GECIs) with in vivo 2-photon calcium imaging. Subsequently, we retrieve the same neurons in brain slices for circuit analysis with LSPS.
In conclusion, L2/3 PCs appear to be a heterogeneous group based on their connectivity patterns, and we currently explore if this is reflected in their visual stimulus preferences.

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Role of Sarm1 in neural-circuit regeneration

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The peripheral nervous system has the ability to regenerate after physical trauma. The clearance of the damaged axons precedes neuronal repair. However, the cellular dynamic that underlie this process is not fully understood. We have investigated this issue in zebrafish. We demonstrate that genetic deletion of the pro-degenerative protein Sarm1 hampers Wallerian degeneration of severed sensory axons, which are maintained independently of Schwann-cell support. The resulting delay in axon clearance does not alter the onset and kinetics of regenerating growth-cone elongation. The functional conservation of Sarm1 from flies to mammals suggests an evolutionary-ancient role in accelerating axonal degeneration, possibly to maximize the fidelity of neural-circuit reconstruction.

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Rapid production of neural progenitor cells from human pluripotent stem cells for the study of Zika virus neuropathogenesis

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Neural progenitor cells (NPCs) play an integral role in early brain development by acting as an intermediate proliferative cell type in the pathway from pluripotent stem cells (PSCs) to neurons and glia. NPC dysfunction has been linked to several neurodevelopmental disorders, including schizophrenia, autism, and more recently, Zika virus (ZIKV)-induced microcephaly. Our understanding of these disorders has been accelerated by advancements in stem cell-derived NPC model systems. Here, we describe a novel 48 hour induction protocol for the production of human PSC-derived NPCs. Using inhibitors of SMAD signaling in combination with lentiviral-based doxycycline-inducible overexpression of the transcription factor neurogenin-2 (NGN2), we are able to generate high purity NPC cultures from both human embryonic stem cells and human induced pluripotent stem cells. These cells, termed *rapid neural progenitor cells* (rNPCs), express several protein markers that are characteristic of early dorsal forebrain neuroepithelial cells. rNPCs are proliferative, multipotent, and able to self-aggregate into neurospheres under low attachment conditions. Importantly, rNPCs are susceptible to ZIKV infection and viral-mediated cell death, which is consistent with the previously reported cell tropism of this enveloped single-stranded RNA flavivirus. Furthermore, ZIKV infection inhibits rNPC neurosphere formation and outgrowth, while also leading to dose-dependent decreases in size. Together, our findings suggest that rNPCs can be used for both two-dimensional and three-dimensional model systems for future investigations of ZIKV neuropathogenesis.

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As fear goes by – how endocannabinoids control fear and anxiety

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In the past decade, endocannabinoids have emerged as a main class of retrograde messengers, which are synthesized and released on demand from postsynaptic sites to travel to presynaptic CB1 receptors, where they mediate a down-scaling of transmitter release. I will provide a brief overview over the involvement of endocannabinoids in controlling fear and anxiety, with special focus on distinct roles of (i) anandamide vs. 2-AG, and (ii) GABAergic vs. glutamatergic neurons. In this context I will emphasize the translational potentials of increased endocannabinoid signaling as a promising strategy for pharmacoenhancement of exposure-based therapies.

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A Compressed sensing framework for dissecting neural circuit in C. elegans

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An important question in neuroscience is how the dynamics of activity in neural circuits modulate behaviors. There are two challenges in answering this question, the first is identifying neurons essential for a behavior. The second is understanding how activity patterns in these essential neurons generate the behavior. Here we developed a framework that sparsity constraints can be used to identify neurons essential for a behavior with far fewer perturbation experiments than conventionally possible, using a combination of optogenetic tools and compressed sensing algorithms. By developing and using a novel stabilization microscope, we validate our discovery by measuring and optogenetically manipulating the dynamics of activity in these neurons in freely moving *Ceanorhabditis elegans* to determine how they drive behavior. The movement of the animal translates spatially varying sensory stimuli into a temporally changing signal profile at the nose tip. This temporally changing signal profile is processed to determine locomotory behavior during food search. Because the speed of the animal determines the translation of space into time, we sought to understand the identity and activity patterns in the essential interneurons in this animal that control speed. We constructed 27 transgenic *C. elegans* lines that expressed the light-gated reverse proton pump archeorhodopsin-3 (Arch) in overlapping subset of neurons under 27 different promoters. We next placed animals on a standard agarose plate and exposed them to green light to activate Arch and inhibit neurons in which it was expressed. By tracking the animals, we measured their speed as they performed chemotaxis. Only 5 of these lines showed statistically different speed distributions from control group as scored by the Kullbach-Leibler divergence. Although this is an underdetermined problem such that number of promoters are less than number of neurons, by imposing sparsity constraints, we were able to identify 3 types of interneurons: AVB, RMG and SIA, important for speed regulations in this animal. To next determine how the activity patterns in these neurons controlled the speed of movement, we built a tracking microscope capable of accurately imaging fluorescence signal from both the soma and processes of multiple neurons in different z-planes for over an hour as the animal moves freely on an agar plate. By selectively inhibiting activity of these neurons we showed that they are causal to speed modulations. Their calcium activity shows that they have 3 different types of behavior such that AVB behaves as rectifier for forward motion which was suggested in literature before. RMG only controls pausing and acts like a switch. SIA is more like continuous control over speed like a knob.

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