Annual Report

2019

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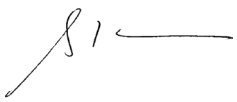
PREFACE

As a neuroethology institute, the research groups at caesar study the neuronal basis of behavior - how the collective activity of the interconnected neurons in the brain gives rise to the diversity of animal behaviors. To this end, our research groups and departments bring a unique combination of experimental and computational approaches together to study behaviors in a wide range of animal species. Our research spans a large range of temporal and spatial scales, from nanometer-scale imaging of brain circuitry to large-scale functional imaging of thousands of neurons in the brain, to the quantification of natural animal behavior. Over the past year we have continued to grow caesar into a unique neuroethology institute by successfully recruiting additional independent group leaders. Dr. Aneta Koseska was awarded a highly competitive Lise Meitner Excellence Program award from the Max Planck Society and selected caesar as the institution to host her new research group. Trained as a theoretical physicist, Dr. Koseska studies the ability of cells to learn and form memories and develops mathematical theories to explain her experimental observations. Two new Max Planck Group leaders, Dr. Monika Scholz and Dr. E. Pascal Malkemper, moved to caesar in 2019 and have taken up their work. Dr. Scholz uses the foraging behavior of the roundworm *C. elegans* to investigate general principles of signal compression, attention and context-dependency in neural systems. Dr. Malkemper works with the subterranean African mole rat to study the neurobiological basis of magnetic orientation in mammals. A further group leader, Dr. James Lightfoot, was also successfully recruited in 2019 when he was awarded a prestigious Open-Topic Max Planck Group Leader position. Dr. Lightfoot comes from the Max Planck Institute for Developmental Biology in Tübingen and studies how a self-recognition system in a predatory nematode prevents cannibalism and enables the worm to focus on appropriate prey. Dr. Lightfoot will begin his work at caesar in January 2021. In total, caesar now houses the largest number of independent research groups in its history, encompassing two scientific departments and seven research groups.

Given the expansion of scientific topics now studied at caesar and the influx of new groups, we held the first caesar scientific retreat with a focus on neuroethology in October to bring together the scientists at caesar to share their scientific work. This was, in particular, an excellent opportunity for doctoral students to gain experience delivering informative seminars. Finally, the new public outreach initiative, the caesar Public Lab, officially opened in 2019. Over the year we invited biology classes from the surrounding schools to spend a day in the public lab learning about the neuroethology research performed at caesar. Students ran experiments and made behavioral observations of the larval zebrafish and learned about how genetic mutations and neuronal circuitry can alter animal behaviors. The Public Lab has been enthusiastically received by teachers and we expect this form of public outreach to only grow in coming years.

We anticipate that the up and coming year at caesar will be as productive as the past year, providing a fruitful and collaborative research environment for studying problems at the interface of the brain and behavior.



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President of the Max Planck Society /
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Scientific Director

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TABLE OF CONTENTS

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OUR RESEARCH

The function of the brain is to coordinate and control animal behaviors. Establishing the link between the brain and behavior is known as 'neuroethology' and requires the combination of research studies focusing on different levels of detail - ranging from the dense anatomical reconstruction of neural circuits to the quantitative behavioral analysis of freely moving animals.

The central goal of neuroethology is to understand how the collective activity of the vast numbers of interconnected neurons in the brain gives rise to the plethora of animal behaviors. A major current challenge in neuroscience is how to integrate findings at these disparate levels of scale so that the behaviors of an animal can be understood in terms of activity on the scale of neural circuits; for it is how an animal makes use of computations in the brain that is ultimately important, not how brain computations are performed in isolation.

What makes this an especially challenging problem is that the link between brain function and behavior can only be studied in a behaving animal, and studying the brain in action poses substantial technical challenges. By overcoming these technical hurdles, both experimental and computational, this approach allows us to address some of the big unanswered questions in neuroscience, such as how the brain maintains a dynamic model of the environment and makes decisions and how the brain enables complex social interactions.

caesar is a research institute dedicated to the neuroethological study of animals. The research groups and departments bring a collectively unique combination of experimental and computational approaches to bear on the question of how the brain controls behavior. Our research spans a large range of scales from the nano-scale imaging of the brain, to large-scale functional imaging of thousands of neurons in the brain, to the quantification of natural animal behavior. Our expertise drives the development of new technological contributions, both in experimental instrumentation as well as in computational modeling and data analysis methods. In addition to the technological breadth of the research groups and departments at caesar, our neuroethological questions are comparative in nature and incorporate a diverse set of species allowing us to study the wide repertoire of behaviors across the animal kingdom.

ABOUT THE FOUNDATION

The institute is operated by a non-profit foundation under private law. The president of the Max Planck Society chairs the foundation board. Trustors are the Federal Republic of Germany, the Federal State of North Rhine-Westphalia and the city of Bonn.

caesar is regularly evaluated by a scientific advisory board. The scientific evaluation, as well as all other scientific measures are being conducted according to the procedures and criteria of the Max Planck Society.

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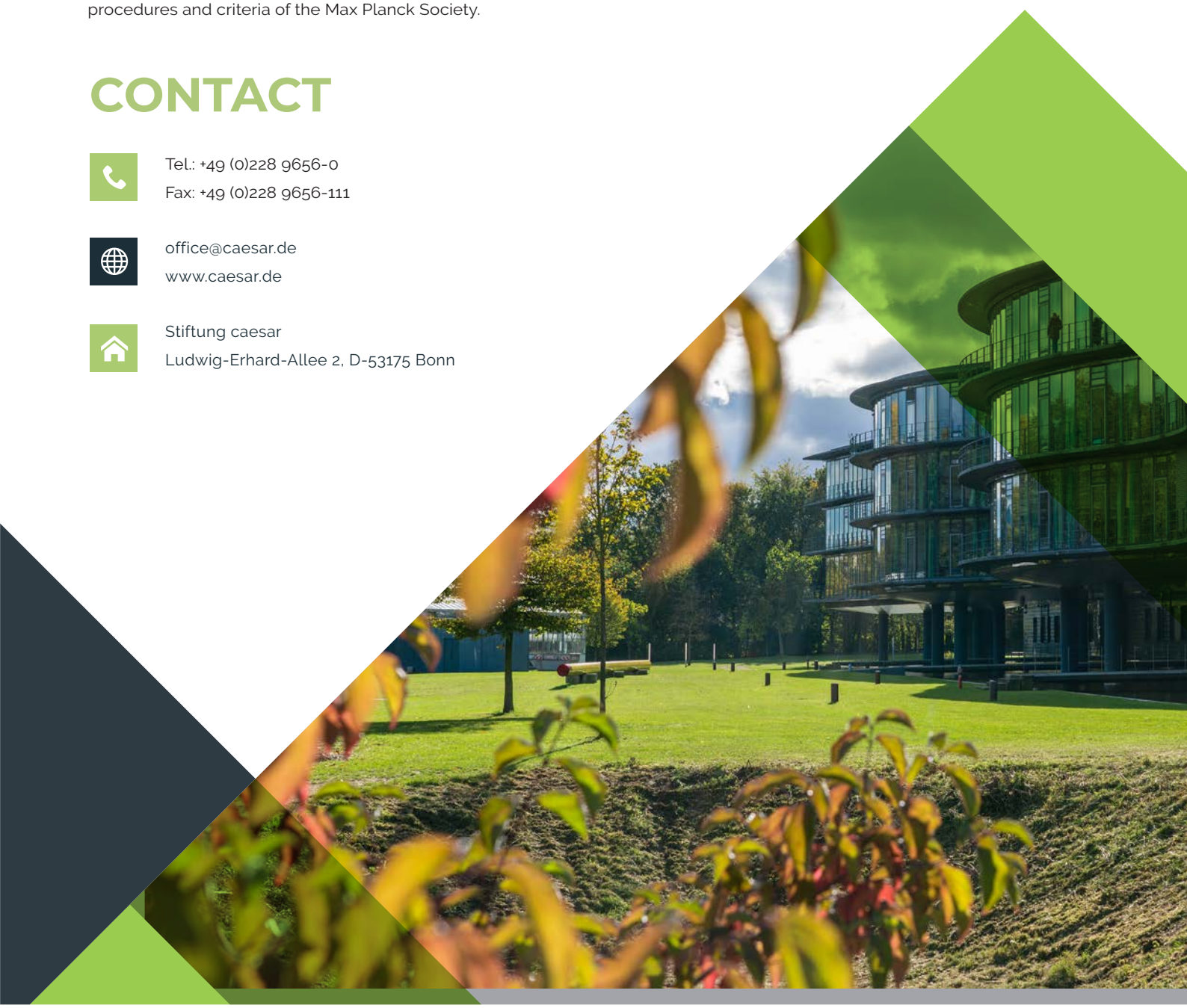

SCIENTIFIC REPORTS

Research groups in 2019

DEPARTMENT OF

COMPUTATIONAL NEUROETHOLOGY

DEPARTMENT OF

BEHAVIOR AND BRAIN ORGANIZATION

MAX PLANCK RESEARCH GROUP

IN SILICO BRAIN SCIENCES

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NEURAL CIRCUITS

MAX PLANCK RESEARCH GROUP

NEURAL INFORMATION FLOW

MAX PLANCK RESEARCH GROUP

STRUCTURAL DYNAMICS OF PROTEINS

EMMY NOETHER GROUP

NEUROBIOLOGY OF FLIGHT CONTROL

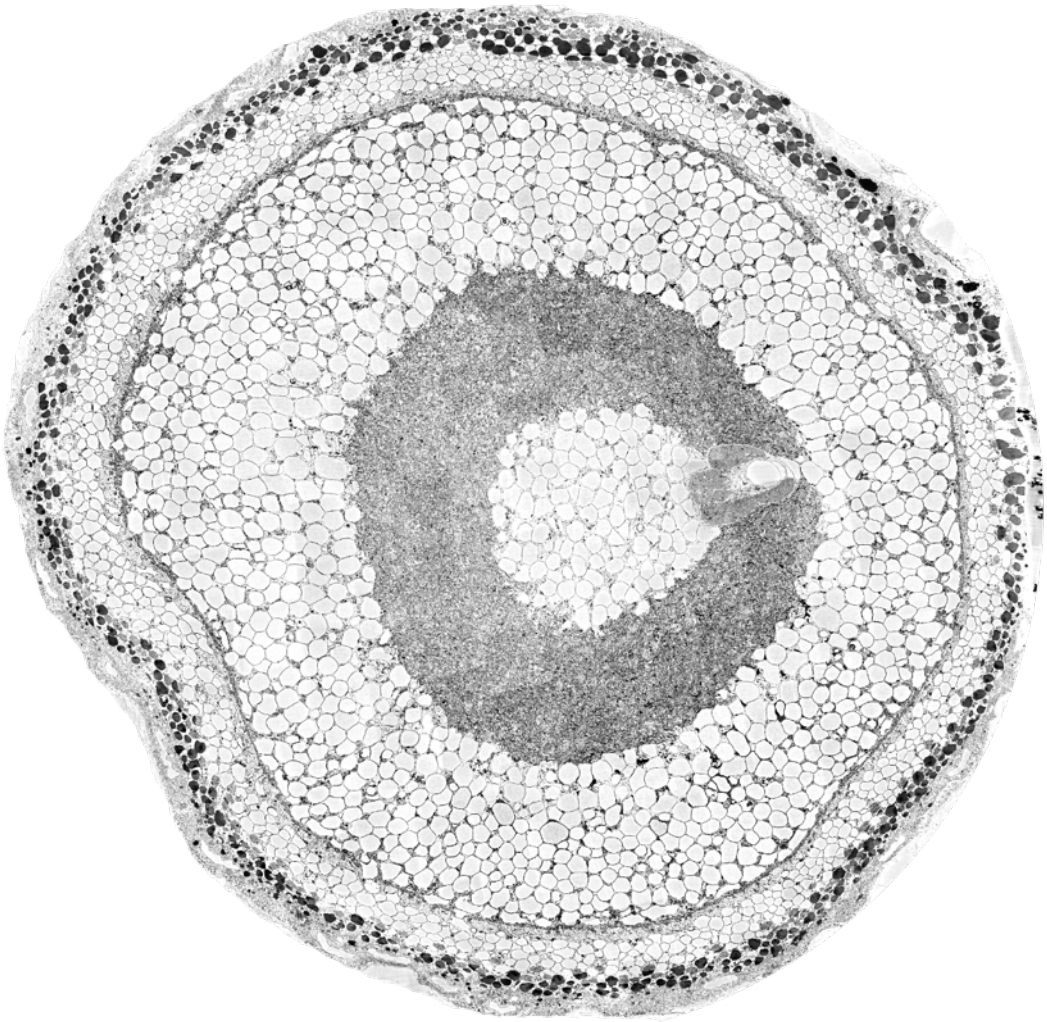
MAX PLANCK FELLOW

CHEMICAL BIOLOGY

Motion encoding in the mammalian retina

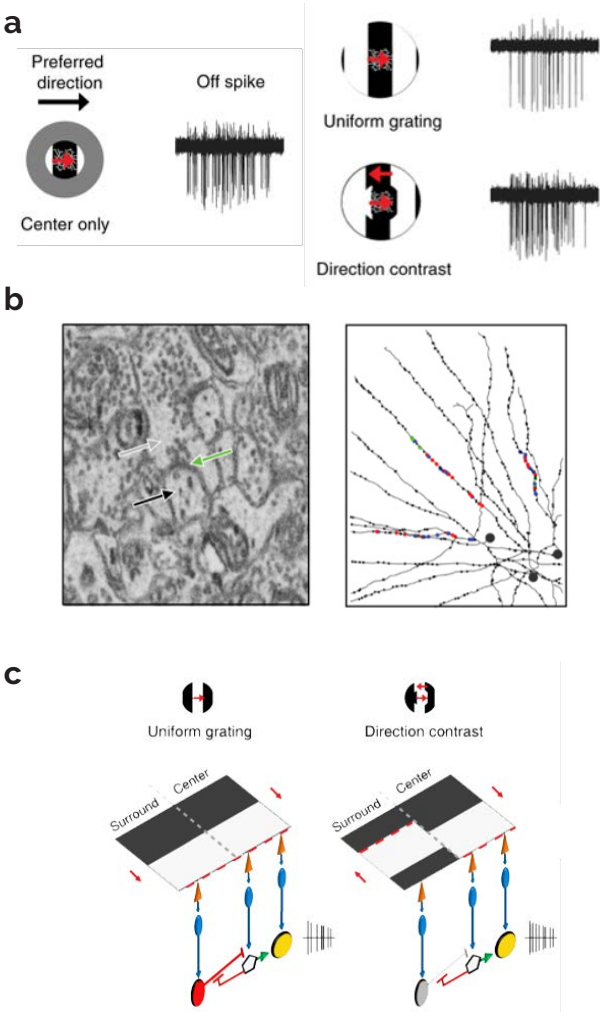
By Kevin Briggman | Computational Neuroethology

Our primary goal is to relate the synaptic connectivity of neural circuits in the central nervous system to the computations they perform.



Electron micrograph of a cross-section through a larval zebrafish eye. The different information processing layers of the retina can be seen from the photoreceptors (outer layer) to the retinal ganglion cells (inner layer). Image acquired by multibeam scanning electron microscopy. Data and image by Kevin Briggman, Silke Haverkamp and Paul Watkins.

Identifying these circuit mechanisms would aid our understanding of how brain networks translate sensory information and respond with appropriate behaviors. We are particularly interested in the initial stages of sensory processing in the visual and olfactory systems. Using a multidisciplinary approach including optical imaging, electron microscopy, electrophysiology and computational modeling, we seek to map the synaptic connectivity of each neuron in a circuit after having previously recorded their functional properties. We take a comparative approach, studying sensory pathways in both the mouse, fish, and frog nervous systems to take advantage of the experimental advantages in each model system. By understanding the wiring in typical wild-type animals, we ultimately aim to provide a baseline of comparison to eventually study wiring deficiencies under pathological conditions.



Direction selective retinal ganglion cells

The direction of motion across the retina is encoded by direction selective retinal ganglion cells (RGCs) that relay motion direction information to the rest of the brain. These neurons increase their electrical activity in response to motion in one particular direction – the 'preferred' direction – and suppress their activity in response to the opposite 'null' direction. The basic mechanism by which the preferred-null axis is established has been previously described and depends on the specific wiring of synapses on direction selective RGCs [1]. However, several aspects of this important neuronal computation remain unexplained at a mechanistic level. In 2019, we identified the synaptic connectivity underlying two key components of direction selectivity: 1) how direction selective RGCs alter their responses based on the surrounding context in a visual scene [2], and 2) how is the velocity of a moving stimulus encoded by direction selective RGCs [3].

Contextual modulation of direction selective RGCs

Direction selective RGCs, like all RGCs, respond most vigorously when a visual stimulus is centered on the neuron, a region referred to as the 'center' of the receptive field. When a visual stimulus extends beyond the center region into the 'surround', responses are often suppressed. This 'center-surround antagonism' serves the purpose of enhancing responses to visual stimuli that match the receptive field of a RGC. It was previously reported that direction selective responses are highly context dependent on the motion in the surround region of direction-selective RGCs [4], however the mechanism and neurons involved were unknown. When surrounding motion is in phase with motion in the central receptive field, directional responses are diminished (Figure 1 a, 'uniform grating').

Figure 1: Contextual modulation of direction selectivity. a) Electrical activity of direction selective retinal ganglion cells is modulated by the pattern of motion surrounding the center. b) Wide-field amacrine cells identified by connectomic reconstruction form continuity detectors. c) When surround and center motion is in-phase, wide-field amacrine cells are activated and direction selective retinal ganglion cell responses are suppressed. When surround and center motion is not aligned, wide-field amacrine cells are not activated. Adapted from [2].

When surrounding motion is out of phase or moves in the opposite direction of motion, directional responses are not affected (Figure 1 a, 'direction contrast').

We collaborated with Dr. Wei Wei's group at the University of Chicago to correlate electrophysiological and pharmacological experiments with a connectomic mapping of the neurons and synapses involved in motion context sensitivity [2]. We identified a key neuron type involved in the computation known as a wide-field amacrine cell (Figure 1 b). These amacrine cells extend across large distances in the retina and are therefore able to carry information from the surrounding motion context to the center of a direction selective RGC. We identified a synaptic wiring pattern that explains why in-phase but not antiphasic or reverse motion direction contexts affect DS – the wide-field amacrine cells form a type of 'continuity detector' that suppress direction selective response when the center and surround motion are spatially aligned. Similar forms of contextual modulation of motion coding are observed in visual circuits throughout the rest of the brain, but it remains to be seen whether similar mechanisms underlie the modulation.

Asymmetric wiring underlies direction selective velocity coding

Direction selective RGCs encode not only the absolute direction of a moving visual stimulus, but also velocity. Our prior work explored the velocity tuning of one class of RGCs, known as 'On-Off' RGCs, that encode motion greater than 1 mm/sec [5]. A second class of RGCs, 'On' RGCs, encode slow velocities less than 1 mm/sec, but the mechanism by which such slow velocities can be encoded was unknown. The slow preferring On RGCs are thought to help stabilize the image of the outside world on the retina during eye and head motion. We collaborated with Dr. Keisuke Yonehara's group at Aarhus University to correlate physiological recordings of the synaptic inputs to On RGCs with connectomic reconstructions of the inputs [3]. Anatomically we mapped a gradient of excitatory inputs arising from different bipolar cell types to the RGCs along the preferred direction axis (Figure 2 a, b). These bipolar cell types have previously been described to release neurotransmitter with different kinetics, such as sustained or transient and slow or fast.

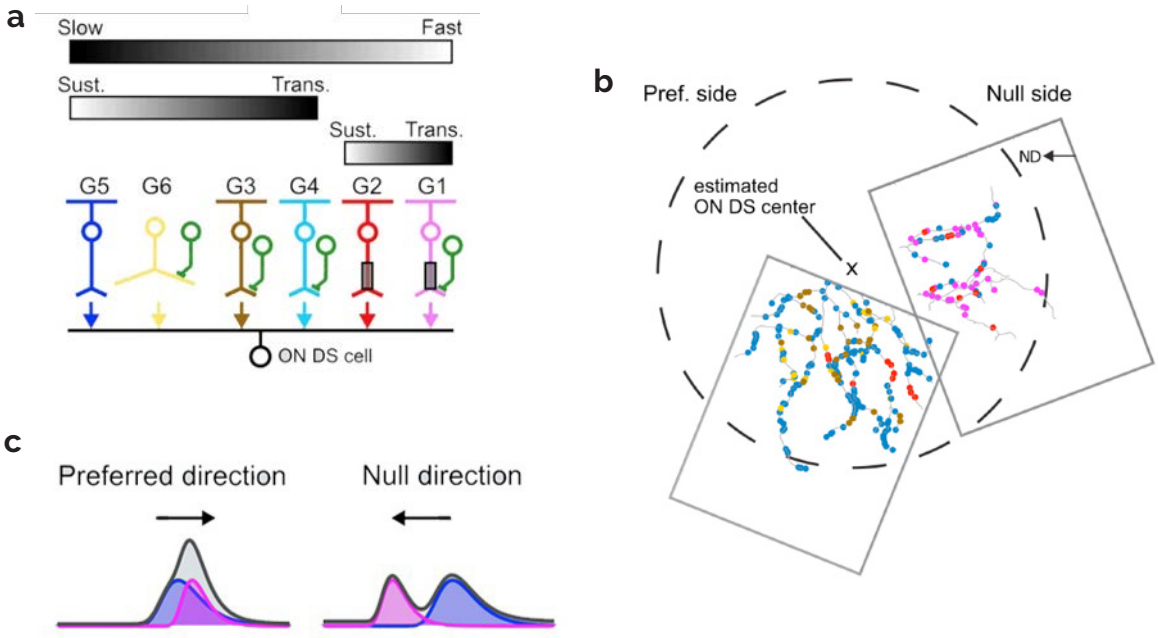


Figure 2: Asymmetric connectivity underlies direction selective velocity coding. a) Physiologically recorded synaptic inputs to On RGCs reveal a gradient of kinetics along the preferred axis (from left to right).. b) Connectomic mapping of synaptic inputs also revealed a gradient of different bipolar cells forming synapses (color-coded dots) onto On RGCs. c) When motion is along the preferred direction, slow input followed by fast inputs are activated allowing an enhanced response. When motion is along the null direction, fast inputs are activated too early in time and responses are suppressed. Adapted from [3].

The physiological recordings also revealed a gradient in neurotransmitter release kinetics along the preferred axis (Figure 2 a). We ultimately were able to map, one-to-one, the five different anatomically distinct presynaptic cell types to the physiologically predicted cell types. Mechanistically the gradient progresses from slow to fast neurotransmitter release along the preferred axis allowing for constructive summation of motion and an enhanced response (Figure 2 c). Motion along the opposite, null axis, does not constructively summate and therefore the response is suppressed (Figure 2 c). A computational model of the observed wiring and release kinetics confirmed that the preferred axis enhancement of activity is effective only at slow velocities. We therefore concluded that a combination of detailed wiring specificity and cell type specific release kinetics underlies slow velocity tuning in On direction selective RGCs, a unique mechanism that is not shared by On-Off RGCs.

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DEPARTMENT OF
COMPUTATIONAL NEUROETHOLOGY

Kevin Briggman
Scientific Director

The goal of the Department of Computational Neuroethology (CNE) is to develop computational models which can predict goal-directed animal behaviors. To acquire the data needed to build biologically plausible models, we develop and utilize a multidisciplinary range of experimental techniques. We record cellular resolution images of neuronal populations from behaving animals. In addition, we use fluorescence microscopy to identify the expression patterns of specific proteins. Finally, we reconstruct synaptic connectivity using 3D electron microscopy.

By combining these methods within individual brains, we aim to discover the relationships between the structure and function of neuronal networks driving behavior. Firstly, we want to understand how sensory stimuli are transformed in the brain to ultimately generate motor decisions. Building on this understanding, we aim to identify the sources of variability during goal-directed behaviors. To address these questions, we compare and analyze neural circuits across mammals, fish and amphibians to determine which aspects of a computation are species-specific and which generalize across species.

"We aim to discover the relationships between the structure and function of neuronal networks driving behavior."

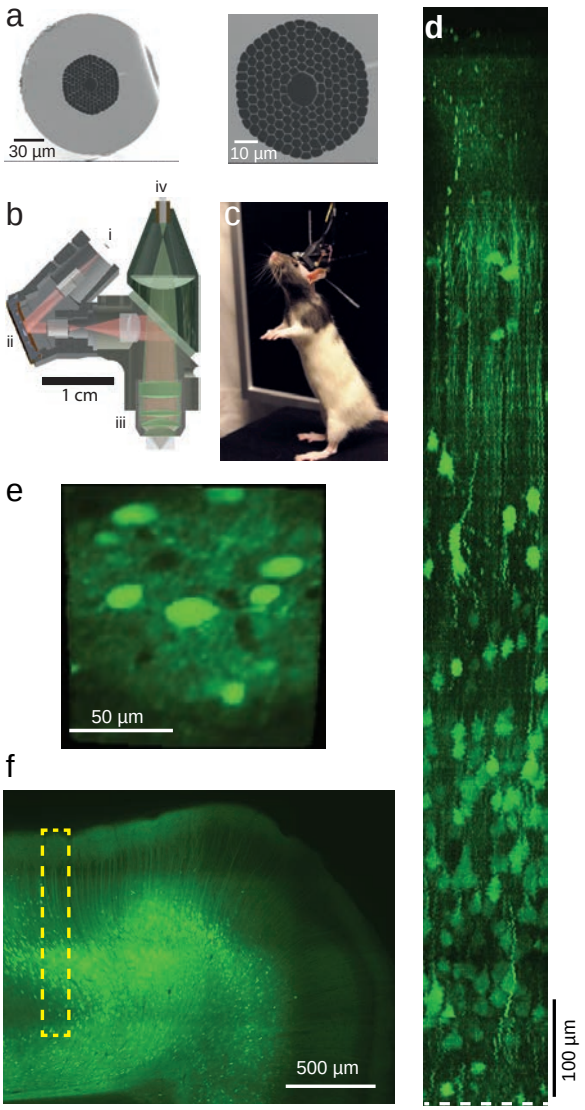
Imaging activity in the freely moving animal

By Jason Kerr | Behavior and Brain Organization

Understanding how animals use their brain circuitry to generate behavior in response to their environment is one of the central goals of neuroscience.

New three-photon fiberscope allows for continuous imaging of neuron populations deep in the cerebral cortex of freely behaving animals. Image: J. Kuhl.

While it is possible to study animal behavior by observing how an animal solves problems, to gain an understanding of the brain's role in generating the behavior the brain circuit activity must be measured at the same time. In 2009, our group, in collaboration with the group of Winfried Denk at the Max Planck Institute of Neurobiology (Martinsried), established a miniaturized head-mounted multiphoton microscope. It enabled recording of activity from neuronal populations located in the upper few cortical layers of freely moving rats, with single cell resolution. While this 'two-photon fiberscope' enabled measuring neuronal activity from freely behaving animals, most of the cortical layers remained out of reach. In collaboration with Philip Russell's group at the Max Planck Institute for the Science of Light, we have now developed a small head-mounted three-photon microscope, capable of imaging all cortical layers in a freely moving rat [1].



Recording cortical activity in freely moving animals allows the relationship between neuronal activity and complex behaviors to be explored while the animal interacts with the environment in a self-determined way. Miniaturized head-mounted two-photon microscopes [2] have enabled imaging of activity from fluorescently labeled neuronal populations in the upper cortical layers, with single cell resolution [3], and recently from single dendritic spines [4], in freely moving rodents. In densely labeled light-scattering tissue, these devices gain subcellular resolution, which is critical for localizing emitted fluorescence to the structure of origin, by restricting excitation to a spatiotemporally confined volume, providing optical sectioning. For two-photon excitation (2PE) imaging, both spatial resolution and efficient fluorophore excitation rely on the spatial, spectral and temporal properties of the near-infrared pulses delivered to the tissue [5]. These pulse properties limit maximum imaging depth of conventional 2PE microscopes [6] and, combined with size limitations of head-mounted microscope optics, at present have restricted functional imaging to the upper cortical layers. This leaves the vast majority of the cortex, including the deep cortical layers that transmit the output of cortical computations to other brain structures, inaccessible to microscopes employing this excitation modality. By comparison, three-photon excitation (3PE) can considerably extend the imaging depth possible in scattering tissue [7, 8] by utilizing longer wavelengths (>1300 nm) that decrease excitation light scattering, as well as virtually eliminating the generation of out-of-focus fluorescence. However, to use 3PE for imaging neuronal activity in freely moving animals while avoiding tissue damage would necessitate delivery of compensated high-energy, ultrashort pulses through a fiber that maintains transmission efficiency and polarization state during fiber bending [3]. To date, these requirements have prevented the development of a

Figure 1: Three-photon head-mounted microscope: design and optical properties. a) electron micrograph cross-section of the HC-PBGF showing the whole fiber (left) and close-up of the central core (right). b) Cross-section engineering schematic of the microscope design showing optical elements, excitation path (red) and emission path (green). i) Attachment point for HC-PBGF. ii) MEMS scanner. iii) objective. iv), plastic optical fiber. c) Photograph of the microscope mounted on a 120g rat, including head tracking posts. d) Side projection of an image stack of GCaMP6s-labeled cortical neurons in posterior parietal cortex (PPC) of an anesthetized rat. e) Image of labeled neurons at a depth of 1120 µm below the cortical surface. f) Post hoc histological section of GCaMP6s-labeled neurons in PPC. Box height shows the attainable imaging depth (1120 µm).

miniature head-mounted microscope using 3PE.

Custom-designed microscope

We designed a hollow-core photonic bandgap crystal fiber (HC-PBGF) [9] with transmission properties tailored for 3PE of functional indicators in freely moving animals (Figure 1). The key advantage of this fiber was that transmission properties were better maintained during fiber bending compared with other types of hollow-core photonic crystal fibers (HC-PCF). Maintaining transmission properties was essential because quantifying changes in emitted fluorescence from labeled neuronal populations requires the intensity and polarization state of the excitation pulses to remain constant as the animal moves. Stable excitation is critical to avoid fluctuations in fluorescence not associated with neuronal activity but instead due to bending-induced changes in pulse properties. Our HC-PBGF design had an inner core diameter of 14 µm, a bandgap centered at 1320 nm and a range covering the excitation spectrum shown to be efficient for 3PE of genetically encoded calcium indicators (1250–1400 nm).

The head-mounted microscope weighed a total of 5.0 g, well suited to cortical imaging in rats (Figure 1). The beam exiting the fiber was collimated with an achromatic lens before the microelectromechanical systems (MEMS) scanner [10]. After the MEMS scanner, a commercial aspheric lens, constituting the scan lens, provided beam magnification in combination with a custom-designed tube lens. The custom-designed water-immersion high numerical aperture objective (NA 0.9, back aperture ⌀5.4 mm, field-of-view ⌀200 µm) was optimized for a telecentric working distance of 1.75 mm. We further increased the working distance of the objective lens by adjusting the distance between the scan and tube lenses for an expanding beam. Altogether, the system allowed frame rates of up to 27.78 Hz at 120×120 pixel resolution with a maximum square field of view (FOV) of side-length 140 µm. Pulse width measured with a photodiode at the objective focal plane with water immersion, after tuning of the dispersion management system, was 52.3 fs (74 fs autocorrelation FWHM, assuming Gaussian pulse shape). The pulses passing through the microscope elicited 3PE of fluorescein. We synchronized the timing of each pixel in the image to the timing of the pulses emitted at the laser, and each pixel contained the fluorescence resulting from excitation by a single pulse. We also gated the photon detection system to integrate only over one-twentieth of the inter-pulse time, which significantly increased the signal-to-noise ratio (SNR).

Reaching all cortical layers

With the microscope mounted on a micromanipulator to explore the achievable imaging depth, we imaged labeled neuronal somata and dendrites down to a depth of 1120 µm below the cortical surface (Figure 1). As we measured the average thickness of the posterior parietal cortex in the area being imaged to be 1343±12 µm (mean±SD), the microscope is capable of functionally imaging 83 % of the cortical thickness (Figure 1) of mature rats (weight 193.5±23.0 g, mean±SD). At a depth of 1120 µm, the infrared (IR) power after the objective was 100 mW. We recorded clearly resolvable spontaneous Ca^{2+} transients at multiple cortical depths down to, and including, the maximum depth of 1120 µm (Figure 1). To estimate single-frame SNR, we compared soma fluorescence during periods of inactivity to the mean of the same number of pixels drawn at random from parts of the frame not containing neuronal somata. The mean single-frame SNR did not systematically change as the imaging depth increased, and the average SNR for all neurons in the most superficial FOV was not significantly different to that of the deepest FOV. To confirm that we could record meaningful functional data, we imaged neuronal populations in visual cortex during presentation of drifting grating stimuli. We found neurons with distinct orientation tuning, as shown previously [11], and continuous imaging (50 min total imaging, 17 neurons) did not alter neuronal orientation tuning, showing that prolonged exposure to 3PE was not detrimental to functional responses.

Behavior

We next performed a series of functional imaging experiments in freely moving animals, again in posterior parietal cortex with neuronal populations expressing GCaMP6s (Figure 2). To quantify the ability of the 3PE head-mounted microscope to stably record functional activity across a range of animal behaviors, we optically tracked the animal's position [12] during explorative, grooming, feeding and chasing behaviors. This approach generated a range of animal velocities, accelerations and head positions over

the continuous imaging periods. Each animal's heading (azimuth), while biased to the directions along the long axis of the track, covered all directions of movement, and the animals covered long distances (mean distance traveled: 22.9±13.5 m). During the behavioral sessions, average head pitch was around -30˚ and head roll around 0˚, both similar to our previous findings [12], indicating that the animal's posture was not substantially influenced by the presence of the microscope. Neuronal populations could be continuously imaged for over an hour. This enabled activity from cortical layer 5 neuronal populations (Figure 2) to be quantified during free behavior (mean imaging depth 736 µm). Imaging at these depths was stable, allowing Ca^{2+} transients to be extracted from individual neuronal somata and dendrites. The lateral image displacement was not correlated with the animal's movement or recording depth. Uncorrectable image displacement in the axial plane occurred infrequently (20 frames in 6000). From motion-corrected image frames, we recorded both isolated stereotypical Ca^{2+} transients, presumably from single spikes or short spiking bursts, and sustained Ca^{2+} transients, presumably from periods of sustained spiking, from both neuronal somata and apical dendrites at all depths imaged. We next assessed the effects on the tissue of the prolonged exposure to 3PE in freely moving animals. Near-IR excitation sources have the potential to cause photodamage [13, 14]. Photodamage induces sudden rises in intracellular Ca^{2+} concentration [13], recorded as progressive increases in both baseline fluorescence, and increases in decay time constant of action-potential-evoked Ca^{2+} transients [14]. We observed no significant difference in mean soma baseline fluorescence between the first and last halves of imaging ses-

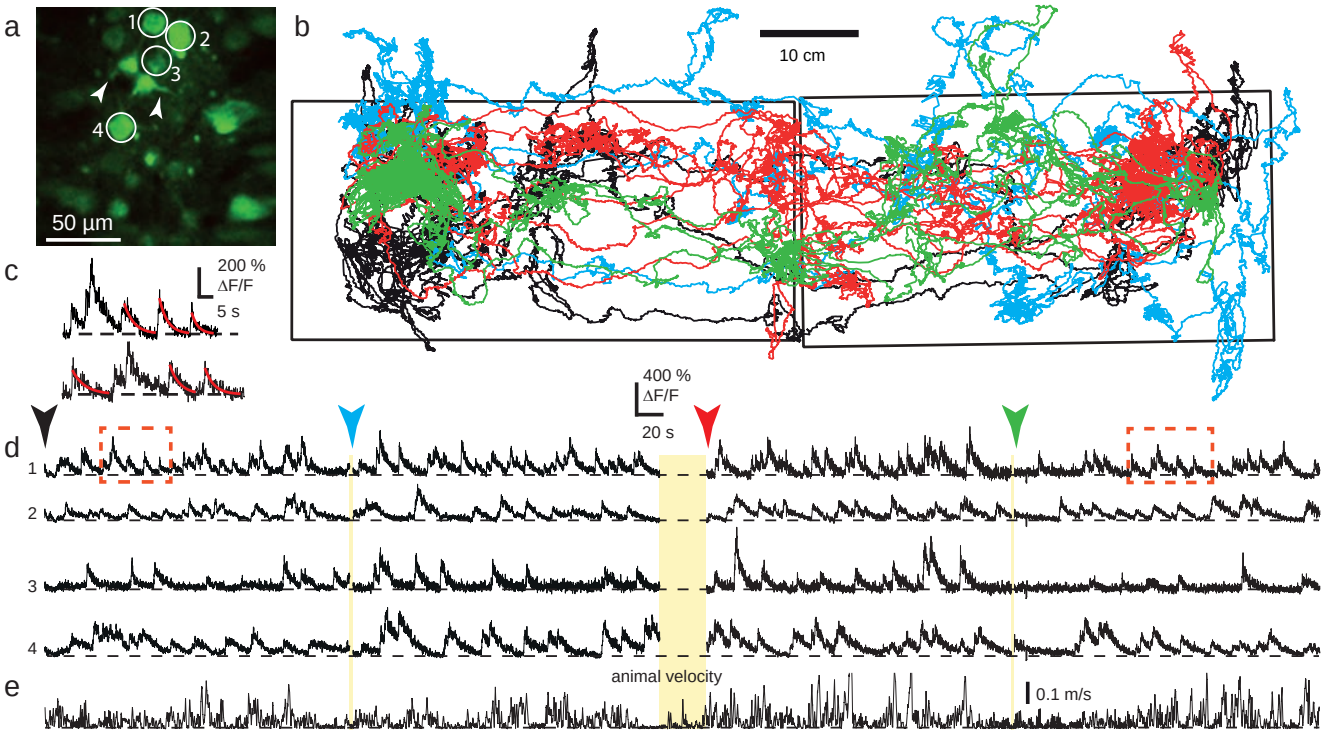


Figure 2: Three-photon imaging of cortical layer 5 neuronal activity in freely moving animals. a) Overview image of GCaMP6s-labeled neurons and dendrites 950 µm below the cortical surface (numbers correspond to calcium traces in d). Note basal dendrites (arrow heads). b) Animal head position during four consecutive ~3.6 min imaging periods overlaid (each color denotes separate period) on an outline of the raised platforms (black rectangles). Note that the animal regularly reached or peered over the edge of the track resulting in the points outside the track boundaries. c) Example Ca^{2+} transients from the initial and last imaging periods (red dashed boxes for Neuron 1 in d)). Exponential fits to the transient decay used to assess the decay time constant as an indication of neuron health (red). d) Ca^{2+} traces from the neurons and dendrites indicated in a). The panel shows four consecutive imaging periods (arrow heads at beginning of each period, colors denote periods in b). Brief pauses between imaging periods were for assessing animal status (yellow boxes). e) Continuous trace of the animal's velocity.

sions. To test for changes in transient decay times, we first identified as many neurons as possible with isolated transients of consistent amplitude. By fitting the decay of the calculated mean transient with a single exponential, we could calculate a decay time constant (τ) for each neuron. We found no significant difference in τ when comparing individual Ca^{2+} transients from the first and last imaging periods from the same neuron.

Outlook

We show here a miniaturized head-mounted 3PE fiber-based microscope capable of imaging activity from neuronal populations in deep layers of the cerebral cortex in freely moving rats. The excitation efficiency from our custom fiber and improved compensation approach allowed continuous imaging from neuronal populations without damaging the tissue. Our microscope design enabled stable imaging and extraction of Ca^{2+} transients during a wide range of animal behaviors, from quiescent to rapid sustained running. We expect this approach to be widely applicable to behavioral research as previous freely moving studies [12] and studies recording neuronal populations during behavior of head-fixed animals on moving treadmills [15] have made major biological findings based on behavioral epochs lasting on the order of a few seconds per behavioral task.

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DEPARTMENT OF

BEHAVIOR AND BRAIN ORGANIZATION

Jason Kerr

Scientific Director

The primary research aim of the Department of Behavior and Brain Organization (BBO) is to gain a mechanistic understanding of how mammals use vision to make decisions. Addressing this question involves developing experimental and computational techniques to quantify both the animal's behavior as well as the underlying computations performed by the neural pathways. As the link between brain function and behavior can only be studied in a behaving animal, and as visually based behaviors involve a combination of head movements, eye movements, and vestibular driven neuronal activity, we study the freely moving animal. We develop head-mounted multiphoton microscopes for imaging neural activity across cortical layers and head, body and eye tracking techniques that can be used on freely behaving animals from a range of species. The use of different species allows, for example, the comparison of how the eye movements of different animals vary in coordination and nature. The overall aim of this approach is to generate a thorough understanding of mammalian vision and the organization of the underlying neuronal circuits.

“We developed a small head-mounted three-photon microscope, capable of imaging all cortical layers in a freely moving rat.”

A sense of direction – Towards understanding a biological GPS

By Elmar Behrmann | Structural Dynamics of Proteins

Many of us have a hard time navigating unknown territories, and our sense of direction is sketchy at best. Instead, we have to rely on technical tools such as GPS. Birds however have a remarkable sense of direction that relies on proteins instead of technical gadgets.

A sense of direction: Birds can detect and follow the Earth's geomagnetic field during migration. We try to find out how proteins allow them to do this astonishing feat. Image: caesar.

A sense of direction

The recently published Google community mobility reports [1] have made apparent how widespread the use of mobile devices for navigational tasks is nowadays. Analog maps, and compasses, are rapidly becoming historic artefacts – or at least tools for very specific settings. Navigating without the help of tools is largely considered either a sport or an oddity. Differently many animals – and even some bacteria – are known to successfully manage complex navigational tasks without employing any tools, neither digital nor analog. Birds are known to migrate for hundreds of kilometers over open ocean on cloudy nights – negating visual landmarks or stellar maps as guiding features. While this is a setting were errors in finding one's route are likely lethal, different from most of our navigational issues that only result in annoying detours or the occasional traffic jam, migratory birds still thrive, implying they have an innate sense of direction. It is established and experimentally validated that in migratory birds this sense of direction is based on magnetoreception. While bacteria likely employ magnetic particles, which would act akin to a nanoscopic compass needle, to detect magnetic field lines there is good evidence that migratory birds, either exclusively or in addition to magnetic particles, feature a purely protein-based sense allowing them to integrate geomagnetic cues into their navigation [2].

A biological GPS

The concept that geomagnetic information could be detected by proteins dates back to a seminal paper by Klaus Schulten in 1978 [3]: if the yield of a photochemically induced radical pair is sensitive to the relative direction of the geomagnetic field, this could stimulate distinct responses in the protein harboring this radical pair. A radical pair corresponds to two uncoupled electrons in any of the proteins amino acid side chains, and it is known that these two radicals can exist in two states: either singlet or triplet. In a simplified view the former corresponds to two antiparallel oriented miniature electromagnets, while the latter is more akin to two parallel oriented ones. Importantly, both states interact differently with external magnetic fields, and can be more or less stable depending on the relative spatial orientation.

Now how much of an energy difference are we talking about? Is the energy brought by the coupling between these two radical pair electrons and the magnetic field enough to determine the molecular fate of a whole protein comprising thousands of atoms and their associated electrons? This of course depends on the strength of the external magnetic field. Regretfully – from the point of view of a protein supposed to sense magnetic field lines - the geomagnetic field of our Earth is rather weak with an average strength of 50 micro-Tesla. How weak is that? It is comparable to the magnetic field produced by a toaster or microwave at a distance of 30 cm, and it is an everyday experience that neither of these household appliances shows strong attractive magnetic forces. All in all, the energy difference is in the same order of magnitude as spontaneous fluctuations caused by the thermal energy at ambient temperatures. So a random thermal event would be as likely a cause of structural changes in the protein as a magnetic event – which would make for a very poor detector of magnetic field lines: imagine placing a magnetic compass needle on a boiling pot of water – this would also make for a poor orientation aid as the thermally introduced noise, that is bubbling, would drown out any magnetic reorientations of the needle.

The trick making the radical pair theory feasible is that the magnetic sensor state of the protein is not a stable ground state but a highly instable state induced by the absorption of a high-energy photon. Peter Hoore has in a beautiful analogy likened this to the example of a fly affecting the position of a block of stone: if that stone is lying flat on its surface the fly has not enough energy to affect anything. However, if the block of stone is tipping on one of its corners – a highly instable state – bumping of the fly onto the surface could affect the falling direction [2].

A candidate for sensing magnetic fields

Much evidence has pointed towards cryptochromes, proteins typically involved in light-induced DNA repair and control of the day/night rhythm, as the sensor protein. Firstly, these are the only vertebrate proteins known to be able to create light-induced radical pairs that are long-lived enough to be affected by magnetic stimuli.

Secondly, a bird-specific isoform of cryptochrome, cryptochrome 4, has recently been detected inside the retina of migratory birds. While the magnetic field permeates all biological tissues equally well, the photo-induction requires a light-transparent localization of the protein, with the eyes being the best suited places. Thirdly, cryptochrome 4 differs from other cryptochromes by a unique and extended C-terminal region – with hitherto unknown structure and function. Still, largely due to the lack of genetic tools for wild migratory birds an associated signalling pathway remains enigmatic. Moreover, on the molecular level we do not know if there is any structural change induced by the formation of the radical pair, and how this would be detected by a putative signalling partner.

Can structure explain function? Tools to study the impact of magnetic fields

As part of a collaborative research centre recently funded by the DFG we set out in 2018 to investigate the molecular architecture of cryptochrome 4 from European robin, a well-established model organism for night-migratory birds. Moreover, we have designed assays to stimulate and investigate if there are structural changes induced by magnetic stimulation. Magnetic stimuli are a well-established parameter in experiments on birds, and the so-called Emlen funnel used to record the starting direction of captive birds has been around since 1966. Helmholtz coils can be used to manipulate the magnetic field experienced by the bird inside the funnel. These techniques are however far from established for proteins. A main issue is that proteins are much smaller than birds and orienting proteins with respect to the coil system, or even just recording their orientation, is much more complicated (Figure 1 a, b). Luckily, cryo-electron microscopy in combination with our recently developed functionalized sample carrier grids (see last annual report) allows us exactly to do this: First, using our molecular tethers we can, at least approximately, orient proteins with respect to the grid surface and thus in a macroscopic controllable way. Second, establishing the orientation parameters of each protein image is an inherent step in any 3D reconstruction scheme in cryo-electron microscopy and single particle analysis (see our 2015 annual report). Third, conformational dynamics in sub-regions of a protein can be detected and visualized by computational methods (see our 2016 and 2017 annual reports).

When using Helmholtz coils to control the magnetic field experienced by the proteins, as established for bird experiments, it is of paramount importance to exclude magnetic or magnetizable instruments from the inside

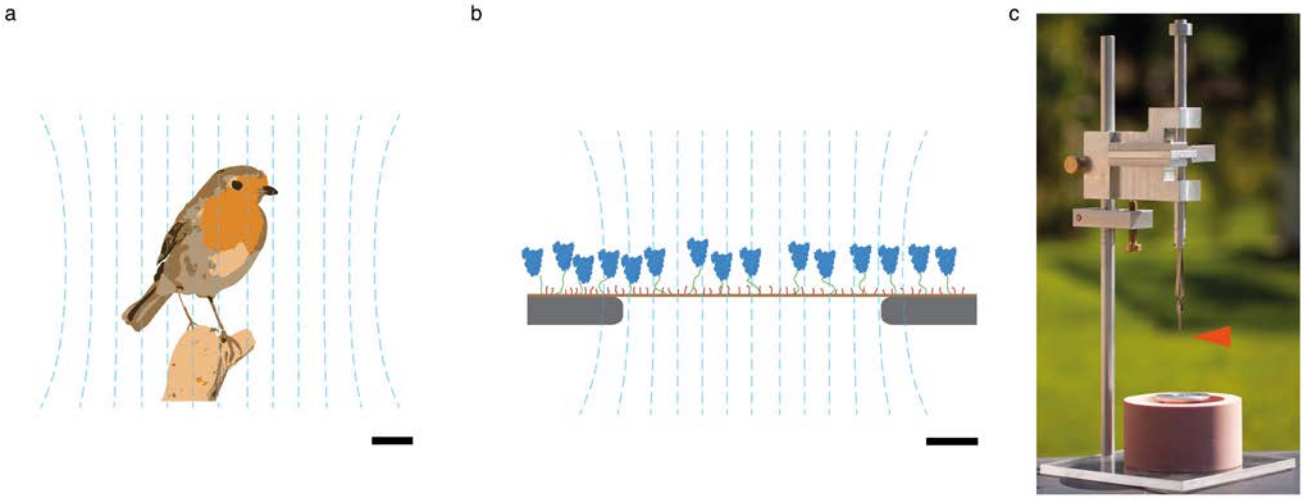

Figure 1: a) Orienting a bird with respect to an externally applied magnetic field, or alternatively recording their relative orientation, is a trivial task given the size of these birds. Scale bar is 2 cm. b) Proteins, illustrated here in blue, on the other hand are objects in the nanometer size range and thus are much harder to manipulate and track. Our functionalized carrier grids, with an activated layer (brown) that is spanning a passive holey support mesh (grey), can orient proteins with respect to the plane of the support grid by relying on molecular tethers (green, red). The grid itself has a diameter of 3 mm (about the size of the bird's eye) and can thus be oriented and manually handled. Scale bar is 200 nm, but proteins are not depicted fully to scale. c) Photograph of our second generation freeze plunger that is compatible with magnetic experiments. Components were machined from high-grade aluminum, and parts in contact with the cryogen from special foam materials. The sample carrier grid is attached at the tip of the forceps, labeled by the orange arrow head.

of the Helmholtz coils – to prevent them from creating stray fields that would interact with the experimentally designed one. Experiments on birds are, where possible, performed in wooden sheds and Emlen funnels are carefully designed from non-magnetic and non-magnetizable materials. Cryo-electron microscopy however requires the use of freeze plungers to prepare the thin liquid layers needed for proper vitrification – that is transferring the protein into the solid, glass-like state important for imaging. Regretfully, commercial freeze plungers are brimming with electronics and ferromagnetic materials, and consequently are ill-suited to work with a putative magnetosensor protein. In cooperation with the central workshop of caesar we could custom-design a freeze plunger comprising only non-magnetic and non-magnetizable materials, and still compatible with the cryogenic temperatures required for solidifying our protein samples. A second generation of this design (Figure. 1 c) has been experimentally confirmed to not interfere with applied magnetic fields, and can be used for vitrifying protein samples.

The road onwards

A main obstacle when working with proteins as small as cryptochrome 4 is to get enough contrast in cryo-electron microscopy images. We have preliminary data on a non-bird cryptochrome, and could find vitrification conditions that allow us to reliably detect the protein on our sample carriers using the Titan Krios electron microscope run by the caesar core facility for electron microscopy and analytics. Still, any technical solution helping us to increase contrast during imaging is going to be an important asset for our project. While contrast enhancement is still an active field of research in electron microscopy, with projects also ongoing at caesar, commercial solutions have reached the market few years ago and are now in a mature state. Accessing microscopes equipped with these would greatly simplify our quest for detecting magnetic-induced structural changes in cryptochrome 4.

Moreover, collaborators within the research consortium have recently uncovered putative signalling partners of European robin cryptochrome 4, among them a long-wavelength-sensitive opsin [4]. Opsins are the central sensor molecules for detecting light, hinting at the possibility of shared signal transduction pathways implying that birds could actually visually experience magnetic field lines. Experimentally, co-expression of this opsin together with cryptochrome 4 would not only allow us to generate a biologically much more interesting protein complex, but would also solve the issue of the small size of cryptochrome as the complex of both proteins is much larger in size.

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MAX PLANCK RESEARCH GROUP

STRUCTURAL DYNAMICS OF PROTEINS

Elmar Behrmann

Max Planck

Research Group Leader

Life is not static and neither are the majority of proteins crucial to the function of our cells. However, our structural understanding of these microscopic machines is often limited to one or at best few static snap-shots.

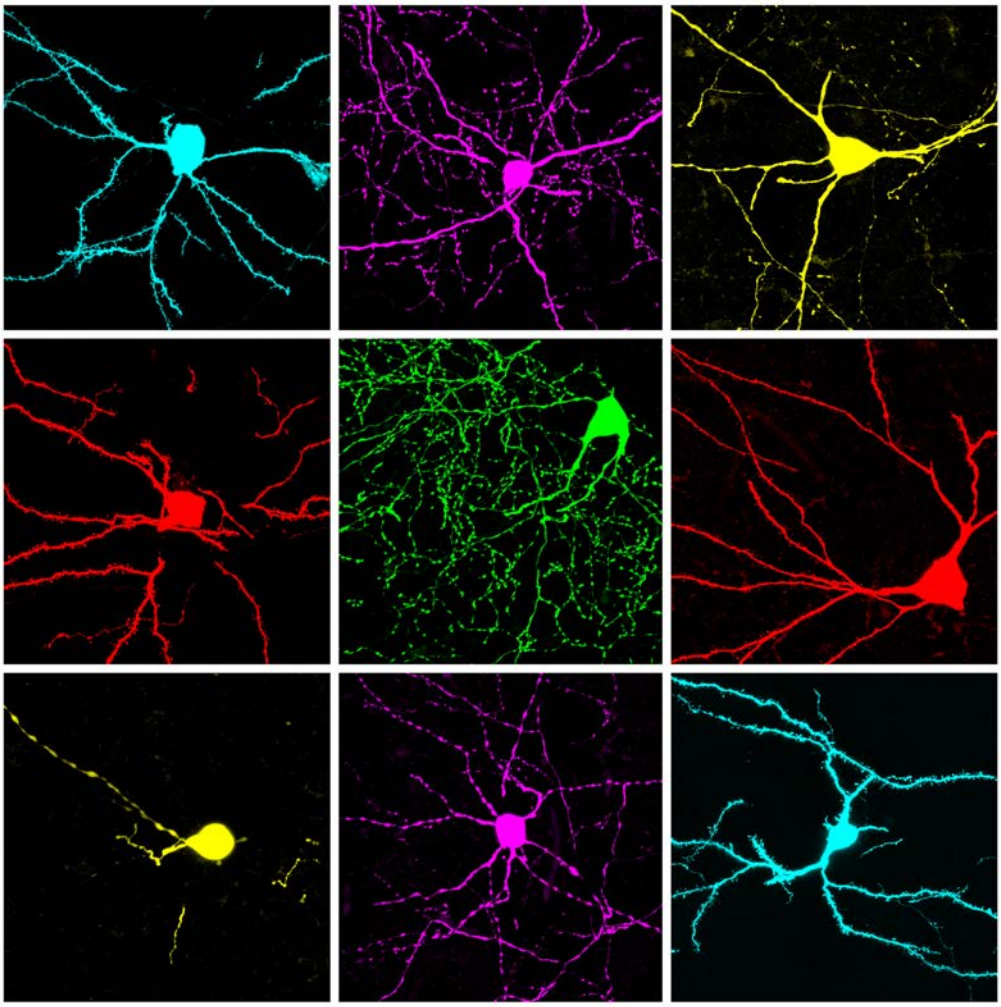
We focus on the application of electron microscopy to visualize such dynamic entities in a native-like environment in order to deduce the structural pathways at the heart of biological processes. Our main interest is in those proteins that are embedded into the lipid membranes of our cells. The structural basis for how proteins can give diverse functions to membranes is still largely lacking, especially with regard to the dynamic inter-play between lipids and proteins. To allow us to visualize such dynamic entities in their native-like environment, we develop sample preparation strategies that will enable us to investigate membrane proteins in defined functional states.

“Life is not static and neither are the majority of proteins crucial to the function of our cells.”

Cortex *in Silico* – Digitizing the brain's networks

By Marcel Oberlaender | In Silico Brain Sciences

In 2019, we made major progress towards our long-term goal of generating anatomically detailed digital representations of the mammalian neocortex. In this report, I will highlight one project that investigates the impact of neuron development on cortical network architectures.



Probing the structure, function & synaptic connectivity of the neocortex. Nine exemplary neurons from rat neocortex whose sensory-evoked synaptic inputs and activity patterns were reconstructed and recorded *in vivo*, respectively. The colors indicate excitatory spiny neurons in layer 4 (cyan) and pyramidal tract neurons in layer 5 (red), as well as inhibitory cell types: basket cells in layer 4 (green), Martinotti (yellow) and neurogliaform cells in layer 5 (magenta). Image: IBS.

Background

Understanding how the brain is able to transform sensory input into behavior is one of the major challenges in systems neuroscience. While recording/imaging during sensory-motor tasks identified neural substrates of sensation and action in various areas of the brain, the crucial questions of 1) how these correlates are implemented within the underlying neural networks and 2) how their output triggers behavior, may only be answered when the individual functional measurements can be integrated into a coherent model of all task-related neuronal circuits.

My group uses the whisker system of the rat for building such a model in the context of how a tactile-mediated percept (e.g. texture) is encoded by the interplay between different cellular and network mechanisms. Rodents, such as rats and mice, actively move their facial whiskers to explore the environment. Our group has developed approaches for generating a digital (i.e., *in silico*) representation of the rodent whisker system. The resultant anatomically and functionally detailed neuronal network models allow performing computer simulations that mimic the *in vivo* streams of whisker-evoked excitation at subcellular resolution and millisecond precision. The simulations provide unique opportunities to investigate how the interplay between different cellular and network properties can give rise to neural substrates that underlie sensory information processing [1], and ultimately sensory-guided behaviors.

The impact of neuron and neurite development on cortical wiring

The structural organization of the neocortex is more complex than that of any other biological tissue. Each cubic millimeter contains hundreds of meters of dendritic, and several kilometers of axonal pathlengths. These neurites originate from hundreds of thousands of neurons with diverse structural, functional and/or genetic properties, and form on the order of a billion synaptic connections. This extremely dense and diversely structured composition of the cortical neuropil is the result of genetically induced programs with different critical periods during embryonic and postnatal development (Figure 1, next page).

Neurogenesis in combination with radial migration and several neurite growth mechanisms guide the neurons' cell bodies (somata), dendrites and axons into specific subvolumes of the neocortex. Rather than instructing a tabula rasa-like cortical sheet, these developmental programs recognize gradients in signaling molecules that partition the neocortex into different areas. As a result, the cellular organization horizontally into cytoarchitectonic layers and vertically into functional columns or maps is highly specific for each area and species. Moreover, dendrite and axon morphologies develop properties that correlate with different features – depending on the cell type – of the areas' characteristic laminar and/or columnar layout.

Once developed, layers, maps and neuron distributions, as well as dendrite and axon morphologies remain largely stable throughout life. Defining the specific structural composition of each cortical area, these features hence provide life-long constraints for which neurites – and of which neuronal populations – can in principle form synaptic connections with each other. However, to what degree the development of neurons and neurites might contribute to the architecture and topology of neocortical networks is unknown. Answering this fundamental question faces several challenges. During development, and to a lesser extent throughout an animal's life, the wiring diagram of the neocortex is constantly remodeled by learning and experience via mechanisms that form, eliminate and replace synaptic connections in an activity-dependent manner. In turn, activity can also be part of genetically induced developmental programs. For example, mechanisms that guide thalamocortical axons, and which thereby contribute to the formation of cortical maps, require both spontaneous and periphery-driven neuronal activity. Moreover, synapses can form based on genetically encoded wiring rules, where molecular recognition between specific pre- and post-synaptic compartments results in connections that depend on subcellular, cellular and/or cell type identity. This plethora of mechanisms involved in the generation and remodeling of synaptic connections represents a major limitation when trying to infer post hoc the origin and relevance of empirically observed wiring patterns: Which patterns could reflect the results of neuron and neurite development, which ones

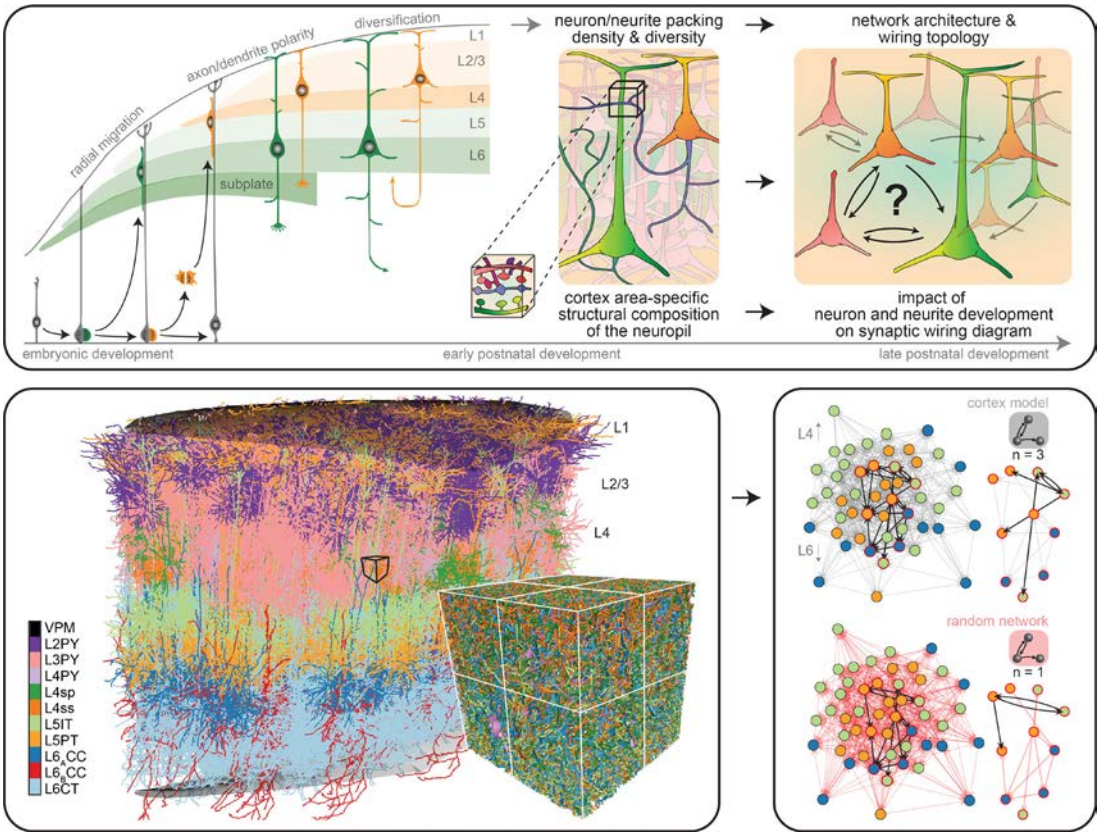


Figure 1: Top: By shaping the structural composition of cortical areas, developmental programs constrain which neurons can form synaptic connections. Bottom left: We generated an anatomically constrained digital model that provides robust estimates of the barrel cortex' dense structural composition. Bottom right: We compute how wiring diagrams would look like if they reflected solely the structural composition. The predictions deviate sustainably from random networks. (Udvary et al., in preparation).

reflect learning, experience or genetically defined cellular identity – and combinations thereof? Finally, connectivity measurements in the neocortex are either limited to sparse reconstructions from pairs or small groups of neurons, or dense reconstructions of very small volumes. Consequently, complete wiring diagrams for sufficiently large volumes, and hence quantitative comparison of cortical architectures across animals and developmental stages, remain presently inaccessible.

We quantitatively addressed these questions and challenges by developing an inverse approach that predicts how wiring diagrams of the neocortex would look like if they reflected solely developmental programs that shape its structural composition – i.e., in the absence of learning, experience and genetically encoded wiring rules. We applied our approach to the whisker related part of the neocortex – the barrel cortex. During the past decade, we had systematically characterized the distributions of neurons [2] and neurites [3] across the rat barrel cortex, the innervation of this cortical area by axons from the primary thalamic nucleus of the whisker system [4] – the VPM, and developed computational approaches for integrating such data from different animals into a common reference frame. We now combined these data and computational approaches to generate a digital model of the barrel cortex' structural composition. We show that the model provides robust and anatomically realistic estimates for cell type-specific soma, dendrite and axon packing density distributions across a volume that is sufficiently large to capture the underlying principles that characterize the barrel cortex' cellular and morphological organization (Figure 1). The model hence allowed calculating all possible wiring diagrams, as well as their respective likelihoods, that could originate from the underlying distributions of pre- and postsynaptic structures. This probability distribution of wiring diagrams – referred to as 'statistical connectome' – revealed direct links between the packing density and diversity of neurites across subvolumes of the neo-

cortex, and the degrees of sparsity, heterogeneity, correlations and recurrence of wiring diagrams that are formed by these neurites. Complex, non-random topologies of networks in the neocortex can hence emerge directly from the dense structural composition of the underlying neuropil, and thereby be defined implicitly by genetically induced programs that shape soma, dendrite and axon distributions during development. This theory predicts properties of the barrel cortex' wiring diagram that are remarkably consistent with the empirical data from sparse and dense reconstructions that is presently available.

Outlook

We are currently developing novel experimental and computational approaches to explore how mammals can generate robust sensory percepts despite variations in stimulus configurations. Specifically, the ability to identify objects regardless of context – such as variations in the object's position, size or orientation – has been observed in the visual and somatosensory systems of both primate and rodent species. The computations that give rise to invariant neural representations remain however poorly understood. We therefore seek to investigate the cellular and circuit mechanisms that underlie context-invariant perception of textures in the rodent whisker system. Tactile texture discrimination is an ideal tractable experimental paradigm by which to study robust perception. Rodents learn rapidly to discriminate arbitrary textures, even if parameters such as position, angle or shape of the presented textures are varied. Moreover, a single facial whisker can provide sufficient information for this behavior, which simplifies analysis of movement, sensory input, and relevant neural circuits. The behavior requires high-level cortical processing – even when the sensory input has been simplified.

We are focusing our investigations on pyramidal tract neurons, which represent the major output cell type of the neocortex. These neurons integrate feed-forward thalamocortical excitation, with recurrent intracortical and top-down corticocortical inputs, and broadcast the results of this integration to the relevant ensembles of downstream targets. Recent [5] and preliminary work from our lab shows that thalamocortical neurons give rise to two separate gating mechanisms that allow pyramidal tract neurons to reliably integrate all synaptic inputs from local and long-range populations that impinge simultaneously onto their proximal and distal dendrites, respectively. We therefore hypothesize that thalamocortical gating enables pyramidal tract neurons to combine information about the current state of their recurrent intracortical and top-down corticocortical input populations with stimulus information, and thereby provide subcortical circuits with an integrated efference copy that reflects the sensory

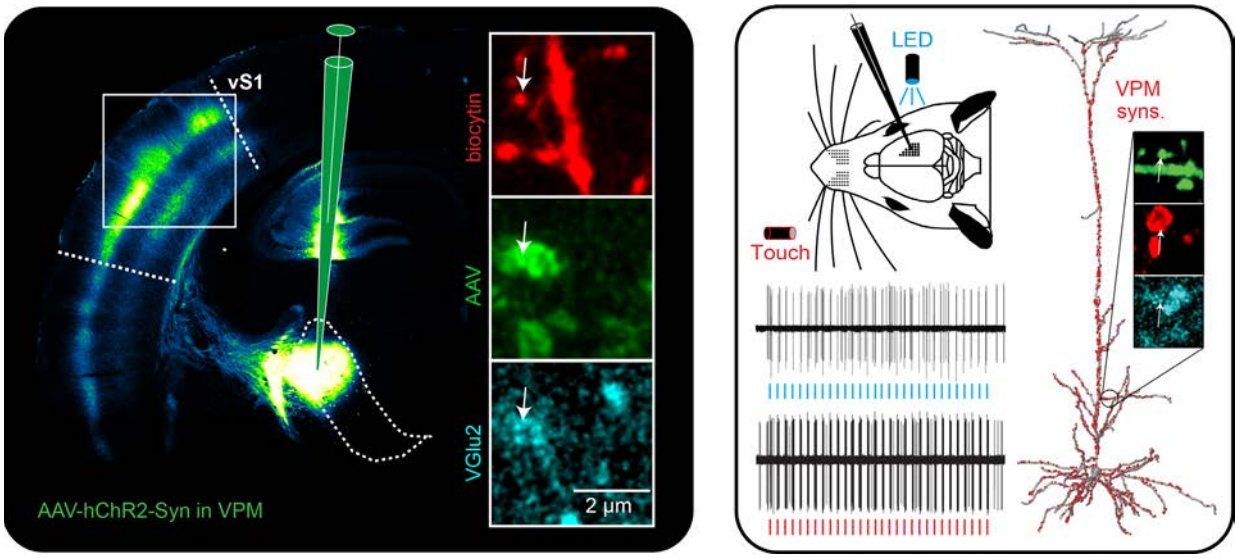


Figure 2: Left: We combine *in vivo* recordings with injections of an adeno-associated virus (AAV) into VPM thalamus to expresses channelrhodopsin and a fluorescent marker within thalamocortical synapses. Neurons are reconstructed and thalamocortical synapses (confirmed by anti-VGlut2) are detected using super-resolution microscopy. Right: Preliminary data of VPM synapses along pyramidal tract neuron dendrites vs. light- and whisker-evoked responses.

input and the context of how it was presented. To test this hypothesis, we are currently characterizing the distributions of thalamocortical synapses along the dendrites of *in vivo* recorded pyramidal tract neurons using an optogenetic approach that we have recently developed in the lab (Figure 2). We will combine these data to further constrain our barrel cortex network model. We will use the model for simulations in which we explore how feed-forward thalamocortical gating can regulate the integration of intracortical with top-down cortico-cortical inputs. Finally, we will test the *in silico* predictions in rats that perform texture discrimination.

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MAX PLANCK RESEARCH GROUP

IN SILICO BRAIN SCIENCES

The overarching goal of our research is to understand how the interplay between biophysical, synaptic, cellular and network mechanisms in the mammalian brain can encode perception and trigger behavioral responses, such as decision making during a sensory-motor task. To pursue this goal our group employs a multidisciplinary approach, combining network anatomy with cellular physiology and computational modelling. The long-term goal of our research is to generate anatomically realistic neuronal network models, and to use these *in silico* representations of the mammalian brain for simulation experiments that mimic *in vivo* conditions, for example during sensory-guided behaviors such as texture discrimination.

Marcel Oberlaender

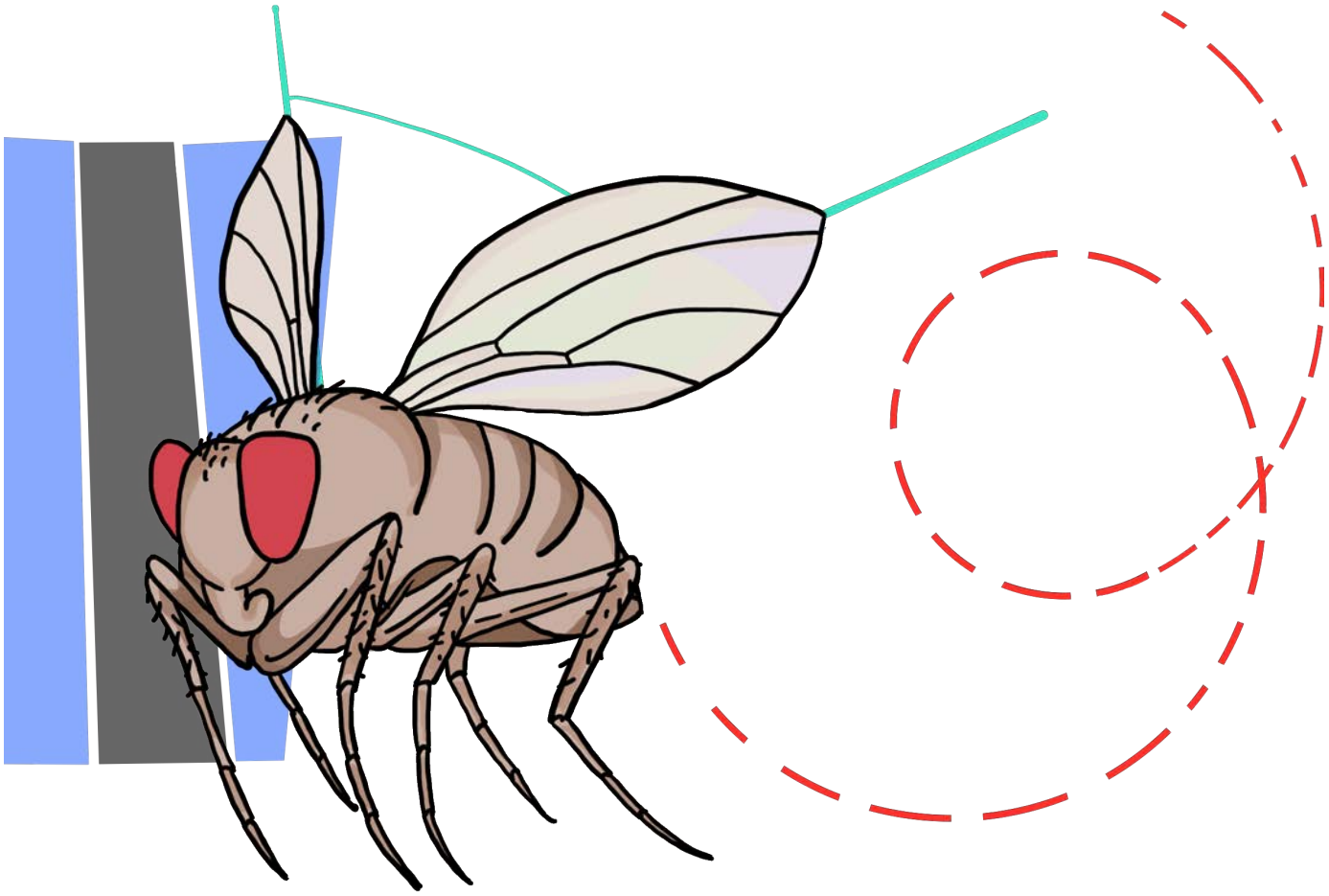
Max Planck

Research Group Leader

Control of saccadic turns in *Drosophila*

By Bettina Schnell | Neurobiology of Flight Control

Using the flight maneuvers of the fruit fly *Drosophila melanogaster* as a model system, we study how behavioral actions are controlled by neural circuits and how the behavioral state of an animal in turn influences the processing of sensory information.



Our group aims to identify and study individual neurons that control steering maneuvers of the fruit fly *drosophila melanogaster* during flight. Image: caesar.

Drosophila as a model system

To navigate through the world, animals rely heavily on sensory stimuli that can indicate e.g. obstacles, food sources, potential mates or predators. How an animal reacts to such stimuli is strongly dependent on its behavioral and motivational state. The main interest of my research group is to figure out how the nervous system selects and controls the appropriate action in response to sensory stimuli. We use the flight maneuvers of the fruit fly *Drosophila melanogaster* as a model system to study that question for the following reasons.

First, steering maneuvers can be measured in head-fixed flies as changes in the parameters of wing motion. Simultaneously we can record the activity of single neurons using the whole-cell patch-clamp technique and thus look for neural correlates of the observed behavior. Second, due to the numerical simplicity of the fly brain – it consists of only about 100.000 neurons – it seems feasible to describe the complete circuits that underlie a specific behavior in *Drosophila*. In particular, individual neurons have been described in the fly that seem to be of high relevance in that they are necessary or sufficient for a specific motor action [1]. Third, the study of such neurons and the circuits they are embedded in is strongly aided by the genetic tool kit available in *Drosophila*, which allows for manipulating the activity of neurons of interest.

The central goal of my current research is to uncover the mechanisms, by which neural circuits control steering maneuvers in *Drosophila*. The peripheral sensory circuits that are involved in processing visual information relevant for course control have been the focus of intense research. Especially large tangential cells within the optic lobes of the fly, the so-called lobula plate tangential cells (LPTCs), have been well studied, because they respond to wide-field motion in a directional-selective manner and are thought to underlie compensatory optomotor responses. However, little is known about how sensory information is integrated into a motor code and transmitted to the motor system and how this process is influenced by the behavioral state of the animal.

Influence of an efference copy on motion-sensitive HS cells

A subgroup of LPTCs, the HS cells, respond to horizontal motion with graded changes in membrane potential in a directional-selective fashion [2]. They are thought to underlie optomotor turning responses to horizontal motion, which serve to stabilize gaze as well as a straight flight path. However, when the fly performs a saccade, i.e. a fast turn to change direction, it needs to make sure that the optomotor system does not interfere and counteract the turn. This is for once achieved by very high turning rates, which do not lead to optimal stimulation of LPTCs. In addition, it has been shown that during spontaneous saccades an efference copy influences the membrane potential of HS cells, which could counteract the response to the visual stimulus elicited by the fly's self-motion [3]. The function of this efference copy, however, has not been conclusively shown.

To study the effect of an efference copy on HS cells and its consequences in more detail, we recorded the membrane potential of HS cells during head-fixed flight, while monitoring turning responses by tracking the difference in wing stroke amplitude between both wings (L-R WSA, Figure 1 a, b, next page). In addition, we presented looming stimuli in either the left or right visual field to elicit evasive turning responses. This stimulus triggers large changes in L-R WSA corresponding to turns away from the stimulated side (Figure 1 c, d, upper panels). To separate effects from the behavior from the visual stimulus itself, we made use of the behavioral variability and compared trials in which flies performed a turn with those, in which they did not. While we found a lot of variability between individual cells, on average, we observed a biphasic response of HS cells with a depolarization during the rising phase of L-R WSA and a subsequent hyperpolarization during evasive turns, which is not apparent or less pronounced if the fly does not react to the stimulus. Note that the visual stimulus is the same in both cases. This effect was stronger for rightward turns (in HS cells on the right side of the brain) compared to leftward turns. This biphasic response is different from what has been observed during spontaneous turns, where the cells either depolarize or hyperpolarize depending on the direction of the turn.

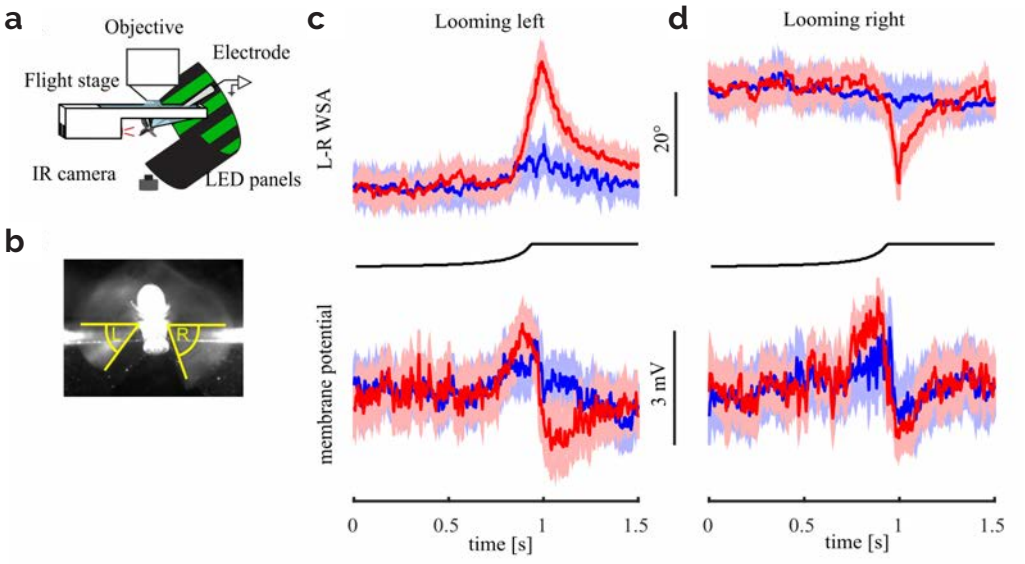


Figure 1: a) Scheme of the setup. b) Image of a fly used for measuring left (L) and right (R) wing stroke amplitude. c) Changes in behavior (L-R WSA, upper panel) and membrane potential of HS cells (lower panel) upon presentation of an expanding stimulus on the left side. Individual trials from all flies were averaged depending on whether the stimulus elicited a saccade (red) or not (blue). Black traces indicate the looming stimulus. N=8. d) Same as c) but for a looming stimulus presented on the right. N=8.

To figure out, why our recordings are so variable, we will now investigate, whether there are differences between the three different HS cells, which differ in the location of their receptive fields (dorsal, medial or ventral), by dye-filling them after recording. Our preliminary findings suggest that different behaviors can have very specific effects on HS cell signaling and that neuronal responses already at the level of sensory systems are very specifically modulated depending on the behavioral task.

To test, whether the effect of the presumed efference copy during evasive maneuvers is strong enough to affect responses to visual stimuli, we combined the looming stimulus with a subsequent presentation of horizontal motion. Again, we compared cases, in which flies performed a turn, with those, in which they did not. For rightward turns, which had the strongest effect, we indeed find that the depolarization of HS cells to rightward motion is initially reduced followed by a later increase (data not shown). Our horizontal motion stimulus does not mimic speeds typically found during saccades, but is optimal for HS cells, which could explain, why the reduction is small. For leftward motion, we find a stronger hyperpolarization in case of evasive turns (data not shown). This suggests that the efference copy is able to influence responses to visual rotation, which might alter behavioral responses to opto-motor stimuli.

Control of saccades by a descending neuron

During my postdoctoral work, using 2-photon Calcium imaging, I have identified a descending neuron, whose activity is strongly correlated with rapid changes in L-R WSA during flight, which likely correspond to free flight saccades [4]. This correlation was apparent for both spontaneous as well as stimulus-triggered turns. While the neuron is not generally activated by large-field horizontal motion, which elicits an optomotor response, its activity can explain some of the observed behavioral variability in response to that stimulus. In cases, when the neuron on one side was active during horizontal motion, this led to a very large behavioral response to motion towards the ipsilateral side. In contrast, the turning response elicited by motion towards the contralateral side were suppressed or even reversed, when the neuron was active. We are now in the process of obtaining more electrophysiological recordings from this and similar neurons during tethered flight to study in more detail under which conditions they are active. We want to know for example, if the neuron shows any activity if the fly does not respond with a turn to a looming stimulus and if it shows inhibition during saccades in the opposite direction. We also want to test responses to olfactory stimuli that can trigger turns. Artificial activation of this neuron during tethered flight, which we achieved by expressing the ATP-gated ion chan-

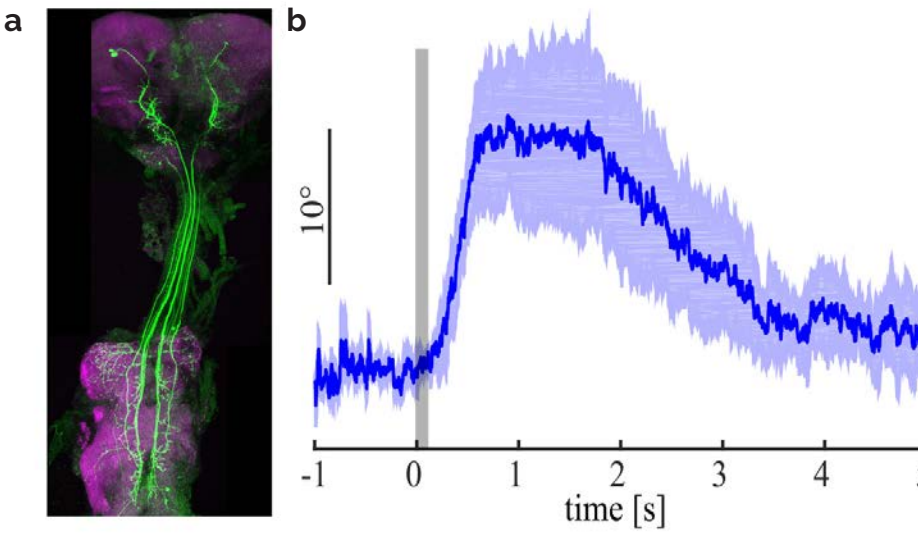


Figure 2: a) Split-Gal4-line labeling the descending neuron correlated with turning behavior (green). b) Baseline-subtracted changes in L-R WSA upon local application of ATP (shaded gray area) onto P2X2 expressing neurons. N=3.

nel P2X2 in this neuron and pressure-applying ATP locally through a micropipette, is sufficient to trigger the expected changes in L-R WSA. We have confirmed this result now using a very specific genetic driver line, which we obtained through a collaboration with Prof. Michael Dickinson (Figure 2). This suggest that individual descending neurons can have a strong effect on flight behavior.

Studying free flight behavior of *Drosophila*

The caveat of the above described activation experiments is that they were done during tethered flight, during which the wing kinematics can be very different from free flight maneuvers [5]. We therefore built a setup, which allows us to track the flight path of flies flying in a circular arena at a temporal resolution high enough to detect saccades. We plan to use this setup to test for the effects of inhibiting or activating this neuron using genetic tools. For the latter, we want to use red-shifted channelrhodopsins like csChrimson, which have to be expressed unilaterally though, to be able to elicited turns to one side. For this, tools are available that allow for stochastic expression of genetic constructs within a genetic driver line. We are currently in the process of testing these tools and of setting up the light stimulus to activate csChrimson. Using this setup, we specifically want to find out, whether activating the descending neuron described above is sufficient to trigger a complete saccade, which typically consists of a banked turn and a subsequent yaw rotation to align the fly with its new flight direction, or whether the neuron only controls a part of this more complex maneuver. In the long run, we can use this setup to test for the effects of inhibiting and activating other descending neurons on flight behavior.

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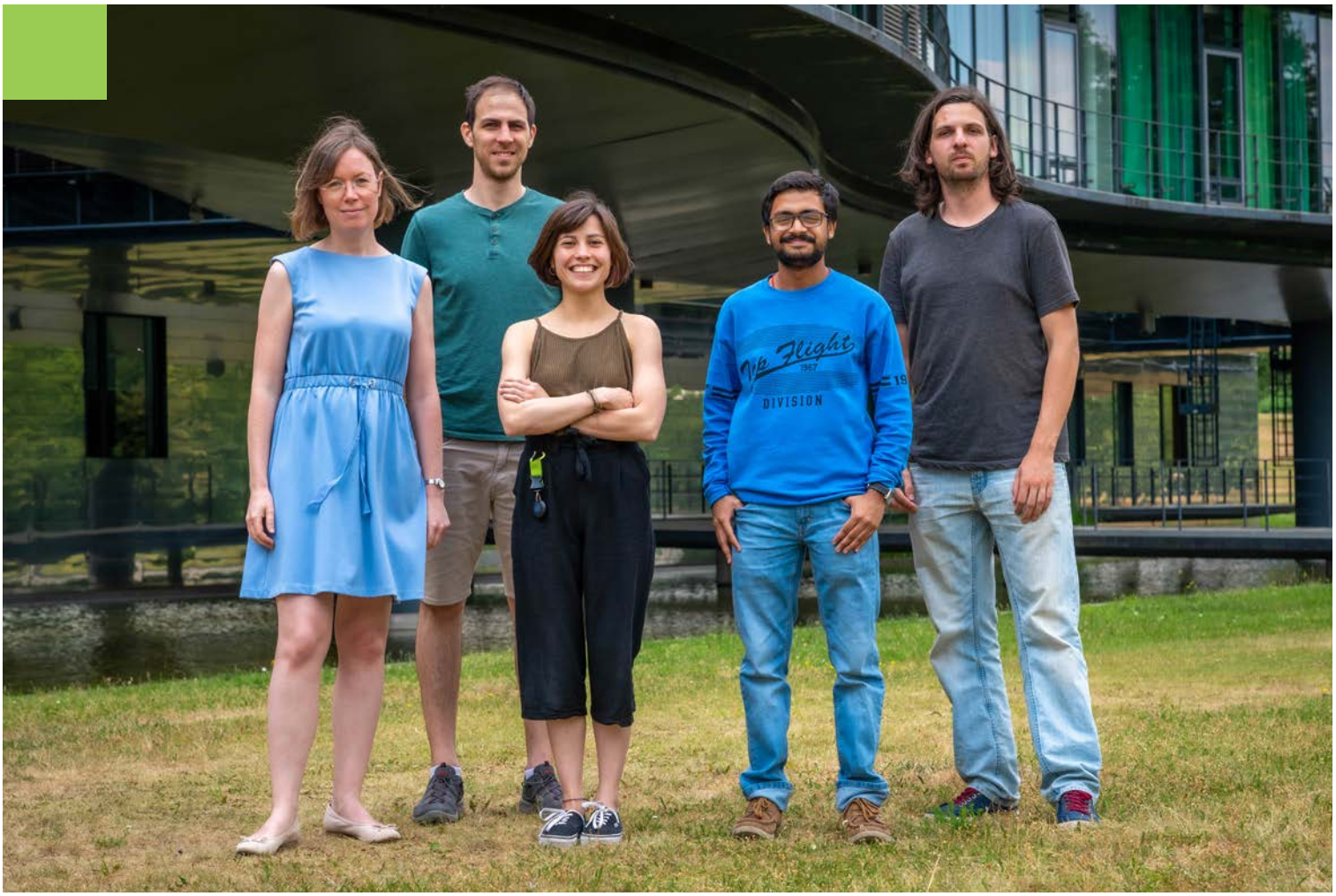


EMMY NOETHER GROUP

NEUROBIOLOGY OF FLIGHT CONTROL

Bettina Schnell

Emmy Noether

Research Group Leader

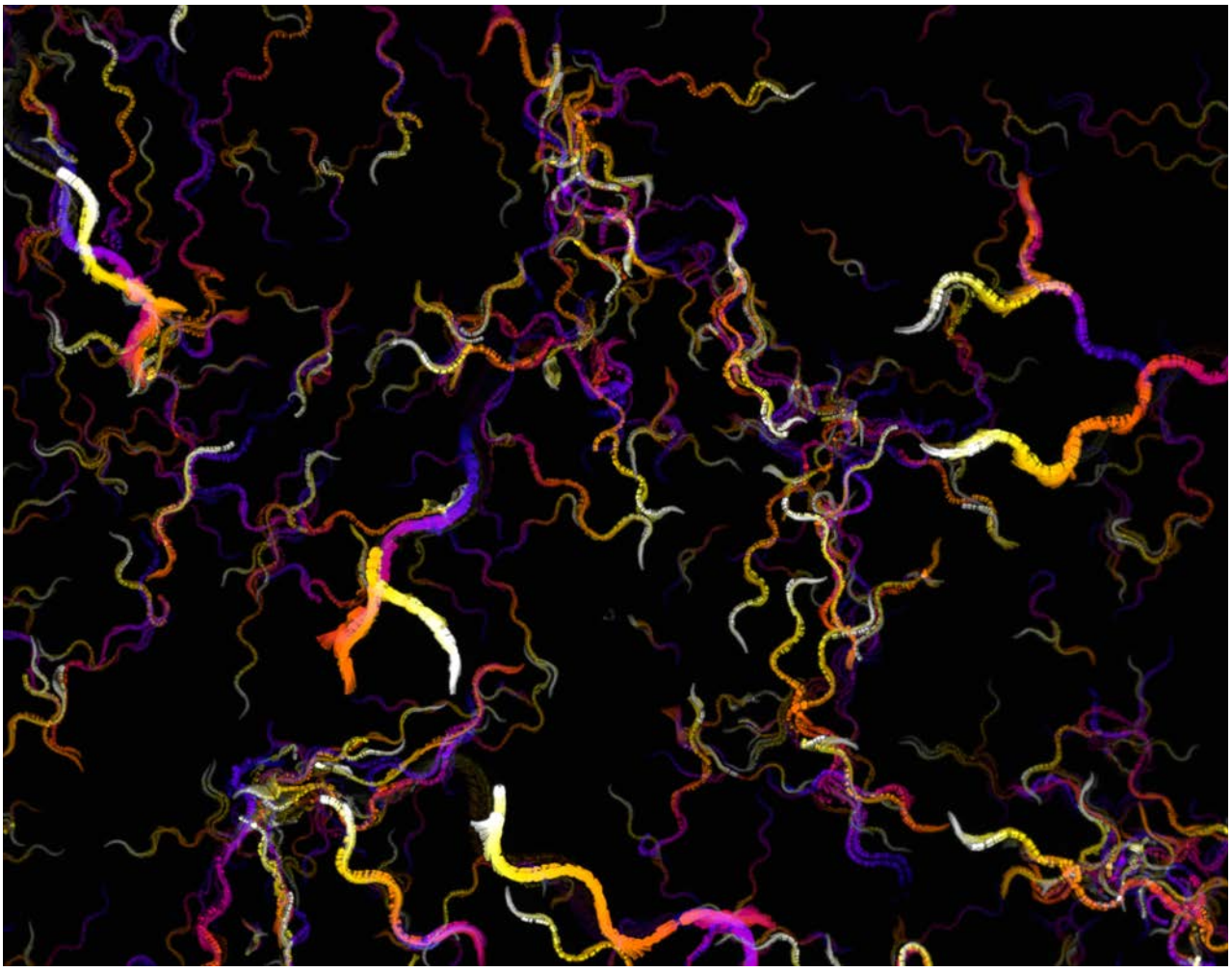
The research group "Neurobiology of Flight Control" is interested in how the tiny brain of *Drosophila* controls complex behaviors guided by sensory information. To answer that question, we make use of recent technological advances, which allow us to measure the activity of single neurons in head-fixed, but flying flies. In addition, we use the elaborate genetic tool kit available in *Drosophila* to manipulate the function of specific neurons. Combining all these techniques we aim to identify and study individual neurons that control steering maneuvers during flight, the circuits they are embedded in, and the computations they perform. We hope that this work will provide insights into general mechanisms of how neural circuits control behavior and make decisions.

"We aim to identify and study individual neurons that control steering maneuvers during flight, the circuits they are embedded in, and the computations they perform."

Finding Food – Following foraging worms

By Monika Scholz | Neural Information Flow

By observing the foraging behavior of the tiny roundworm *C. elegans*, we investigate how multiple behaviors can be coordinated through shared information. High-throughput imaging allows us to record the behavior of many animals at the same time as they search for food.



Like a dog's nose: Tracks of many worms looking for bacteria. The animals use their sensitive sense of smell to find new food sources. Image: NIF.

Foraging for food is essential for surviving a challenging world

Animals have access to a wide array of possible behaviors and need to decide which one is appropriate at any given time. One such behavior is foraging, where animals switch between exploring their surroundings and exploiting food sources. The timing of locomotion and feeding, the two behaviors underlying foraging, must be tightly controlled. Otherwise, mistimed foraging leads to starvation in food-scarce conditions, with dramatic consequences for the animal's survival.

We use the foraging behavior of the roundworm *C. elegans* to study how animals temporally coordinate locomotion and feeding behavior. The worm is an ideal model system to study fundamental questions about behavior because the biological constituents are largely known. After the connectome was published in 1986, full neurotransmitter, receptor, and a single cell transcriptome maps have followed. These data are a solid basis for understanding behavior at all scales, from the functions of molecular receptors on the actions of individual neurons to complex behaviors.

C. elegans feeds on bacteria

C. elegans is a bacteriovore that forages in patchy environments, where nutrient-dense spots of bacterial colonies are interspersed with large distances without food. To survive in these complex environments, animals need to seek patches and feed only when they are within a food-rich environment, since feeding has a small but non-zero cost [1]. For example, worms alter their locomotion rapidly upon encountering a patch of dense food, and adapt their feeding rate to the amount of food present. It remains unclear in which order the slowdown upon food encounter and upregulation of feeding rate occur, and how they are controlled in efficient foraging. To understand how these two behaviors are connected, both need to be observed simultaneously.

Measuring feeding in a moving animal

C. elegans feeding relies on the pumping action of the pharynx. We have previously developed assays to measure pumping in immobilized animals [2]. Studying foraging, however, requires the observation of feeding in a moving animal. Observing both feeding and locomotion is challenging due to the large range of time and length scales involved: worms forage over centimeters in minutes, while the feeding organ is displaced by only 10 µm per contraction.

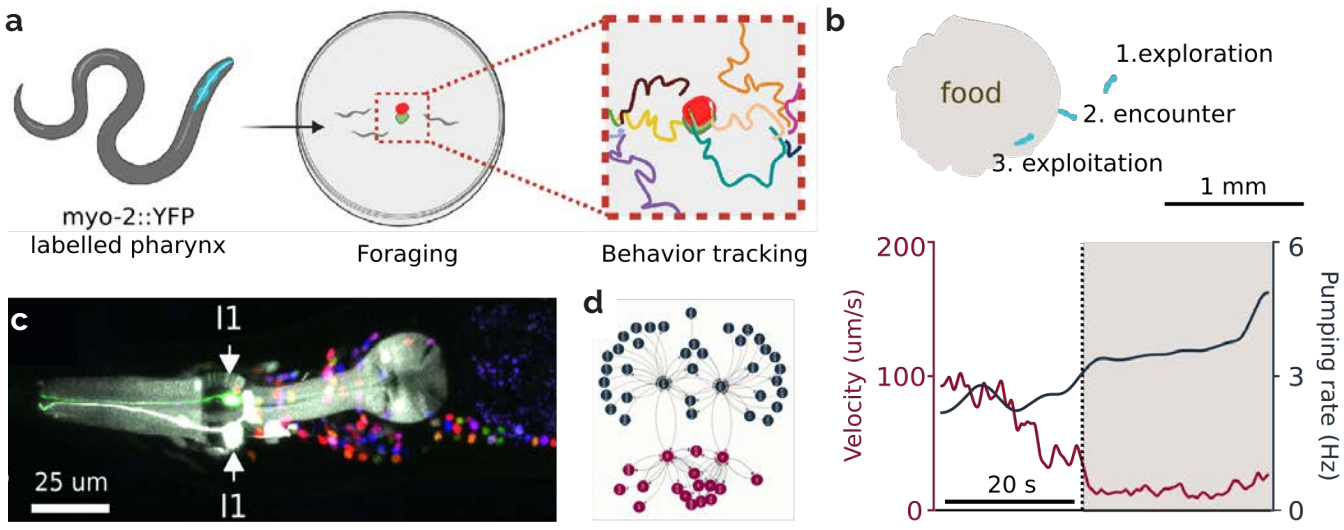


Figure 1: Automated detection of feeding and locomotion in foraging animals. a) Schematic of a foraging assay for *C. elegans*. Worms are placed in a foraging arena with a millimeter-sized food source and imaged during foraging. b) An animal progressing through the stages of foraging, exploration, encounter, and exploitation. Velocity and feeding rate of animals entering a dense spot of bacteria. At the time of encounter (black, dashed line), animals slow down and increase their feeding rate. c) Scan of all neurons of the worm brain, with the bottleneck between pharynx and somatic networks highlighted (white text). d) Schematic of the bottleneck.

To overcome this challenge, we use animals that express a fluorescent reporter in the pharyngeal muscle (Figure 1 a). We developed a battery of cost-efficient microscopes with fast 4k cameras that allow us to synchronously observe as many as hundreds of worms per day. Our fully automated tracking software allows us to accurately detect feeding in these behavior recordings (Figure 1 b). This allows us to quantify not only the mean rates of food intake, as is common in the field, but full distributions and inter-animal variation as well.

Perturbing the neural network

We can therefore detect contractions in freely moving animals while tracking their locomotion in a field of view the size of multiple animal body lengths (Figure 1 a). To systematically study the neural circuit underlying foraging control, we are currently developing a library of genetic tools to specifically target the implicated neurons (Figure 1 c). Thus far, we have created strains that lack components of olfactory processing to modify how a food stimulus is detected through the known olfactory neurons AWA, AWB, and AWC. We have also created animals lacking a type of gap junction to remove the connection between the pharyngeal and somatic neural networks. Using these perturbations, we will be able to find out how the smell of food travels from the sensory neurons, through the brain and to the pharynx - telling it 'here is food, start eating'.

Two subnetworks control locomotion and feeding

Foraging is an interesting behavior to observe when trying to understand how information travels in the brain: locomotion and feeding are controlled by two entirely distinct neural circuits. Yet, these two circuits need to communicate effectively during foraging. These two subnetworks are only connected by a bilateral pair of gap junctions, forming a bottleneck between them. Intriguingly, sensory information is still transmitted between the networks, e.g. a touch detected by the touch-sensory neurons of the somatic network stops feeding in the pharynx. Bottlenecks are common both in biological networks as well as artificial networks, and are often the crucial ingredient for separating relevant information from irrelevant information. How information is transmitted and how it is compressed in this particular neural bottleneck is a key question in my group (Figure 1 d).

Looking to the future

Moving forward, we will systematically step through the circuit from the sensory periphery to the pharyngeal motor output using our behavioral assay to measure motor coordination. We will use wholebrain calcium imaging to observe neural activity in foraging worms directly [3], which will allow us to compare the measured performance of the nervous system with theoretical predictions based on control theory. This project will inform how neural systems share both sensory and proprioceptive information to coordinate two distinct motor programs.

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MAX PLANCK RESEARCH GROUP

NEURAL INFORMATION FLOW

Monika Scholz

Max Planck

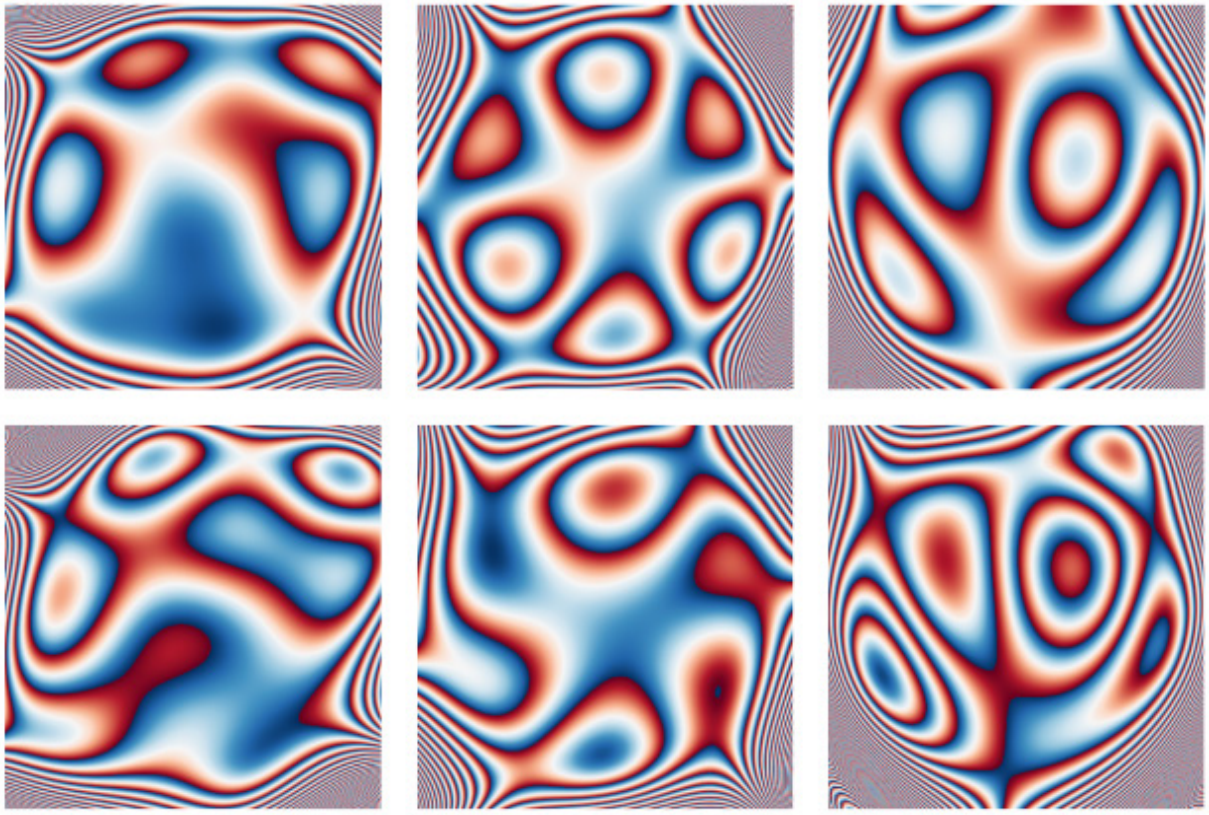
Research Group Leader

The goal of the group 'Neural Information Flow' is to understand how animals integrate multiple sources of information to guide their behavior. We study foraging in the roundworm *C. elegans*, a behavior that is essential for survival. The worms' brain is particularly interesting, as locomotion and feeding are controlled in two distinct neural circuits connected by a bottleneck of only a few neurons. How information is transmitted and how it is compressed between these two circuits is a key question in my group. We follow the information flow in the brain experimentally using behavioral assays, neural activity imaging, optogenetics, and genetics. We expect that this approach will yield insight on decision making and attention in a small invertebrate brain.

Adaptive optics with reflected light and neural networks

By Johannes Seelig | Neural Circuits

Scattering and aberrations limit optical imaging in biological samples. We develop an approach for correcting such aberrations using machine learning and reflected light.



Phase aberrations predicted with a neural network (top row) and target patterns (bottom row). The neural network is trained to extract such phase patterns from scattered light images to correct aberrations in microscopy. Adapted from [4].

Scattering and aberrations in biological tissue

Many biological samples, such as the brain or other tissues, that are investigated with optical microscopes are only partially transparent and the quality of recorded images therefore degrades as the plane of observation moves deeper into the sample. With many commonly used optical techniques this limits imaging to the first few hundred micrometers from the sample surface and therefore in many cases invasive procedures are required to clear the optical access to a structure of interest. This is for example the case for the fruit fly *Drosophila*. Once the brain of the fly is exposed, it is actually well suited for optical imaging thanks to its small diameter of less than half a millimeter and neurons throughout the brain can be imaged. However, in an intact fly the brain is hidden under a non-transparent cuticle and additional layers of scattering tissue that need to be at least partially removed to access the brain. While such clearing procedures can be performed in a way that behavior can still be reliably studied in head fixed animals [1], the lifetime of this kind of preparation is typically limited to less than a day. In particular studying neural activity across multiple timescales, for example from fast dynamics of neural networks that underlie visually guided behavior, to activity modulations that occur over long wake and sleep phases, requires a different approach.

While less invasive procedures have been developed for such long-term imaging [2], one ideally would like to investigate the brain in an entirely intact animal to minimally affect the brain as well as the animal's behavior. With this goal in mind, we are developing techniques that aim to allow imaging through strongly scattering samples such as the cuticle and tissue surrounding the fly brain.

Correction of excitation and detection aberrations

Techniques to correct for scattering in optical imaging where originally developed in astronomy, where light scattering in the atmosphere distorts starlight detected with terrestrial telescopes. These astronomy approaches take advantage of the point-like nature of distant stars, so called guide stars. The way in which the recorded image of such a guide star is distorted compared to its expected point-like nature, allows inferring a correction of the distortions encountered in the atmosphere. An optical device inside the telescope can then compensate for these distortions by changing the optical path in a way that is complementary to the distortion encountered in the atmosphere and inverts it (similar to glasses that correct for the aberrations encountered in the eye). Such corrections are typically applied using a spatial light modulator (SLM) which can display suitable changing corrections at high frame rates resulting in a well-focused image in the focal plane.

Similar methods have also been adapted for optical microscopy and different approaches have been developed over the years. Many of these approaches use fluorescent contrast for improving image resolution. While this works well in many situations, it comes with the problem that the fluorescence signal can be very dim, in particular in strongly scattering samples. Additionally, this approach only works for fluorescently labeled samples and not for methods that use intrinsic sample contrast for imaging. Using reflected light instead of fluorescence for making corrections can provide better signal levels and is independent of sample labeling, but introduces a new problem: aberrations that accumulate on the path towards the focus and aberrations of the reflected light back from the focus accumulate and cannot easily be separated, since different from fluorescence, they both have the same wavelength. But to form a focus inside the sample, we need to separate these two aberrations, since for example for fluorescence imaging the excitation correction is required for correcting the image. Approaches have been developed that can separate excitation and detection aberrations (see [3] for review), but they rely on performing a large number of measurements to computationally extract the different corrections. Such long measurements times are however in conflict with another challenge encountered in biological samples: these samples are dynamic and aberrations can change at the timescale of milliseconds.

Machine learning approach for excitation and detection correction

To address the problems mentioned above we developed an approach that takes advantage of machine learning to disentangle excitation and detection corrections [4].

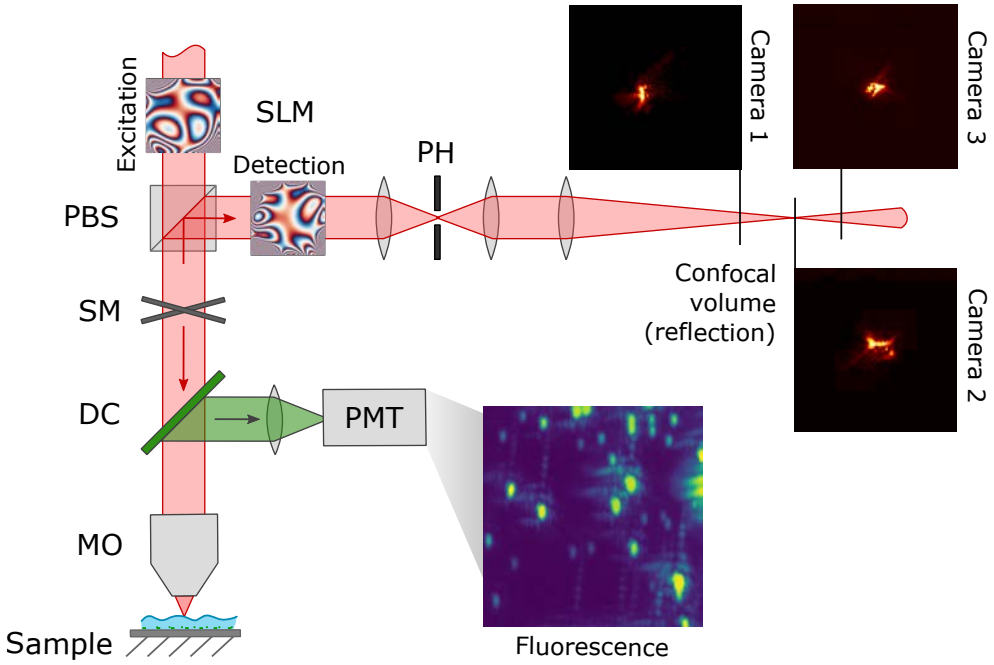


Figure 1: Setup schematics. A two-photon microscope is combined with detection of reflected light. Both, the excitation and the reflected light can be independently phase-modulated. MO=microscope objective, DC=dichroic mirror, SM=scanning mirrors, PBS=polarizing beam splitter, SLM Excitation=spatial light modulator in excitation pathway, SLM Detection=SLM in detection pathway, PH=pinhole, PMT=photomultiplier tube. Adapted from [4].

Machine learning relies on large datasets for finding statistical regularities that cannot easily be extracted in other ways. In particular, for image datasets multilayered neural networks have proven to be very suitable. To take advantage of this approach, we therefore first generated a large dataset of excitation and detection aberrations simulating a variety of potential scattering samples that could be observed in experiments. These aberration images were generated by placing a SLM in both, the excitation and detection pathway of a microscope and by positioning a reflector at the sample plane (Figure 1). At the same time, we recorded the resulting distorted focus patterns with multiple cameras. After generating several hundred thousand of such images, we trained multilayered neural networks to recognize the excitation and detection correction patterns based on the reflected light focus distributions. We showed that these artificial patterns were sufficiently similar to patterns recorded from actual scattering samples to correct for scattering. For this we distributed fluorescent beads on a reflecting surface and placed an aberrating layer of vacuum grease on top (Figure 2). The reflected distorted focus images then allowed us to find excitation and a detection corrections and the excitation correction was used to correct for fluorescence imaging with a two-photon microscope. Importantly, this approach does not require a guide star.

Scattering guide star for excitation correction

In some situations, however, it might be useful to introduce scattering guide stars, such as small metallic particles, into a sample. Compared to biological tissue they offer the advantage of scattering light very strongly and are therefore easy to detect. In this situation, similar to astronomy, due to the point-like nature of the scatterer only one correction is required. Similar to the approach described above, we generated a large training dataset of aberrated guide star images and corresponding aberrated focus images. After training a multilayered neural network on these data, corrections were again found when imaging in scattering samples with embedded guide stars and we showed that in this was aberrations could be corrected.

Outlook

In the future we will develop this approach towards *in vivo* applications. While we are particularly interested in imaging in the fly, these methods will more broadly be applicable to any scattering sample.

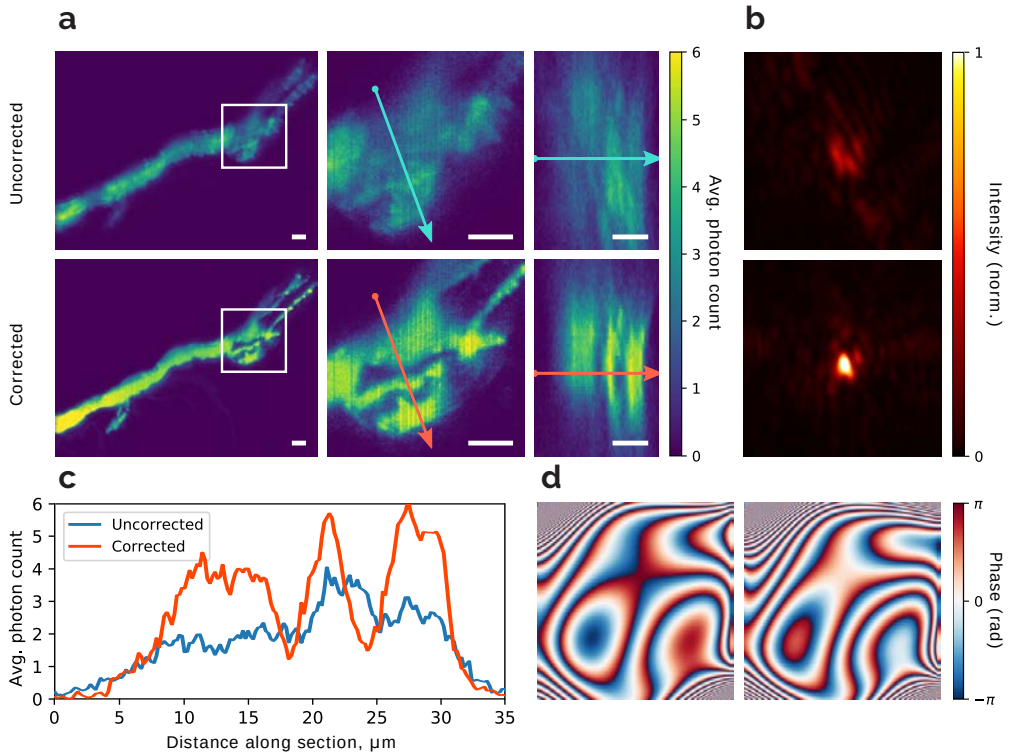


Figure 2: Example of aberration corrections through a scattering layer of vacuum grease of fluorescent beads distributed on a reflecting surface. a) Left: example of corrected and uncorrected image of fluorescent beads distributed on reflector surface imaged through a layer of vacuum grease. Center: white frame in left figure. Right: axial cross section through lines in center figure recorded in a z-stack with 1µm step size. b) Top: uncorrected focus at center of field-of-view in a). Bottom: corrected based on reflected light. Color scale is saturated in the corrected image, so that aberrations in the uncorrected image are visible. c) Cross sections for uncorrected (blue) and corrected (red) images along the lines indicated in figure a), center. d) Left: excitation and Right: detection phase mask. All scale bars are 5µm. Adapted from [4].

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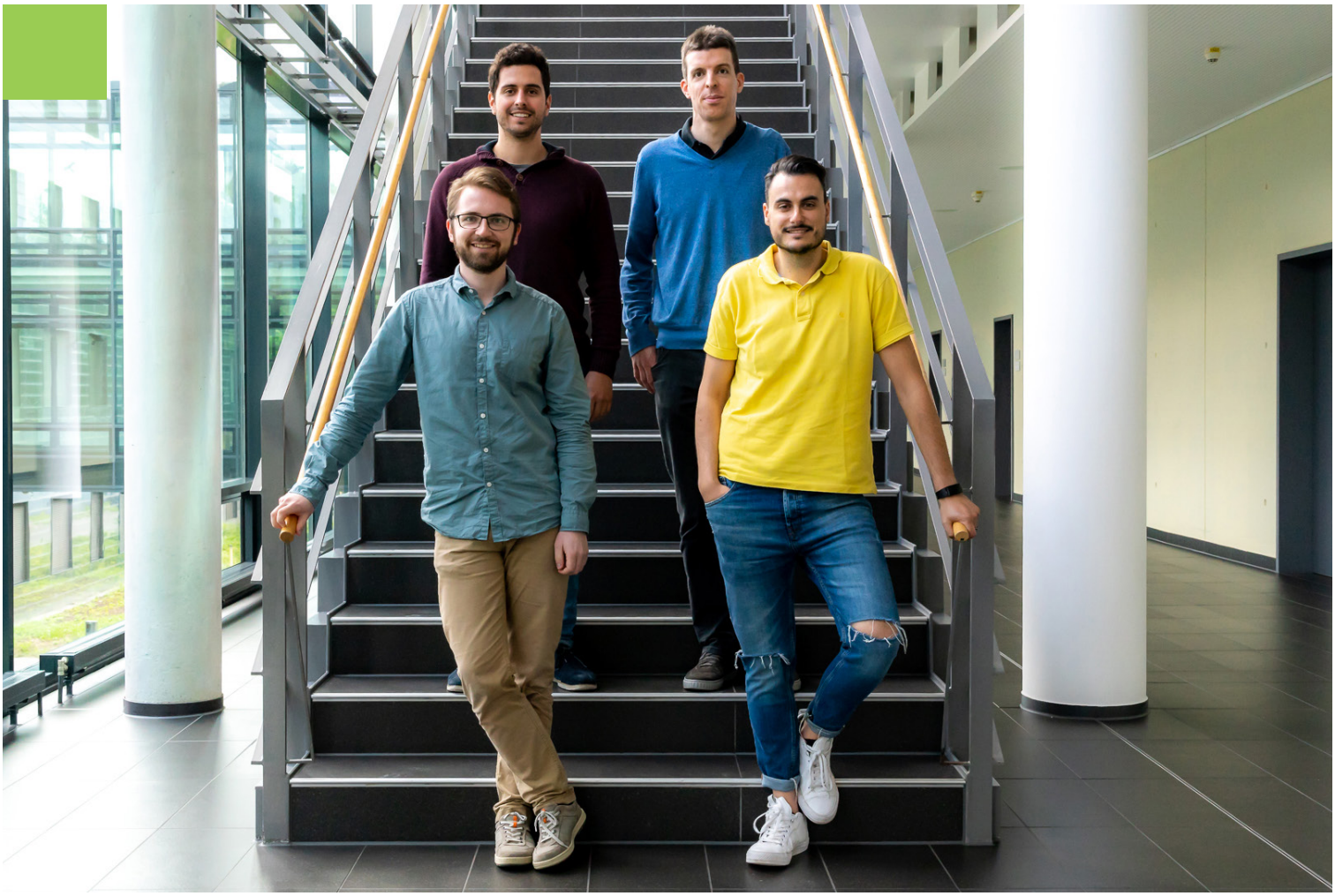


MAX PLANCK RESEARCH GROUP

NEURAL CIRCUITS

Johannes Seelig

Max Planck

Research Group Leader

We use optical microscopy techniques to understand how neural networks in the brain change over time and how these changes relate to behavior. For this we monitor neural circuits at multiple timescales, from the fast neuronal dynamics to changes that occur over multiple days and nights, and in particular during sleep. Our research focuses on the model organism *Drosophila melanogaster* where genetically identified, comprehensive neural networks can be studied. We interpret and guide these experiments using computational modeling of neural circuit dynamics.

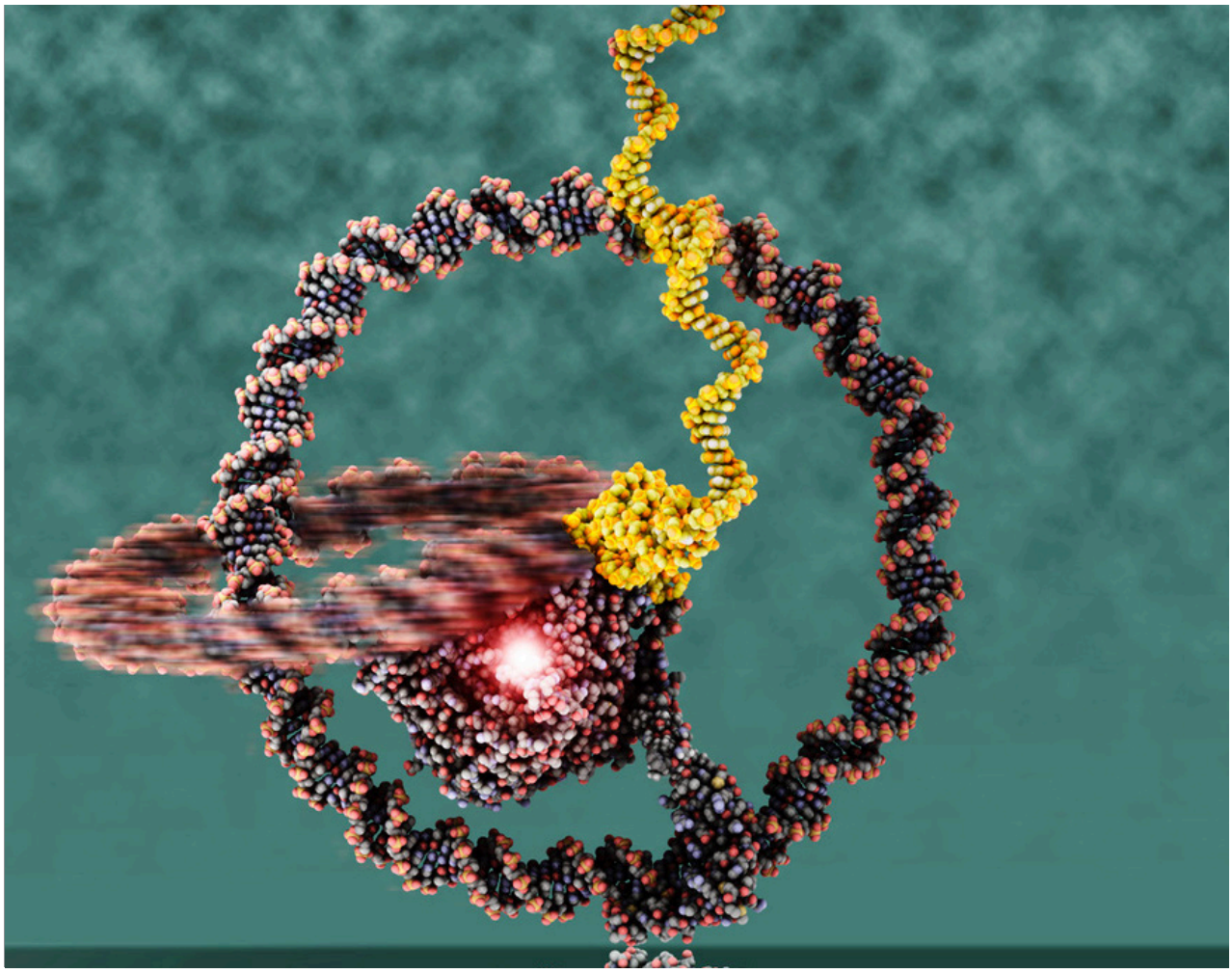
In a second line of research we use machine learning in combination with optical microscopy with the aim of optimizing the temporal as well as spatial resolution of functional imaging in animals engaged in virtual reality behavior.

“We monitor neural circuits at multiple timescales, from the fast neuronal dynamics to changes that occur over multiple days and nights, and in particular during sleep.”

Analysis and control of molecular assemblies

By Prof. Dr. Michael Famulok, Dr. Anton Schmitz
| Chemical Biology

As chemical biologists we use chemical tools to investigate, modulate or generate biological structures including naturally occurring proteins as well as synthetic nucleic acid-based nano-architectures.

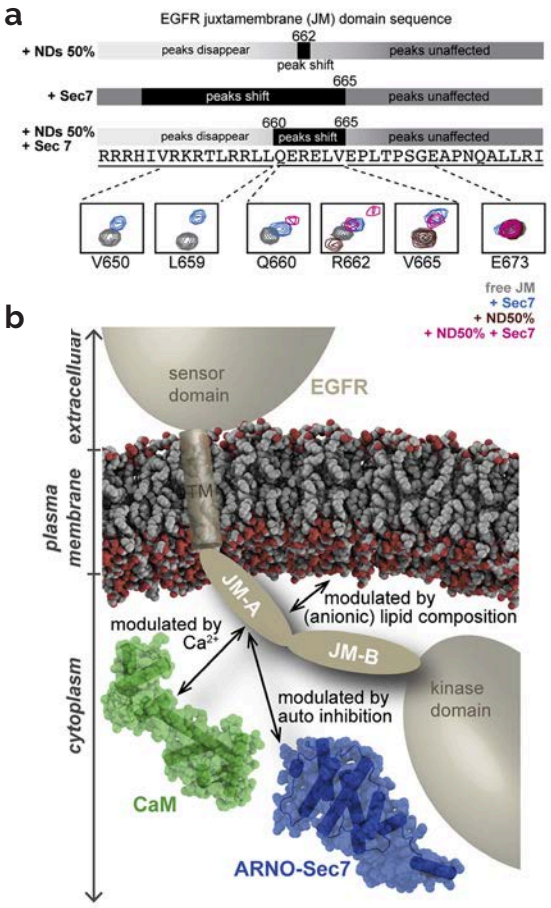


The DNA catenane consists of the stator ring with the bound T7 RNA polymerase, propelling the rotation of the rotor ring. This produces a single strand of RNA (yellow), which is connected to the nano machine and allows for movement along a predetermined path on a nanotube (not pictured). Image: CB.

Almost all biological processes are executed by macromolecules, primarily proteins and nucleic acids. In most cases, these macromolecules assemble into large complexes composed of two to hundreds of identical or different subunits forming intricate regulatory networks. The strength of chemical biology lies in its ability to provide tools, typically small molecules which are accessible to chemical synthesis, to analyse or control these large assemblies or to equip them with new functions.

Analysis of an epidermal growth factor regulatory network

The flow of information between and in cells, called cellular signaling, relies on the transient assembly of signaling complexes. The assembly and activation of these complexes is controlled by their integration into larger regulatory networks. We wanted to analyze the regulation of a particular signaling complex assembled from two copies of the epidermal growth factor receptor (EGFR). A critical structural element for the assembly and activation of the complex is a short segment of 38 amino acids, the so-called juxtamembrane or JM segment. It is located directly at the inner face of the cell membrane and couples the extracellular signal-receiving domain of the receptor to its intracellular signal-transmitting kinase domain (Figure 1). Due to this central function in the activation process of the EGFR the JM segment is targeted by several regulatory proteins. To elucidate the structure of the interfaces between the JM segment and these regulatory proteins we combined biophysical and molecular biology techniques. Using microscale thermophoresis and mutational analysis we could identify the protein ARNO as a potential novel regulator acting on the JM segment of the EGFR. By nuclear magnetic resonance spectroscopy we structurally characterized the interaction of ARNO and the JM segment. Surprisingly, we found that ARNO and the known regulatory protein calmodulin use an almost identical binding site on the JM segment suggesting that these two proteins affect the activation of the EGFR in a similar but competitive manner. Moreover, we found that phospholipids of the cell membrane bind to a site of the JM segment that is partially overlapping with the binding site of the two proteins. As binding of the JM segment to the cell membrane inhibits EGFR activity the competition of this binding by ARNO or calmodulin could provide the structural basis for an activating function of ARNO or calmodulin. Taken together, our data establish the JM segment as a central hub integrating several regulatory inputs affecting EGFR activity [1].



Control of a DNA walker by light

DNA nanomachines are molecular assemblies constructed from DNA which are able to generate some kind of molecular motion. Typical examples are nanoengines driving the rotation of a ring or propeller (see title figure) [2]. Other molecular devices move in a linear fashion and thus can move from one place to another. The prototypic example of the latter is the so-called DNA walker (Figure 2, next page). We constructed a DNA walker and replaced some of the natural nucleotides with small molecule substitutes such that the walker can be controlled by light.

Figure 1: Hypothetical cytoplasmic regulatory network of EGFR activation. The JM segment of the EGFR acts as a central hub integrating the inputs of several cytoplasmic regulatory factors. The interaction of the JM segment with phospholipids of the plasma membrane stabilizes the auto-inhibited state of the EGFR. When calmodulin or ARNO bind to the JM segment its inhibitory interaction with the plasma membrane is weakened and thus the formation of the active EGFR dimer is facilitated.

The walker consists of two legs held together by a short body. Each leg contains two distinct stretches of bases which can bind to complementary stretches on the path (governed by the same principle of base pairing as the formation of the DNA double helix). The stretches in the legs contain two distinct photoswitchable small molecules (azobenzene derivatives) replacing several bases. Therefore, by choosing the wavelength of irradiation the operator can dictate where the walker has to move to. Thus, the DNA walker is non-autonomous. Each step has to be triggered by the operator and can be controlled by the operator. The energy for the movement comes from the energy of the base pairing. As a consequence, in addition to the thermal energy present in the system energy input by light is required to break the base pairings to allow the next step. The DNA walker represents an approach for a nano-transporter. The walker is not well suited for long distances but it is intended for transport tasks where precisely controlled short-distance movements are required. Accordingly, one could imagine as a future application a nano-scale assembly platform where several distinctly controlled walkers bring together in a spatio-temporally ordered manner chemical building blocks which then react to a defined product [3]. These reactions can be catalyzed by enzyme-like DNAs, so-called DNAzymes, as for example our light-regulated peroxidase-mimicking DNAzyme [4].

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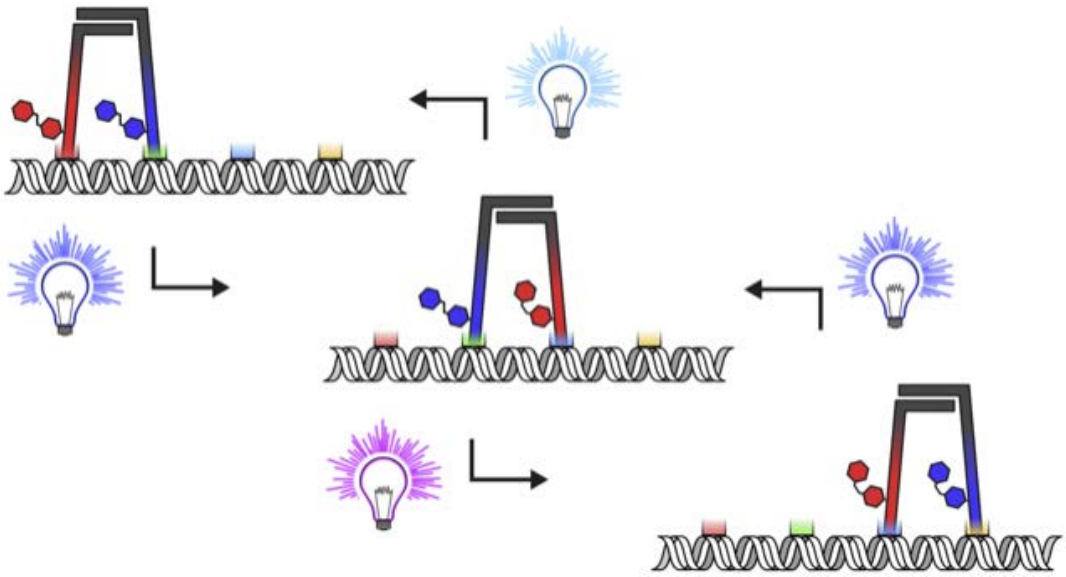
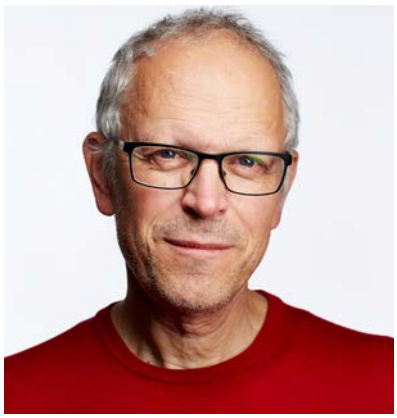


Figure 2: A non-autonomous DNA walker can processively move two steps back and forth on a defined path, powered exclusively by light-irradiation in an orthogonal fashion. Simply by irradiating at a defined sequence of different wavelengths precise control over the motion and position of the walker at every step is achieved. The non-invasive regulation of multi-step processive motion is a first critical achievement towards nanoscale robotics.

MAX PLANCK FELLOW GROUP

CHEMICAL BIOLOGY



Michael Famulok

Max Planck Fellow

The major activities of the Max Planck Fellow group Chemical Biology can be split into two fields of interest. On the one hand we use chemical tools to provide analytical reagents to study biological processes in a broad sense. These tools range from small organic molecules to nucleic acid-based aptamers and are obtained by chemical synthesis, by biochemical enzyme-catalyzed synthesis or by a combination of both. On the other hand, we use chemical tools to construct nucleic acid-based nanoarchitectures and nano-machines as well as bio-hybrid nanoengines. A particular focus is to design these nano-devices in a way that their properties and functions can be controlled by light.

PUBLICATIONS

With caesar affiliation | 2019

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Dissertations

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CAESAR INSIGHTS

News from the IMPRS for Brain and Behavior

By Ezgi Bulca | IMPRS coordinator

The IMPRS for Brain and Behavior completed its fourth full year of operation in 2019. The program's aim is to recruit outstanding doctoral students and immerse them in a stimulating environment that provides novel technologies to elucidate the function of the brain, from molecules to animal behavior.

The graduate school is the first transatlantic cooperation in neuroscience between two Max Planck connected neuroscience institutions (caesar – associated with the Max Planck Society – and the Max Planck Florida Institute of Neuroscience (MPFI)) and two universities (University of Bonn and Florida Atlantic University (FAU)). The program is funded predominantly by a Max Planck Society IMPRS grant with additional support for student positions, research costs and overheads by the participating institutions, and grants to individual investigators. caesar and MPFI host the coordination offices for the school in Bonn and Florida respectively. The call for applications ran from September 1 to December 1 this year, with a response of 381 applications of which 198 were complete and forwarded to the Selection Committee for review. This was the first year that the intake was split according to the location the

applicants were interested in pursuing their PhD. Those applicants, who indicated a preference for Florida, were invited to the Symposium in Florida, and those who preferred Bonn were invited to the Symposium in Bonn. The selection symposia were held on March 7-8, 2019 (Jupiter, FL) and March 12-13, 2019 (Bonn). 20 applicants were invited to the Selection Symposium hosted at caesar, of which 17 applicants attended. From this Bonn Selection, 10 admission offers were extended, and 5 applicants accepted the offer to join the IMPRS. All 11 applicants invited to the Selection Symposium at MPFI accepted the offer to attend, 7 admission offers were extended, and 3 applicants accepted the offer to join the IMPRS. Our application to acceptance ratio was 1:22 for the fourth year.

Our fourth incoming class is composed of 8 students: 1 at FAU, 2 at MPFI, 2 at University of Bonn, and 3 at caesar. Our students are from 5 countries with 1 from Argentina, 1 from China, 1 from Indonesia, 2 from the U.S., and 3 from Germany. This class is made up of 5 males and 3 females. In total, there are 31 students in our IMPRS coming to us from 15 different countries: 17 males, 14 females.

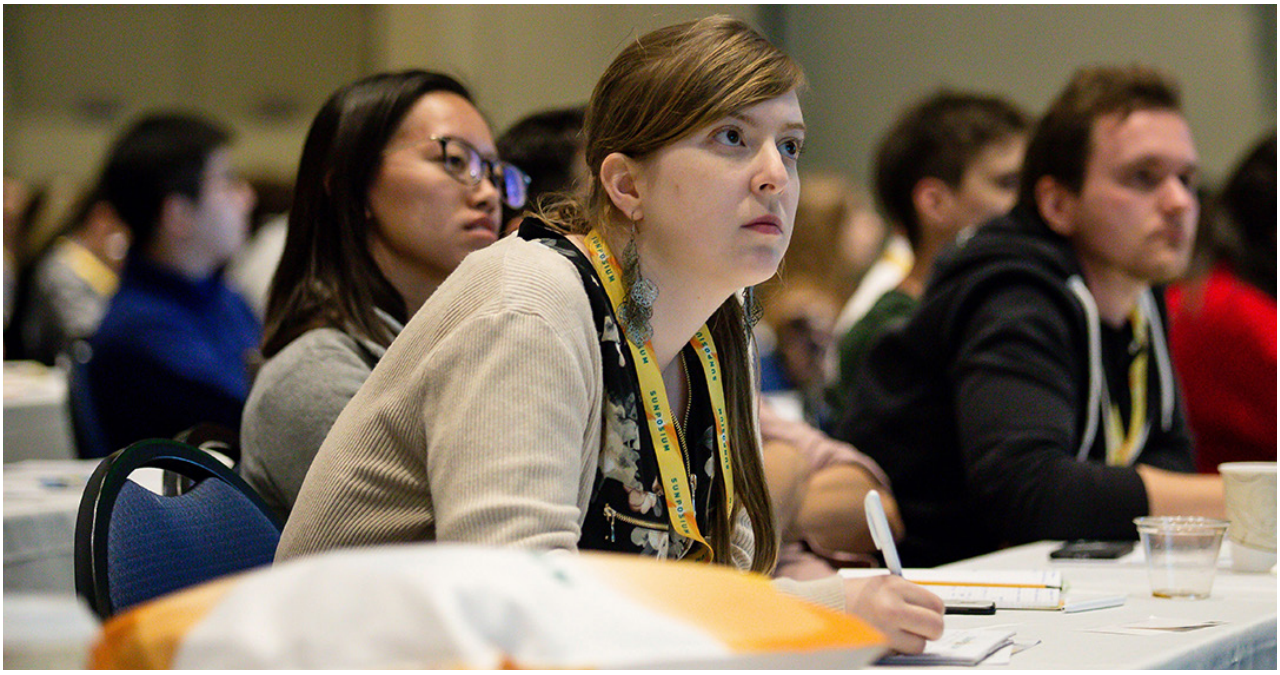
In 2019, Kuo-Sheng Lee (MPFI) became the third IMPRS PhD student to successfully defend his doctoral thesis and obtain his PhD degree. Kuo is now a postdoctoral fellow in Daniel Huber's lab at University of Geneva. Sarah Kruessel (MPFI) transferred out of the IMPRS to Johns Hopkins University's PhD program following her advisor Hyungbae Kwon in his move from MPFI.

The curriculum of the program comprises both theoretical and practical hands-on training elements. To maximize the benefits of international education, students take workshop courses and attend symposia at both caesar and MPFI, allowing students to interact with a broad group of international scientists.

In 2019 MPFI hosted a series of events hosting 25 IMPRS PhD students from Bonn and Jupiter. All 25 students participated in a two-day IMPRS Short Course on Optics and Advanced Light Microscopy organized by the IMPRS Coordinator at MPFI. The course featured practical training from light microscopy experts and MPFI complemented by theoretical lectures from Nobel Laureate Dr. Stefan Hell (MPI for Biophysical Chemistry). The IMPRS PhD students also participated in a student retreat featuring "lightning" research talks and opportunities for informal interactions, attended and presented their research findings at Sunposium 2019 along with 400 other scientists, and participated in the first IMPRS Selection Symposium held in Jupiter.

IMPRS students in Bonn also organized a 2.5-day student retreat at Forum Vogelsang IP where they practiced giving "elevator pitches" of their research project. IMPRS Coordinator Ezgi Bulca gave talks on "Life after PhD" and "Mental Health in Academia" which started fruitful discussions about these topics. IMPRS students in Bonn also enjoyed an exclusive workshop on "Starry Night Sky of Eifel National Park – Artificial and Natural Light at Night" by astronomer Dr. Harald Bardenhagen. The addition of two new Coordinators with previous neuroscience research experience in 2018 was pivotal in planning and executing these scientific training activities.

In September, IMPRS held a "Career in Academia" workshop where caesar directors Kevin Briggman and Jason Kerr explained how their careers led to their current positions and answered questions from the students. IMPRS students in Bonn also organized the "Promoting Better Skills in Neuroscience" event at caesar together with the BIGS Neuroscience students from the University of Bonn. 2 IMPRS students and 2 BIGS Neuroscience students gave creative chalk talks about their PhD projects in a casual environment.



Students exchanged feedback while strengthening their connections within the Bonn neuroscience community. IMPRS students in Bonn together with the coordinator also formed a team of 7 and joined the Bonn Company Run.

In 2019, IMPRS shifted their advertising focus to generate a greater online presence. In addition to the social media platforms, Twitter, Instagram, Facebook, and LinkedIn, online advertisements on the following sites were used: DAAD, MPG, MPG – PhDNet, NENS, NeuroSchools-Germany. For the first time, a social media campaign was booked through Academic Positions for three weeks in October. The campaign ad was seen by over 91.000 users and resulted in over 1000 clicks to see the detailed offer page. 76 people ended up applying because of this ad campaign. Included in the social media campaign, the IMPRS ad was also placed on the regular Academic Positions portal and resulted in another 400 users to end up on the application page. In November, at the annual meeting of the Society for Neuroscience (SfN) in Chicago, IL the IMPRS for Brain & Behavior coordinators staffed the Neuroscience Research in Germany and MPFI booth in the main exhibit hall. Ezgi Bulca was also present at the "German Neuroschools" booth at the Grad School Fair. In addition, IMPRS materials were on display at all booths throughout the SfN meeting, and IMPRS promotional items were given away to SfN conference attendees.

Scientific, PR and Outreach Events at caesar

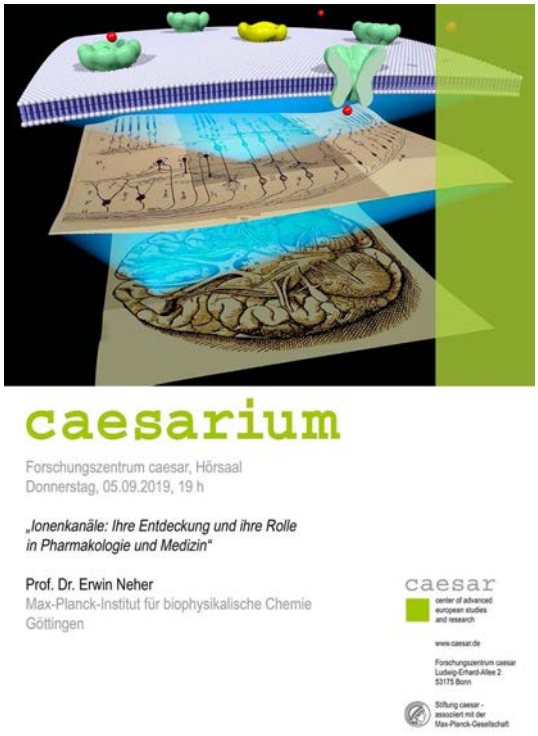
By Julia Schlee and Sebastian Scherrer | Outreach Officer and PR Officer

Scientific Events

caesarium

Since 2008, caesar has presented a lecture series (caesarium) for the local scientific community as well as the general public. Well-known scientists give a comprehensive public talk about their research field and their findings to a lay audience. The seminars stimulate discussions and questions within the public and enjoy great popularity.

On September 5th 2019, the Nobel prize laureate Prof. Dr. Erwin Neher spoke about ion channels, their discovery and role in pharmacology and medicine. The talk attracted more than 300 guests and resulted in very positive local press coverage.



caesar seminar series

To make more effective use of the expertise available in the field of biosciences, and to foster cooperation and information exchange, caesar hosts a regular talk series featuring the following scientists and presentations:

08.01.2019
Prof. Dr. Henning Urlaub,
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
"Current development in quantitative MS-based proteomics"

18.01.2019
Dr. Nereo Kalebic,
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
"Molecular mechanisms of neocortex development and pathogenesis"

25.01.2019
Dr. Takeshi Yoshimatsu,
University of Sussex
"Retinal circuits for seeing colours in nature: Functional studies of the inner and outer retina in zebrafish"

30.01.2019
Dr. Michael Frosz and Dr. Richard Zeltner,
Max Planck Institute for the Science of Light, Erlangen
"Fabrication and applications of photonic crystal fibers"

04.02.2019
Timothy J. Lee,
Georgia Institute of Technology
"Batch processing of brain tissue sections for millimeter-scale serial section transmission electron microscopy connectomics"

07.03.2019
Prof. Samuel Young,
Department of Anatomy and Cell Biology, University of Iowa
"Molecular mechanisms regulating presynaptic calcium channel subtype levels to drive neuronal circuit function"

14.03.2019
Prof. Gil Westmeyer,
Technical University Munich/Helmholtz Center Munich
"Molecular neuroimaging across scales: Towards calcium sensors for optoacoustics & multiplexed gene reporters for electron microscopy"

04.04.2019
Dr. Monika Rutowska,
TMC Science and Technology & IMEC Institute for Nanoelectronics, Leuven, Belgium
"From photonics crystal fibres, through optical fibre bundles, to SiN waveguides"

09.04.2019
John Tuthill,
University of Washington, Seattle, USA
"Neural mechanisms of leg proprioception and motor control in *Drosophila*"

10.05.2019
Dr. Aneta Koseska,
Max Planck Institute for Molecular Physiology
"Towards theory of non-Turing biochemical computation and learnability"

07.06.2019
Dr. Christian Puller,
Carl von Ossietzky University Oldenburg,
"Network motifs of electrical coupling between retinal ganglion cells"

25.06.2019
Dr. Nina Vogt,
Nature Methods
"Publishing high-impact research: Perspective of a Nature Methods editor"

04.07.2019
Dr. James Lightfoot,
Max Planck Institute for Developmental Biology, Tübingen
"Killing, cannibalism and self-recognition behaviors in predatory nematodes"

16.07.2019
Dr. Jeffrey S. Diamond,
National Institute of Neurological Disorders and Stroke, Bethesda, USA
"Diverse synaptic signaling within a single retinal amacrine cell type"

04.09.2019
Dr. Armin Bahl,
Harvard University
"Neural circuits for evidence accumulation and perceptual decision-making in larval zebrafish"

30.10.2019
Dr. Georgina Fenton,
Department of Neuroscience, Psychology and Behaviour, University of Leicester
"Physiological basis of noise-induced hearing loss in a tympanal ear"

13.11.2019
Jun.-Prof. Dr. Benjamin Risse,
University of Münster
"Is science mostly driven by ideas or by tools? Interdisciplinary research at the interface of behavioral biology and computer vision & machine learning"

05.12.2019
Dr. Ann Clemens,
Bernstein Center for Computational Neuroscience,
Humboldt University Berlin
"Neural mechanisms of kinship behavior"

Neuroscience seminar series

caesar is also regularly inviting speakers from other neuroscience laboratories, research institutes or universities for seminars as well as informal scientific exchanges, These talks feature short presentations and discussions with caesar researchers. The aim is to exchange ideas and techniques and foster possible collaborations, especially giving caesar graduate students and postdoctoral fellows the opportunity to interact with the guest speakers.

11.03.2019	Dr. David Fitzpatrick
08.04.2019	Prof. David Berson
29.04.2019	Dr. Misha Ahrens
20.05.2019	Prof. Adam Cohen
27.05.2019	Prof. Florin Albeanu
19.06.2019	Dr. Adam Hantman
04.11.2019	Prof. Richard Hahnloser
11.11.2019	Markus Knaden
09.12.2019	Andrés Bendesky

PhD and Postdoc seminars

On a regular basis, PhD students as well as Postdocs give short presentations on their research and discuss progress as well as potential issues. The aim is an exchange of ideas and techniques as well as forming a network for students and postdocs. These seminars are also a forum to discuss new research ideas and draft applications for potential future projects.

24.01.2019	Mythreya Seetharama
31.01.2019	Matthew Schiel
07.02.2019	Andres Flores
14.02.2019	Philippe Fischer
21.02.2019	Artur Speiser
14,03.2019	Jan-Mattis Lückmann
21.03.2019	Marta Pallotto
25.04.2019	Daniel Udvary
02.05.2019	Fernando Messore
09.05.2019	Rajeevan Narayanan Therpurakal
23.05.2019	Carl Holmgren
06.06.2019	Kristina Barragan-Sanz
13.06.2019	Federica Roselli
04.07.2019	Anja Günther
19.09.2019	Giacomo Bassetto
10.10.2019	Mike Guest
17.10.2019	Fabian Svara

07.11.2019	Daniel Udvary
14.11.2019	Kara Fulton
21.11.2019	Aman Maharjan

2019 scientific retreat

Between September 30th and October 2nd, 2019, the caesar scientific retreat took place in Oberems, deep in the Taunus forest. 75 scientific employees of caesar experienced three highly interesting days, tightly packed with scientific insight. The retreat was a great occasion to gain an overview of all the excellent research done at caesar, especially for incoming group leaders Monika Scholz, Pascal Malkemper and Aneta Kosesaka, who also presented their own research. A beautiful hike through the mists of the Taunus forest, as well as a highly competitive pub quiz were other high points of the event.

iGEM spring festival

Between May 3rd and 5th 2019, caesar hosted the annual European iGEM spring conference at the institute. 200 MINT students from 8 countries filled the hallways and the auditorium, bringing student research from a plethora of disciplines together. As part of the lecture program, Prof. Jason Kerr presented caesar's key discipline, neuro-ethology.

The program also included workshops, a poster session, and social events, such as a visit of *Rhein in Flammen* and the caesar science slam.

Public Relations

One of caesar's goals as an institute for basic research is to educate the public about its research. The mission: to bring awareness and visibility, and to convey the institute's scientific accomplishments.

Press releases

In 2019, the institute published 9 press releases to journalists, media outlets and interested individuals. The releases were also published on the caesar website. To enhance the impact of these press releases, they were also distributed utilizing platforms such as IDW ("Informationsdienst Wissenschaft") and a network of direct journalistic contacts. The press is also regularly invited to caesar events such as the caesarium, and responds readily to these invitations (e.g. the caesarium by Nobel laureate Prof. Erwin Neher). As a result, 50 news items (print, internet and radio) were published about caesar in 2019.

04.01.2019	Cilia as the brain's supply pump
12.02.2019	GBA2 in the nervous system: lipids and gait imbalance are intertwined
25.03.2019	Nano walker follows the path of light
06.05.2019	Bessel rays illuminate structures in 3D
09.07.2019	Students explore the world of neuroscience
01.10.2019	How does the brain coordinate behavior? New research group at caesar
19.11.2019	New research group on magnetoreception in animals
26.11.2019	Traffic lights for perception
29.11.2019	Jason Kerr appointed professor for „Behavior and Brain Organization"

News releases

Our press releases are complimented by shorter news articles on our relaunched website. All relevant news are reported on our website and further distributed via our social media channels.

In 2019, we published 19 news official releases.

04.01.2019	Cilia as the brain's supply pump
12.02.2019	"Female scientists should trust themselves, to have faith that they will make it"
25.03.2019	Nano walker follows the path of light
28.03.2019	Scientist for a Day! (Girls' and Boys'Day at caesar)
04.04.2019	Successful premiere for caesar public lab
03.05.2019	Full house for science
05.05.2019	Jason Kerr presents neuroethology research to iGEM students
06.05.2019	Bessel rays illuminate structures in 3D
10.07.2019	Research center caesar new partner of the zdi network of the Rhine-Sieg-District
15.08.2019	Fifty young physicists introduced to neuroethology research
12.09.2019	Nobel laureate visits research center caesar
25.09.2019	Local school teachers visit caesar public lab
04.10.2019	New research group: Neural Information Flow
22.10.2019	caesar participates at the inaugural "Pint of Science" in Bonn
23.10.2019	Neuroscience Seminar Series returns
19.11.2019	New research group on magnetoreception in animals
27.11.2019	Traffic lights for perception
27.11.2019	August-Macke-Hauptschule visits caesar public lab
29.11.2019	Jason Kerr appointed professor for „Behavior and Brain Organization"

Internet presentation

The caesar website was relaunched in early 2019. It now features a modern, responsive design and is streamlined to address scientists, PhD students and the public directly. It presents the science at the institute, as well as and upcoming events at caesar.

With the 2019 relaunch, our website obtained the new capability to post news articles that convey current developments at our institute. Another feature of the website is a timeline on the front page, highlighting upcoming events, and an event database that includes past and future events. The pages for the individual research groups gained new capabilitites, too. Repositories of data can now be hosted, also multimedia data sets. Jobs and news are now also directly associated with the group pages, so vacancies can be immediately seen. It is possible to subscribe to the newsletter and receive all the latest information about caesar.

Social media

caesar is present on the following social media platforms: Twitter, Facebook, Instagram, YouTube, LinkedIn, ResearchGate.

The twitter account @caesarbonn reaches more than 5.000 followers. On caesar's YouTube channel (around 900 subscribers), the institute has published 46 self-produced videos on research topics, professional recordings of all events and video trailers to promote upcoming events and capture impressions from past events. The Facebook presence (1.218 followers on Dec 31st, 2019) is a source of information about current activities at caesar and features additional information about aspects of neuroethology and neuroethological research. caesar also has a LinkedIn presence and caesar researchers are present with their work on ResearchGate. To reach a general audience with regular mailings, caesar runs a MailingList using the MailChimp network. Here, caesar currently has 1.482 subscribers.

Publications

caesar's annual report is distributed to a large number of people interested in the research at caesar. The report consists of scientific articles for the general public. The annual reports, starting with the year 2011 are available for download on the caesar webpage, in both German and English.
In 2019, the institute changed the format of the annual report to an electronic version, to decrease the ecological footprint of the report.

Outreach

Visitors

In 2019, there was a continuous flow of visitors at caesar, in particular of school classes. On request, caesar offers presentations and guided tours.

Schools

At caesar, we aim to engage with teachers and school children at all ages, using hands-on activities linked to our research in the field of neuroethology. caesar has established an internship program for outstanding high school students in cooperation with schools of the region and the German youth science competition "Jugend forscht". Students can apply for internships in the labs. Additionally, several other programs are offered, including e.g. the Boys' and Girls' Day. caesar is also involved in a program for talented students together with the CJD Christophorusschule Königswinter. In the framework of this program, caesar, with its electron microscopy facility, offers a special 4-day-program "Journey into the nanoworld".

Moreover, caesar has a modern equipped public lab and offers workshops for students from class 8-12.

Public Lab

caesar's newly established public lab offers insight into the life of a scientist, with hands-on activities under the umbrella of neuroscience. caesar offers interactive lab-based sessions for secondary school pupils and their teachers, with the aim to provide a realistic and fun experience in STEM education. Our goal is to foster enthusiasm for research and science.

Experiments are designed to answer specific scientific questions via a hands-on approach. Participants work within a team as well as independently, following detailed instructions, but also by having space to individually 'develop and try out' other methods and tools. The workshops can be offered in German and English.

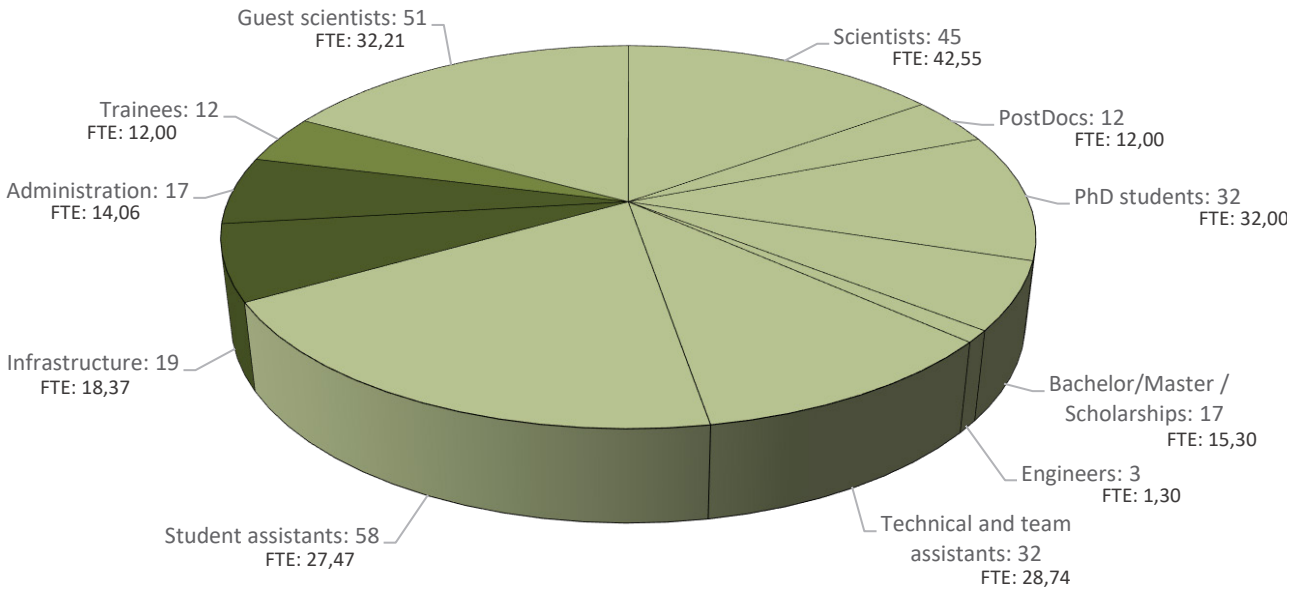
The current workshop is titled "Underwater Sensation". As part of this workshop, students perform comparative studies in zebrafish larvae at different developmental stages, as well as getting an insight into various aspects of neuroscience by performing simple behavioral experiments. Thus, it is possible to demonstrate how mechanosensory information is perceived, processed within a neuronal network and results in a change of animal behavior. Activities such as constructing an electric circuit enables the pupils to understand the signal transduction within a neuron. caesar also offers workshops for teachers to experience the workshops themselves.

The public lab is certified by "Zukunft durch Innovation.NRW" (zdi), a joint initiative to support and foster young scientists and engineers in North Rhine-Westphalia. The zdi initiative is supported at state level by several ministries (science, schools and business); the Ministry of Science is a main stakeholder. The zdi state office advises and supports the centers and student laboratories in their work and coordinates the allocation of funds.

In 2019, the following school classes have visited us:

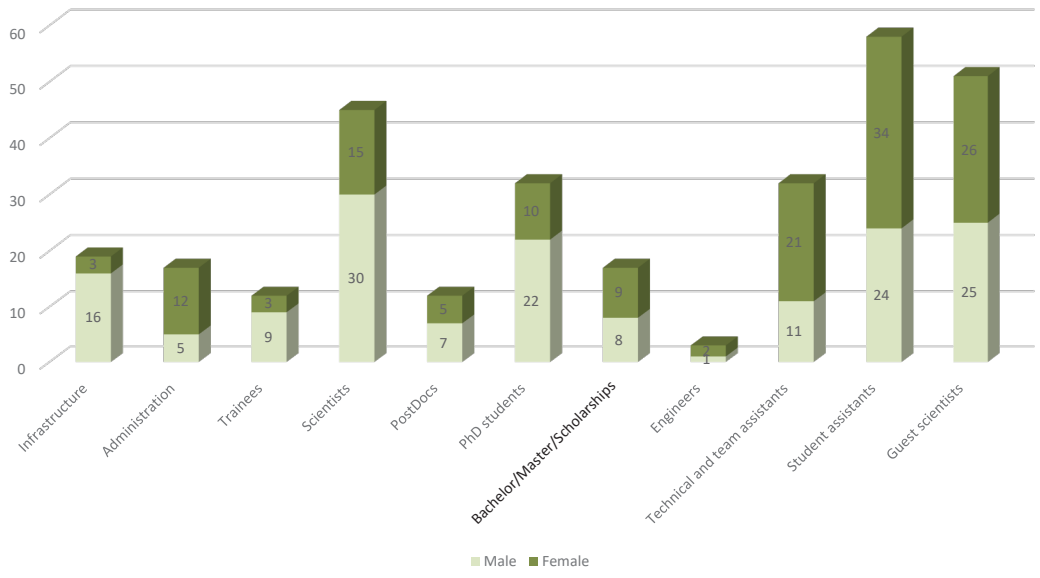
21.02.19	Amos Comenius Gymnasium	8 participants
04.04.19	Amos Comenius Gymnasium	8 participants
10.07.19	Gesamtschule Lohmar	20 participants
27.11.19	August-Macke Schule	19 participants

Facts and Figures



FTE = full time equivalent

As of December 31st, 2019, research center caesar employed 235 personnel in total and hosted 51 guest scientists. The distribution of the employees within the departments and functions is deductible from the above graph. Converted into full-time positions there are 14.06 people working in the administration and 18.37 in the infrastructure. Furthermore, caesar instructed 12 trainees in 5 departments: electrician, tool mechanic, laboratory assistant in biology and chemistry, administration and animal care.



Finances

Basis of the caesar foundation

caesar was established as a non-profit foundation under private law by the Federal Republic of Germany and the state of North Rhine-Westphalia in accordance with the Bonn-Berlin compensation act.

The foundation's assets amount to 383,4 Mio. € with 6,6 Mio. € added property value, which was entrusted to caesar by the city of Bonn.

286.3 Mio. € of the foundation's capital were invested in the capital market on a long-term basis. The revenue funds the research of caesar.

ASSETS 2019	
Tangible assets	82,870,404.74 €
Financial assets	301,736,604.26 €
Miscellaneous	26,978,341.77 €
Total assets	411,585,350.77 €

INCOME 2019	
Income from sales revenues and promotions	5,479,490.60 €
Income from securities and interest	14,653,383.63 €
Total income	20,132,874.23 €

EXPENSES 2019	
Personnel expenses	9,579,915.42 €
Material expenses	5,399,242.80 €
Amortisations	3,653,615.20 €
Total expense	18,632,773.42 €

Balance sheet

Final balance as of December 31st, 2019

ASSETS	31.12.2018	31.12.2019
A. Capital assets		
I. Intangible assets	**105,007.91 €**	**154,946.71 €**
II. Tangible assets		
Property and construction	68,769,854.29 €	67,570,221.68 €
Various investments	13,868,728.82 €	13,899,832.62 €
Advance payment & investment in construction	939,967.55 €	1,400,350.44 €
Total of II.	**83,578,550.66 €**	**82,870,404.74 €**
III. Financial assets		
Assets	330,000.00 €	330,000.00 €
Securities of capital assets for investment of foundation capital	290,575,021.51 €	290,575,021.51 €
Securities of capital assets for reacquisition of property	10,831,582.75 €	10,831,582.75 €
Total of III.	**301,736,604.26 €**	**301,736,604.26 €**
Total of A.	**385,420,162.83 €**	**384,761,955.71 €**
B. CURRENT ASSETS		
I. Inventory stock	**382,325.04 €**	**538,130.81 €**
II. Accounts and other assets		
Trade accounts receivables	485,069.87 €	514,049.26 €
Receivables from affilliated companies	0 €	0 €
Miscellaneous assets	1,587,700.70 €	1,696,046.34€
Total of II.	**2,072,770.57 €**	**2,210,095.60 €**
III. Securities	**0 €**	**0 €**
IV. Cash assets, Federal bank balances, credit balances and checks	**21,618,691.65 €**	**23,843,411.85 €**
Total of B.	**24,073,787.26 €**	**26,591,638.26 €**
C. Deferred income	**189,282.78 €**	**231,756.80 €**
Total capital	**409,683,232.87 €**	**411,585,350.77 €**

Final balance as of December 31st, 2019

LIABILITIES	31.12.2018	31.12.2019
A. Capital assets		
I. Foundation assets		
Financing capital	286,323,453.48 €	286,323,453.48 €
Investing capital	97,145,457.43 €	97,145,457.43 €
Support City of Bonn	6,681,051.01 €	6,681,051.01 €
Apropriation reserves	1,283,956.17 €	1,283,956.17 €
Total of I.	**391,433,918.09 €**	**391,433,918.09 €**
II. Reserves		
Free reserves in accordance with § 58 Nr. 7a AO	8,716,423.79 €	10,216,524.60 €
Maintenance reserves	4,943,788.62 €	4,943,788.62 €
Total of II.	**13,660,212.41 €**	**15,160,313.22 €**
III. Result		
Annual surplus / deficit	0 €	0 €
Total of III.	**0 €**	**0 €**
Total of A.	**405,094,130.50 €**	**406,594,231.31 €**
B. Exceptional items for investment subsidies	**2,770,722.39 €**	**2,348,225.35 €**
C. Provisions	**831,496.05 €**	**631,573.77 €**
D. Payables		
Received payables	175,000.00 €	6,201.26 €
Trade account payables	681,690.75 €	1,872,417.37 €
Other payables	129,806.00 €	132,701.71 €
Total of D.	**986,496.75 €**	**2,011,320.34 €**
E. Deferred income	**387.00 €**	**0 €**
Entire assets	**409,683,232.69 €**	**411,585,350.77 €**

Foundation bodies

Foundation board

As of Dec 31st, 2019, the foundation board consisted of:

Chairman

- Prof. Dr. Martin Stratmann
  President of the Max Planck Society for the Advancement of Science e.V.,
  Munich

Members

- Prof. Dr. Philippe Bastiaens
  Max Planck Institute for Molecular Physiology,
  Dortmund

- Prof. Dr. Jens Brüning
  Max Planck Institute for Metabolism Research,
  Cologne

- Prof. Dr. Veronika von Messling
  Head of department 6 in the Federal Ministry of Education and Research,
  Berlin

- Prof. Dr. Winfried Denk
  Max Planck Institute for Neurobiology,
  Martinsried

- Prof. Dr. Pascal Fries
  Director of the Ernst Strüngmann Institute gGmbH,
  Frankfurt a.M.

- Prof. Dr. Bill Hansson
  Vice President of the Max Planck Society for the Advancement of Science e.V.,
  Max Planck Institute for Chemical Ecology,
  Jena

- Prof. Dr. Michael Hoch
  Rector of the Rheinische Friedrich Wilhelms Universität Bonn,
  Bonn

- Prof. Dr. Regine Kahmann
  Max Planck Institute for Terrestrial Microbiology,
  Marburg

- Tankred Schipanski
  Member of the German Bundestag,
  Berlin

- Ulrich Schüller
  Head of department 4 in the Federal Ministry of Education and Research,
  Bonn

- Karl Schultheis
  Member of the Landtag North Rhine-Westphalia,
  Düsseldorf

- Ashok Sridharan
  Mayor of the Federal City of Bonn,
  Bonn

- Annette Storsberg
  State secretary in the Ministry for Culture and Science of North Rhine-Westphalia,
  Düsseldorf

- Prof. Dr. Heinz Wässle
  Max Planck Institute for Brain Research,
  Frankfurt a.M.

- Dr. Norbert Windhab
  Evonik Nutrition & Care GmbH,
  Darmstadt

Executive board

As of Dec 31st, 2019, the caesar executive board consisted of:

- Dr. Kevin Briggman
  Managing Director
  Director of the Department "Computational Neuroethology“

- Prof. Dr. Jason Kerr
  Director of the Department “Behavior and Brain Organization“

- Helmut Kolz
  Administrative Director

CONTACT

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