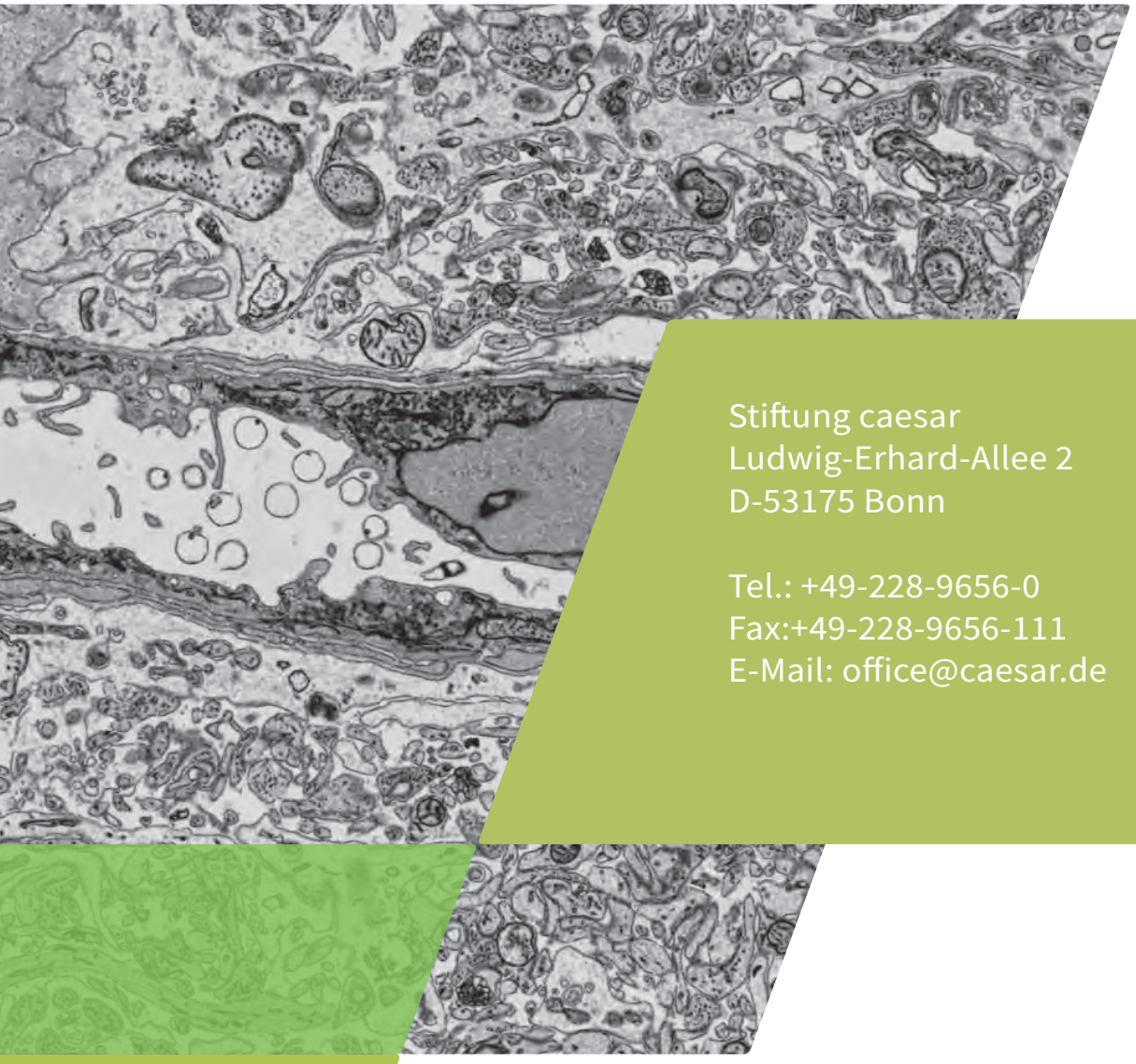
Annual Report
2017

caesar

associated with the Max-Planck-Society



Stiftung caesar
Ludwig-Erhard-Allee 2
D-53175 Bonn

Tel.: +49-228-9656-0
Fax:+49-228-9656-111
E-Mail: office@caesar.de

Preface

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. 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 our researchers 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.

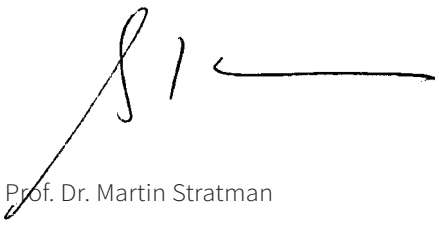
caesar is a research institute dedicated to the neuroethological study of animals. The research groups and departments bring a collectively unique combination of experimental and computational approaches to bear on the question of how the brain controls behavior. The research done at caesar spans a large range of scales from nano-scale imaging of the brain, to large-scale functional imaging of thousands of neurons in the brain, to the quantification of animal behavior. This 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, the neuroethological questions are comparative in nature and incorporate a diverse set of species (i.e. small mammals, birds, amphibians and flies), allowing us to study the wide repertoire of behaviors across the animal kingdom. While setting up an institute to house and care for such a wide range of species is challenging, the guiding principle is the wellbeing of the experimental animals and adherence to the principles of animal ethics. This principle not only drives the development of minimally invasive experimental techniques, but also ensures that our staff undergo regular training and further education keeping caesar at the forefront of animal care.

In 2017 Dr. Kevin Briggman was appointed as a scientific director at caesar. His Depart-

ment "Computational Neuroethology" (CNE), established in June, has the overall goal to develop computational models which can predict goal-directed animal behaviors that take into account behavioral variability. To this end Dr. Briggman incorporates behavioral analysis, cellular resolution imaging of neuronal populations, 3D electron microscopy and computational modelling to understand how circuits in the nervous system control animal behavior. Turning caesar into a modern neuroethology institute within the Max Planck Society is a goal he shares with his co-director Dr. Jason Kerr. He is head of the Department of "Behavior and Brain Organization" which aims to generate a thorough understanding of mammalian vision at the neuronal circuit level.

In addition to the scientific departments, caesar is currently hosting the following Max Planck research groups: "Neuronal Circuits" led by Dr. Johannes Seelig, "In Silico Brain Sciences" led by Dr. Marcel Oberlaender, "Neural Systems Analysis" led by Prof. Dr. Jakob Macke, and "Structural Dynamics of Proteins" led by Prof. Dr. Elmar Behrmann. Furthermore, Dr. Bettina Schnell established her Emmy Noether research group "Neurobiology of Flight Control" at caesar in 2017. Using *Drosophila* as a model organism, she is studying the neuronal mechanisms allowing the flies to navigate. We are also hosting the emeritus group of Prof. Dr. U. Benjamin Kaupp who retired from his position as director at caesar in August 2017.

Again, 2017 proved to be another outstanding year for caesar and we are already well into the planning for 2018, where we envisage another year of growth and changes to realise our aim of becoming a modern neuroethology institute enabling a vibrant research environment to conduct curiosity-driven research in this exciting field.



Prof. Dr. Martin Stratman

President of the Max Planck Society
Chairman of the Foundation Board

Dr. Jason Kerr

Managing Director

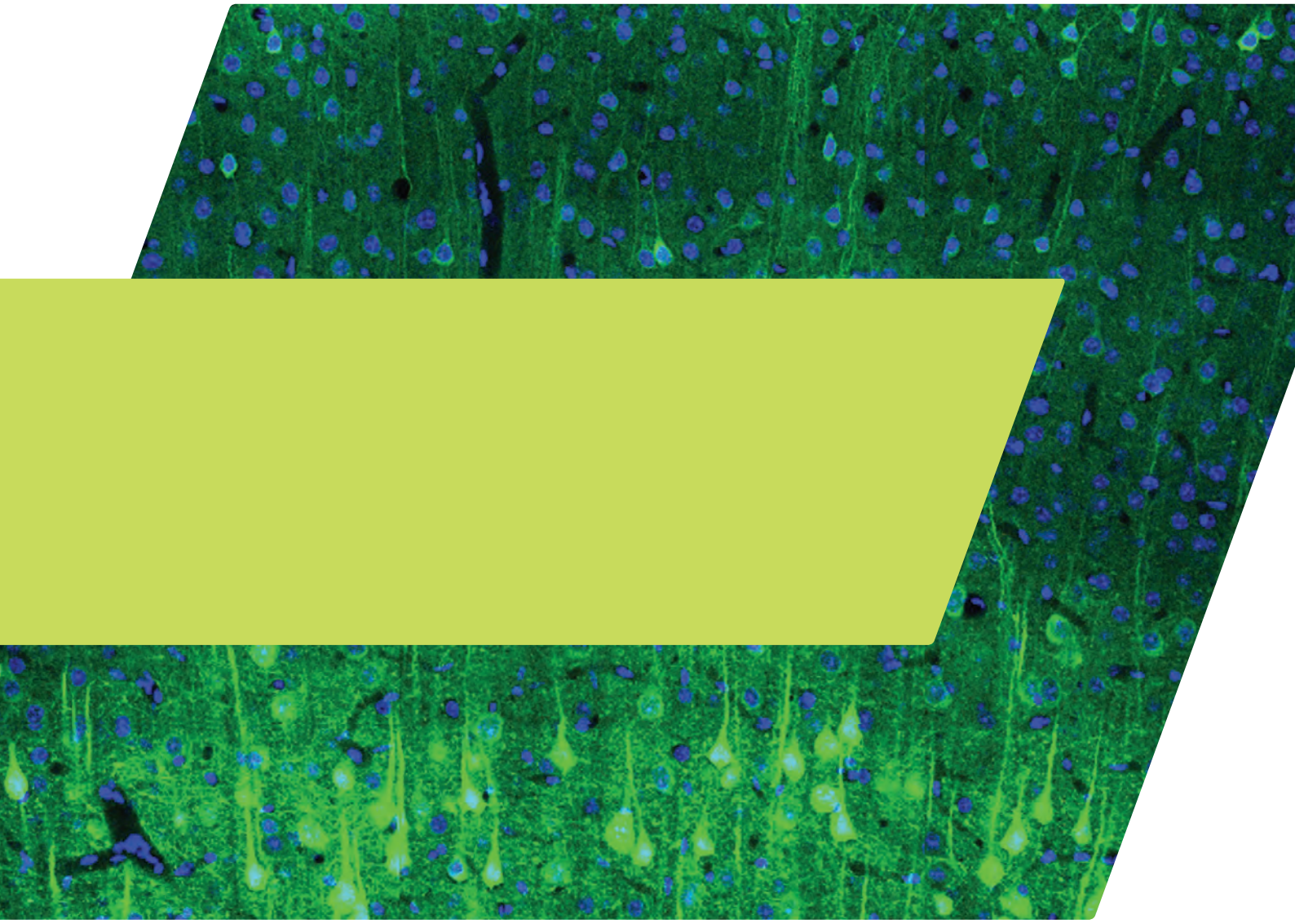
Dr. Kevin Briggman

Director

Gertrud Bilski

Administrative Director

Content



Prof. Dr. Martin Stratmann

President of the Max Planck Society /
Chairman of the Foundation Board

Dr. Jason Kerr

Managing Director

Dr. Kevin Briggman

Director

Gertrud Bilski

Administrative Director

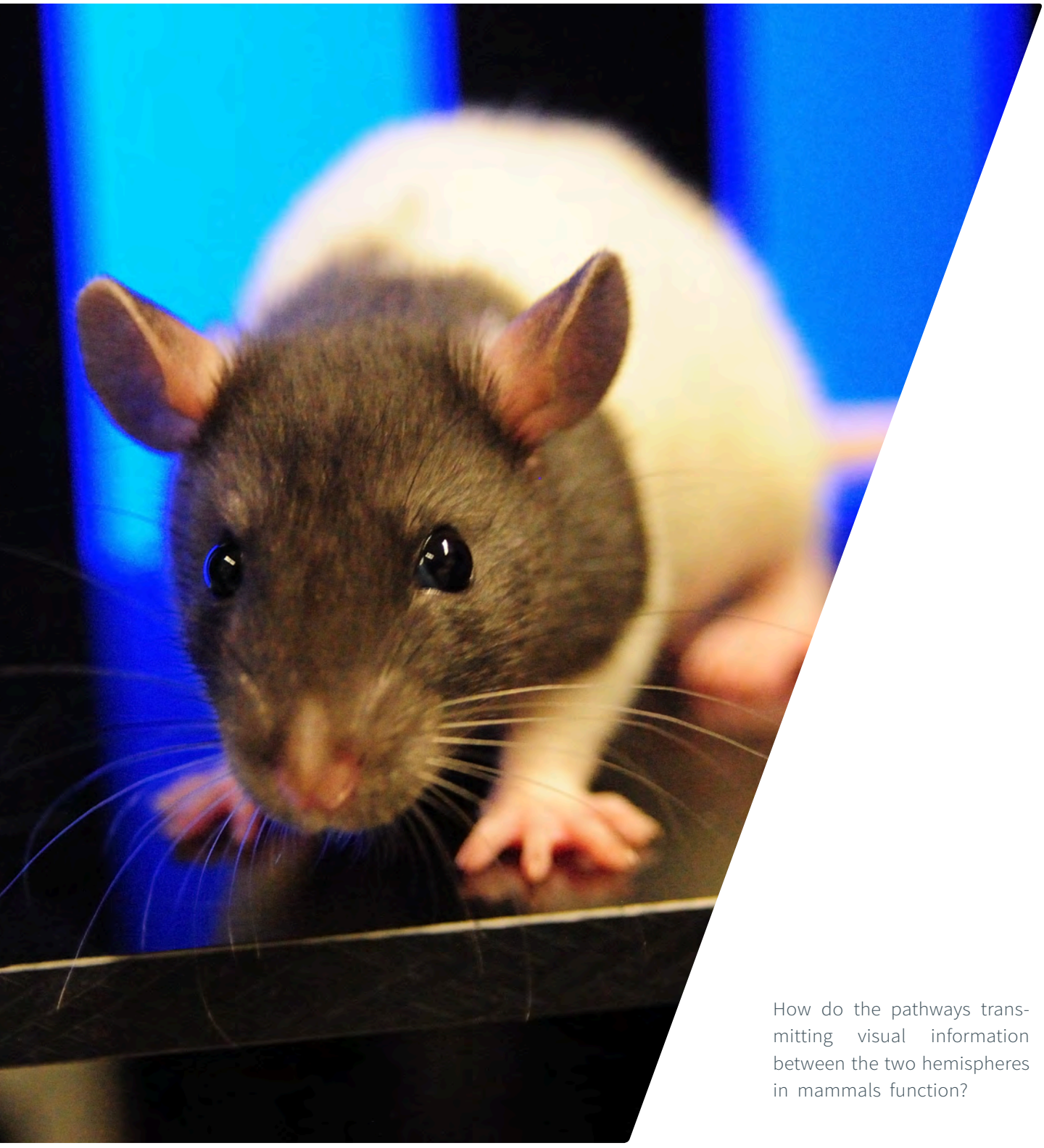
Stiftung caesar

caesar is a neuroscience research institute associated with the Max Planck Society.

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 and 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.

RESEARCH REPORTS



How do the pathways transmitting visual information between the two hemispheres in mammals function?

The visual cortex is a gateway with many paths

Mammals have a complex and highly interconnected visual system which includes a large and characteristic nerve tract connecting the left and right visual cortices, the function of which is largely unknown. We investigated some of the functional principles through which the tract operates.

by Dr. Jason Kerr | Department of Behavior and Brain Organization

The left and right visual cortices in mammals are commonly interconnected by a population of neurons that project their axons to the visual cortex in the contralateral hemisphere. These neurons are termed callosal projection neurons after the nerve tract (the corpus callosum) through which the axons travel. Not all of the visual cortex, which is topographically organized based on the visual space encoded by each eye, has this reciprocal projection, but the area that does represents the space directly in front of the animal. Performed in cats, early studies looking into the function of this pathway showed that this projection targets the retinotopically matched region of contralateral visual cortex [1], that is it connects regions in each visual cortex coding for the same part of the visual space, the space directly in front of the animal. As this also corresponds to the space where the left and right visual fields overlap, accordingly, it was suggested that the callosal pathway had the function of joining the representations of these overlapping regions in the left and right hemispheres [1, 2]. These studies, in cats and primates, subsequently also led to the suggestion that this projection was critical for binocular processing and stereopsis [3]. However, visual discrimination experiments in cats using random dot stereograms provided evidence to the contrary, showing that the callosal projection contributes to but is not essential for their stereoscopic vision [4], with animals being rendered incapable of stereoscopic vision only after sectioning both the corpus callosum and the optic chiasm. Since, very few studies have investigated the role that this pathway plays in visual processing and its role remains largely unclear. While results from some studies [5, 6] can be interpreted as indicating that the callosal pathway drives responses in neurons in the

contralateral visual cortex there have been no studies examining the functional contributions of the callosal projection to visual processing with single cell resolution. In this study we have investigated this in rodents. The questions we set out to address were 1) can the callosal projection independently drive activity in neurons in contralateral visual cortex in the rodent and 2) how does this pathway contribute to visual cortex processing of visual inputs?

Localization of contralateral projection neurons and effects of optogenetic inactivation

We first identified the region of the left visual cortex representing the visual space directly in front of the animal using intrinsic optical signal imaging (a technique in which a visual stimulus is positioned in the region of visual space of interest, and the corresponding region of visual cortex identified by the changes in light absorption which intrinsically accompany neuronal activity). We then used a modified adeno-associated virus to initiate expression of the fluorescent indicator yellow fluorescent protein (YFP) and the optogenetic inhibitor eArchT3.0 in a large population of neurons within the identified region (Fig. 1 a). Using the YFP as an anterograde tracer revealed the region of the visual cortex in the opposite hemisphere targeted by the cortical projection neurons (Fig. 1 a & b). We next used intrinsic optical signal imaging to target a small injection of red fluorescent marker (CTB-Alexa-594) into the representation in right visual cortex of the same region of space in front of the animal. The CTB-Alexa-594 injection was found to be located within the region targeted by the anterogradely labelled callosal projection neurons (Fig. 1 b), showing that the projection targets regions in the opposite hemisphere with matching retinotopic alignment. We next investigated the effects of optogenetic inactivation of callosal projection neurons on visually-evoked responses in neurons in the contralateral visual cortex.

This finding therefore may indicate that the callosal pathway can independently drive activity in a subpopulation of visual cortex neurons, without requiring activity in the ipsilateral LGN.

The optogenetic inhibitor eArchT3.0 is a light-activatable proton pump which when illuminated with blue light provides a strong hyperpolarizing current that inhibits neuronal activity, allowing reversible silencing of neuronal populations. Using conventional recordings of electrical activity evoked in neurons in the infected region by visual stimuli we could confirm that even neurons present in the deepest cortical layers furthest from the surface could be effectively silenced. We next used the eArchT3.0 to investigate how the activity of the callosal projection neurons in the left visual cortex contributes to the activity of neurons in the right visual cortex (Fig. 1 c). We recorded the activity of neurons in the upper layers of the right visual cortex *in vivo* by labelling them with the activity indicator OGB-1 (a fluorescent Ca^{2+}-sensitive indicator

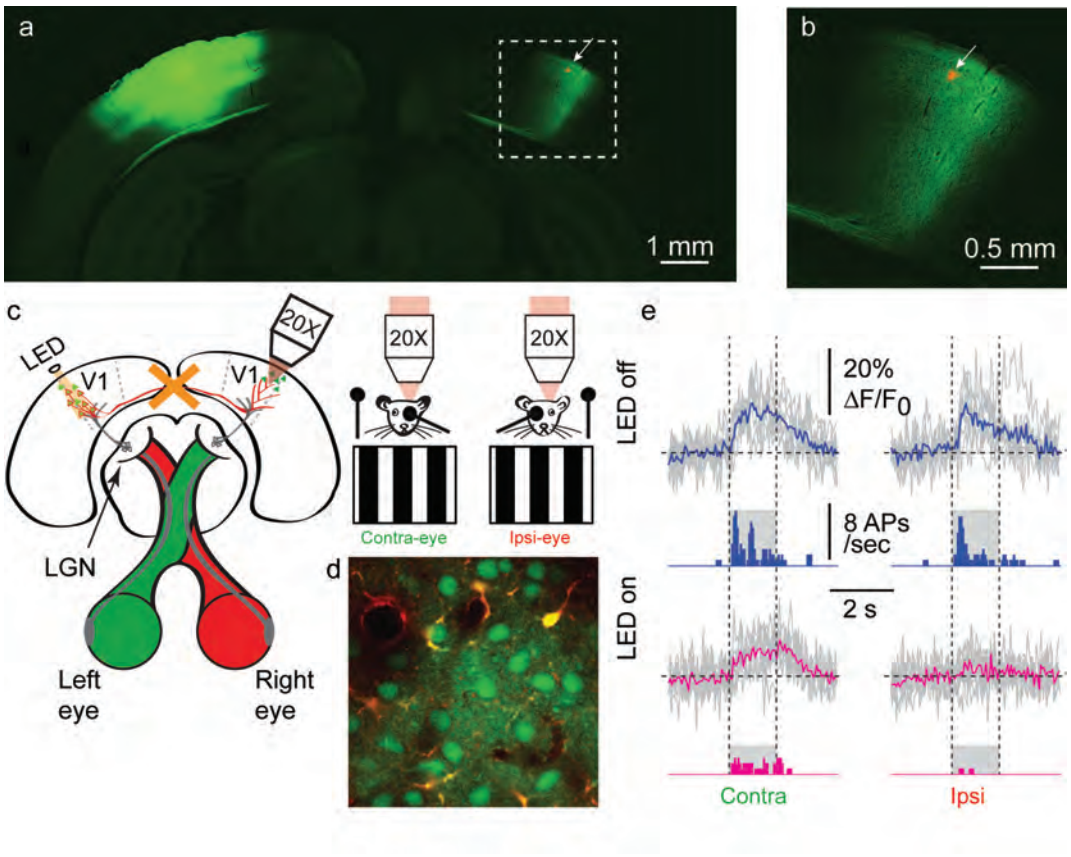


Figure 1: The callosal pathway in rat visual cortex and effects of optogenetic silencing. a. Coronal section showing neurons in the visual cortex of the left hemisphere expressing yellow fluorescence protein (YFP). b. Enlarged image of the region in the right hemisphere outlined in a. A small injection of CTB-Alexa-594 (red) was made in the region responsive to the visual space in front of the animal. c. Schematic of the main visual pathways from eye to visual cortex in the rat (left), showing also the experimental configuration for contra- and ipsilateral visual stimulation (right). Multiphoton recordings were made from neurons in the right visual cortex, with and without silencing of the callosal projection in the left hemisphere (denoted LED). The left eye (contralateral to the neuronal recordings) and associated projection to visual thalamus (lateral geniculate nucleus or LGN) is shown in green, the right eye (ipsilateral) and associated projection is shown in red. The uncrossed pathways are shown in gray. d. Example multiphoton image of neurons in right visual cortex labelled with OGB-1 (green), with astrocytes counterstained with sulforhodamine-101 (red). e. Example Ca^{2+} kinetic traces showing responses to contra- and ipsilateral visual stimulation for one neuron in the absence (LED off) and presence (LED on) of silencing of the callosal projection.

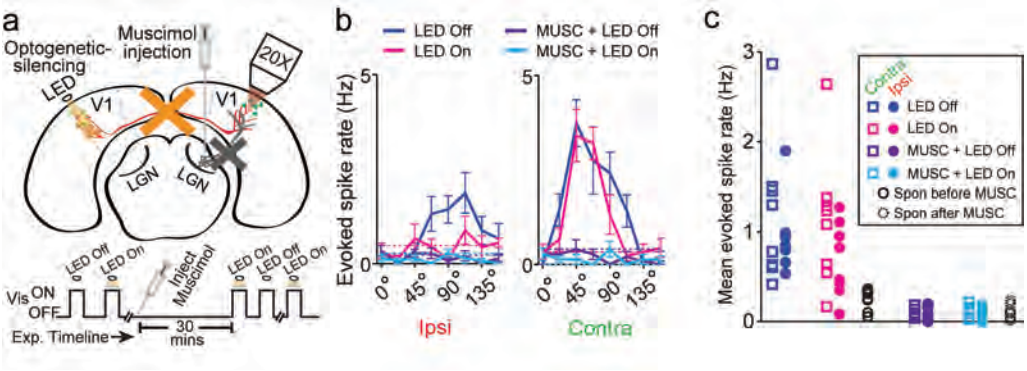


Figure 2: Effects of silencing the ipsilateral visual thalamus. a. Schematic of the experimental setup and design. b. Example average visual responses evoked in one neuron in the right visual cortex by multiple oriented visual stimuli for contra- and ipsilateral visual stimuli. Responses were recorded in the presence (LED on) and absence (LED off) of silencing of callosal projection neurons in the left visual cortex, and in the presence and absence of pharmacological silencing of the ipsilateral LGN with muscimol (MUSC). Dashed lines indicate mean rate of spontaneous activity. c. Group data of the experiment described in b, but showing the mean of responses to all stimulus orientations. Each marker denotes responses from one neuron. Mean of spontaneous rates in the presence and absence of silencing of visual thalamus also shown.

that increases its brightness as Ca^{2+} enters when a labelled neuron becomes electrically active) and monitoring the fluorescence of the labelled neurons using multiphoton imaging (Fig. 1 d). We recorded neuronal responses to visual stimuli presented to either the left eye (contralateral to the labelled neurons in the right visual cortex) or right eye (ipsilateral), both with and without optogenetic inactivation of the callosal projection neurons. Silencing of the callosal projection neurons resulted in a significant modulation of visual responses in 46% of the binocular neurons from which recordings were made. In 27% of the modulated neurons, responses to stimulation of the ipsilateral eye were reduced to be not significantly different to spontaneous activity (Fig. 1 e). There are at least 2 pathways that may account for the responses observed in these neurons after stimulation of the ipsilateral eye, one through the large projection to the left visual cortex (the crossed pathway) via the left visual thalamus (the lateral geniculate nucleus or LGN) then through the callosal pathway to the right visual cortex and the other through the much smaller projection directly to the right visual cortex (the uncrossed pathway) via the right LGN (Fig. 1 c). This finding therefore may indicate that the callosal pathway can independently drive activity in a subpopulation of visual cortex neurons, without requiring activity in the ipsilateral LGN. In summary, callosal projection neurons make a substantial contribution to visual responses in neurons in primary visual cortex, and for a subpopulation of binocular neurons the response to the ipsilateral eye is dependent on activity in the callosal projection neurons.

Dependence of responses on activity in visual thalamus

This result suggests activity in the callosal projection neurons may by itself be sufficient to drive activity in neurons in the visual cortex of the opposite hemisphere. If this were the case, then visual stimuli presented to the ipsilateral eye should still drive activity in neurons in the right visual cortex when the pathway through the ipsilateral LGN is pharmacologically silenced (Fig. 2 a). To test this, we first recorded responses from neurons in the right visual cortex as before. In this dataset, as in the previous one, responses to stimulation of the ipsilateral eye in a subpopulation of binocular neurons could be completely prevented by optogenetic inhibition of the callosal projection neurons in the left visual cortex (Fig. 2 b & c). Pharmacological inhibition of the ipsilateral LGN prevented all visually evoked responses, both those from stimuli presented to the contralateral and those presented to the ipsilateral eye (Fig. 2 b & c). So, while for some binocular neurons responses evoked by stimulation of the ipsilateral eye depend on activity in the callosal projection neurons, this projection alone is not sufficient to generate visually-evoked responses, but instead also requires activity in the ipsilateral LGN.

Comparison of responses in projection and non-projection neurons

Finally, to directly compare the visual-response characteristics of the callosal projection neurons with neighbouring non-projection neurons we recorded visually-evoked responses from neurons in the left visual cortex in which the cortical projection neurons had been labelled by injection of the retrograde tracer cholera toxin β subunit into the corresponding region of the right visual cortex (Fig. 3 a & b). The cholera toxin β subunit, tagged with a red fluorescent label, enters neuronal axons at the site where it is injected, and is subsequently transported throughout the neuron. Within the recorded population, 37.26 ± 2.79 % (mean ± SD, N = 3) of the neuronal population were found to be projection neurons. Neither spontaneous firing rates nor mean stimulus evoked firing rates were different between the two populations (Fig. 3 c). There was also no difference in the sharpness of tuning to visual stimuli (Fig. 3 d), nor in any other stimulus response characteristic that we analysed. Thus we found no evidence that callosal projection neurons respond differentially to visual stimuli compared to their non-projecting neighbours.

Summary

In this study we have demonstrated that visual responses in a subpopulation of binocular neurons in rodent visual cortex depend both on input from callosal projection neurons in the contralateral visual cortex and on input from the ipsilateral LGN. In addition, we also show that callosal projection neurons do not respond differentially to visual stimuli and that the projection targets retinotopically aligned regions in the contralateral visual cortex.

The finding that all ipsilateral eye visually-evoked responses observed in neurons

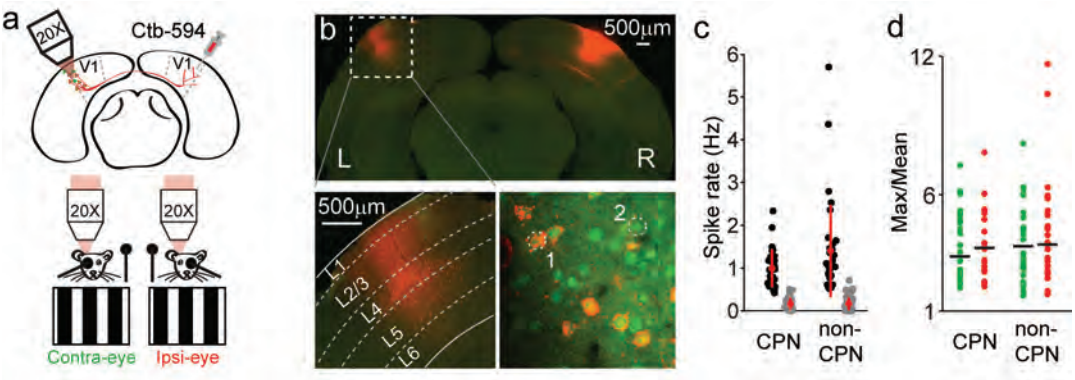


Figure 3: Visual responses in projection and non-projection neurons. a. Schematic of the experimental set-up. b. Coronal section of rat brain showing neurons in left visual cortex retrogradely labelled by injection of cholera toxin beta subunit (CTB) into right visual cortex (upper). Lower left image shows an enlarged image of the region outlined in the upper panel. Lower right image shows an example overview image of neurons in left visual cortex labelled with OGB-1 (green), with and without co-labelling with CTB (red puncta). c. Pooled data plot showing maximum visually-evoked response rate (black spots) and spontaneous firing rates (grey) for cortical projection neurons (CPN) and non-projecting neurons (non-CPN). Mean and standard deviation shown in red. d. Indication of stimulus tuning sharpness as measured by the maximum divided by the mean stimulus-evoked response rate for the same dataset as shown in b. Responses to contralateral stimulation shown in green, ipsilateral in red.

in visual cortex are abolished upon inactivation of the ipsilateral LGN, including responses in cells which could also be abolished by CPN silencing, forces a re-evaluation of the potential functions of the callosal pathway and the underlying neuronal mechanisms. Callosal projection neurons apparently act cooperatively with the projection from the visual thalamus, a requirement that until now was completely unknown.

References

1. Choudhury, B.P., Whitteridge, D., and Wilson, M.E. (1965). The Function of the Callosal Connections of the Visual Cortex. Q J Exp Physiol Cogn Med Sci 50, 214-219.

2. Hubel, D.H., and Wiesel, T.N. (1967). Cortical and callosal connections concerned with the vertical meridian of visual fields in the cat. J Neurophysiol 30, 1561-1573.

3. Blakemore, C. (1969). Binocular depth discrimination and the nasotemporal division. J Physiol 205, 471-497.

4. Ptito, M., Lepore, F., and Guillemot, J.P. (1991). Stereopsis in the cat: behavioral demonstration and underlying mechanisms. Neuropsychologia 29, 443-464.

5. Blakemore, C., Diao, Y.C., Pu, M.L., Wang, Y.K., and Xiao, Y.M. (1983). Possible functions of the interhemispheric connexions between visual cortical areas in the cat. J Physiol 337, 331-349.

6. Schmidt, K.E., Lomber, S.G., and Innocenti, G.M. (2010). Specificity of neuronal responses in primary visual cortex is modulated by interhemispheric corticocortical input. Cereb Cortex 20, 2776-2786.

About the Department of Behavior and Brain Organization

The primary aim of the Department of Behavior and Brain Organization (BBO) is to understand how mammals use vision to make decisions and what the underlying neural processes are. BBO combines imaging, computation, behavioral analysis, electrophysiological recordings, and anatomical mapping to explore the connection between behavior and neuronal activity. The research of BBO can be divided into two broad regions. The first develops tools and techniques, which have single cell and single action-potential resolution, for recording and analyzing neuronal activity from large populations of cortical neurons in the awake and freely moving mammal. The second is focused on understanding the neuronal mechanisms underlying vision-based decision making in freely moving mammals. This involves the development of special multiphoton microscopes and of optics-based head and eye tracking techniques that can be used on freely behaving animals from a range of mammalian 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.

How do the various visual systems of animals perceive the same speed of a moving object, despite a vast range of differently sized retinas?

Secrets of the retina: Eye size and the perception of speed

Amongst mammalian species, the diameter of the eye varies by more than an order of magnitude. We investigated how the detailed synaptic wiring of the retina has evolved to accommodate differences in eye diameter and the detection of moving objects.

by Dr. Kevin Briggman | Department of Computational Neuroethology

The detection of moving objects is critical for the survival of most animals with a developed visual system. For example, animals must detect the motion of a predator or prey and then make an appropriate behavioral response. A key part of this computation is the ability to distinguish not only whether something is moving in the visual space, but also the direction of a moving object. In many species, this computation – distinguishing whether an object is moving up, down, left or right – is performed early in the visual system at the level of the retina, the photosensitive neural tissue located at the back of the eye.

We and many other research groups have studied precisely how this computation is performed by the various types of neurons in the retina. This effort has been successful at discovering which neurons are involved in computing direction and how the precise connection pattern between the neurons enables this computation.

Circuit secrets

The neural circuit that computes the motion direction is often referred to as the retinal direction selective circuit. A subclass of the retinal output neurons relay visual information to the rest of the brain – termed direction selective retinal ganglion cells, or DSGCs – encode the direction of motion along four visual axes (up, down, left, or right) [1]. These neurons receive inhibitory synapses from a class of interneurons, starburst amacrine cells (SACs), so named because of their unique radially symmetric morphology [2].

While it was known for some time that the inhibitory synapses formed by SACs onto DSGCs was essential for the computation of direction selectivity [3], it was only recently described that the biophysical properties of SACs and a very precise wiring pattern underlie the computation [4, 5].

The problem of eye sizes

Our recent work has focused on a problem that arises in the animal kingdom related to the substantial variability in eye diameters across species and how these differences effect the computation of direction [4]. For example, the eye of a mouse measures approximately 3 millimeters in diameter, while larger mammals, such as rabbits, have eyes that are about 15 millimeters in diameter – a factor of 5 times larger. The diameter of an eye and the lens determines how large an object in visual space projects onto the surface of the retina. An object that spans 10 degrees of visual space projects onto an area of approximately 1500 micrometers on the surface of a rabbit retina, but only 300 micrometers on the surface of a mouse retina (Fig. 1). This difference extends to the velocity of moving objects – an object moving at an angular velocity of 10 degrees/sec traverses the surface of a rabbit retina at a linear velocity five times faster than the mouse retina.

This difference poses a dilemma about how different mammalian species can detect the same ranges of angular velocities. If the direction selective circuits in the retinas of rabbits and mice are identical, it would imply that the two species detect different angular velocity ranges of visual motion (i.e., shifted by a factor of five). If, on the other hand, an evolutionary goal was to allow the two species to detect similar angular velocity ranges, then the implication is that the circuits must be in some way different to compensate for the difference in eye diameters. These two competing hypotheses drove us to compare the detailed anatomy of the rabbit and mouse retinas and look for any differences in the wiring of the direction selective circuits.

> The differences in eye size pose a dilemma about how mammalian species can detect the same ranges of angular velocities. The circuits must be in some way different to compensate for the difference in eye diameters.

Examining the retina

We first collected a large three-dimensional electron microscopy dataset from a mouse retina using serial block-face scanning electron microscopy [5]. We then mapped the locations of excitatory and inhibitory synapses formed on the dendritic branches of SACs within the data. We noticed a striking difference in the organization of the synaptic inputs onto mouse SACs compared to previously reported reconstructions of the synaptic inputs onto rabbit SACs [6]. The inhibitory inputs were clustered along dendritic locations proximal to the somas of mouse SACs, whereas they are more distally distributed along the dendrites of rabbit SACs (Fig. 2).

To explore the effect of shifting the locations of inhibitory inputs, we then constructed a computational model of a network of SACs.

Given the detail of our anatomical reconstructions, we were able to substantially constrain our network model, including using realistic dendritic diameters, synapse locations and inter-SAC connectivity statistics. We then examined the direction selective properties of our model by simulating a bar of light sweeping across the model at different linear velocities. When we wired the model to reproduce the pattern of connectivity observed in the rabbit retina, it produced direction selective responses in the SACs above about 1 millimeter/sec. However, when we shifted the location of inhibitory connections to reproduce the mouse wiring pattern, the velocity range of directions selective responses shifted to lower speeds down to around 0.2 millimeter/sec. In other words, simply changing the location of the inhibitory synapses along the dendrites of SACs shifts the velocity range of direction selective responses by close to a factor of five – nearly the same difference in eye diameter noted above.

Subtle wiring changes

Our data therefore favor the hypothesis that retinal direction selective circuits have evolved to allow mammalian species to see, and respond to, objects moving at similar angular velocities. The retina achieves this by altering the wiring in a relatively subtle way that we were only able to detect by large-

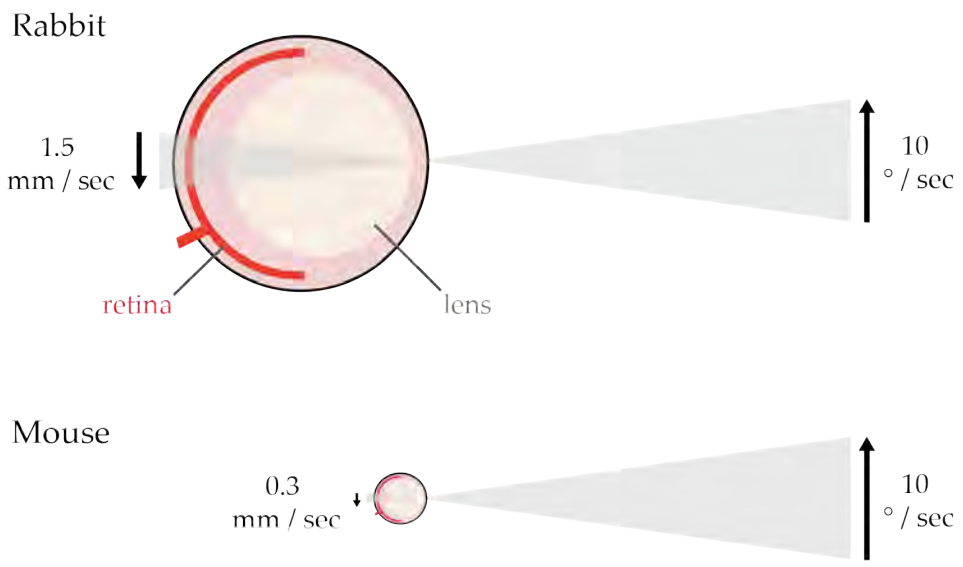


Figure 1: The diameter of the eye determines the velocity of an object projected onto the retina.

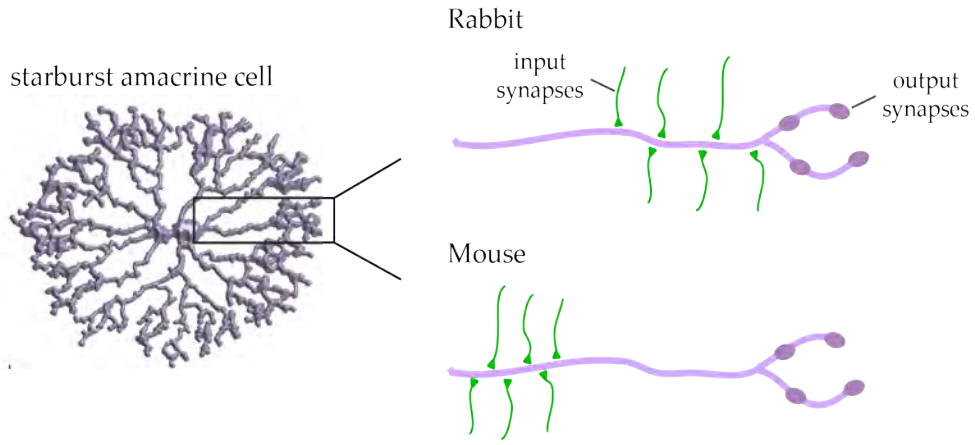


Figure 2: An adaptation in the detailed synaptic wiring of starburst amacrine cells (SACs) between the mouse and rabbit retinas can compensate for the difference in projected velocities.

scale anatomical reconstructions at the nanometer scale. If our hypothesis is correct, we would now expect the differences in wiring patterns to generalize to additional species with even larger or smaller eye diameters – a task we are now undertaking. More generally, this study demonstrates the benefits of comparing the detailed connectivity of the brain across multiple species. In our case, this type of comparative approach ('comparative connectomics') allowed us to probe which features of a neural circuit are essential for a given computation and which details are species specific.

References

1. Barlow, H.B., Hill, R.M., and Levick, W.R. (1964). Retinal Ganglion Cells Responding Selectively to Direction and Speed of Image Motion in the Rabbit. J Physiol 173, 377-407.

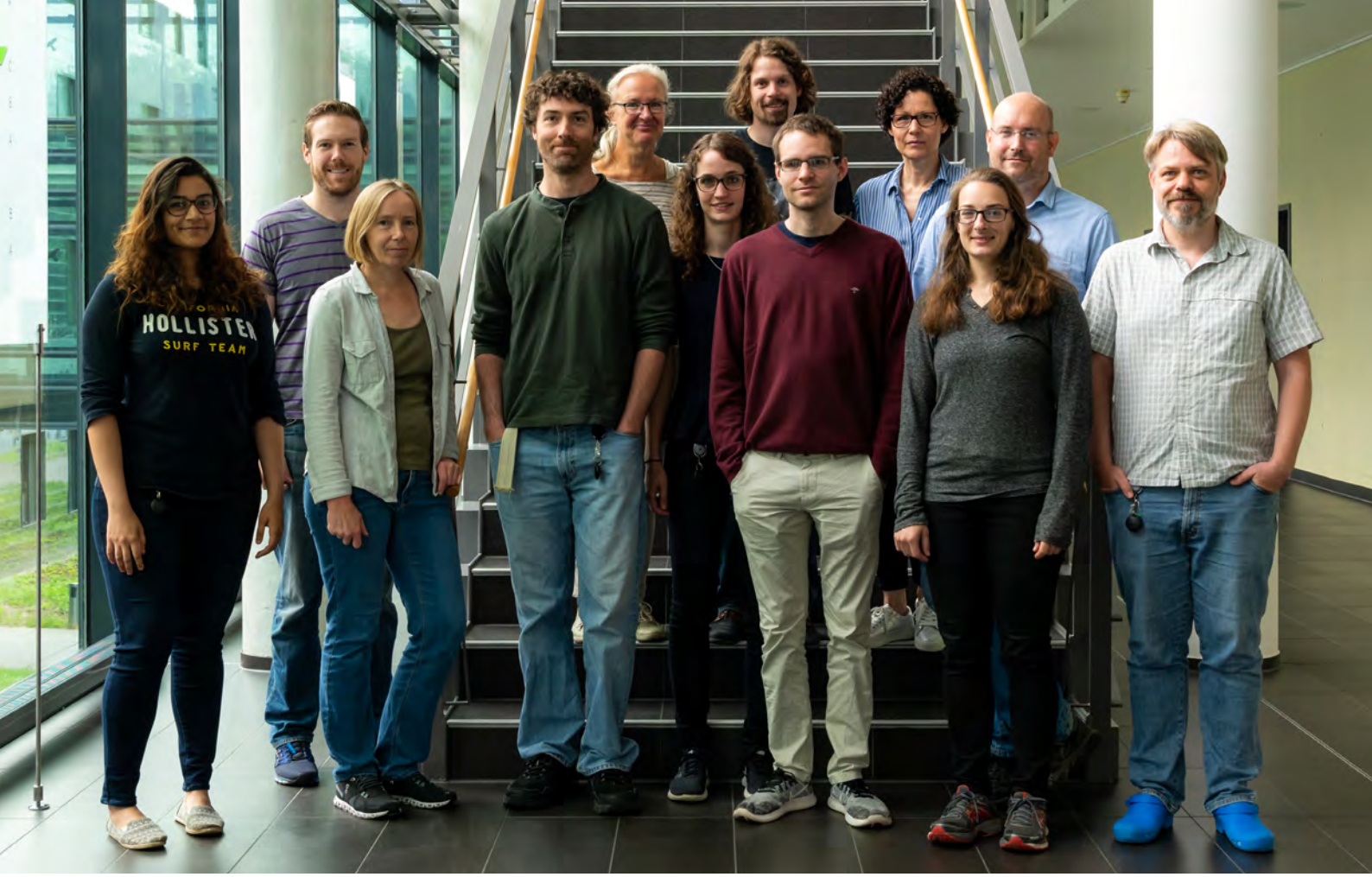
2. Euler, T., Detwiler, P.B., and Denk, W. (2002). Directionally selective calcium signals in dendrites of starburst amacrine cells. Nature 418, 845-852.

3. Briggman, K.L., Helmstaedter, M., and Denk, W. (2011). Wiring specificity in the direction-selectivity circuit of the retina. Nature 471, 183-188.

4. Denk, W., and Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol 2, e329.

5. Ding, H., Smith, R.G., Poleg-Polsky, A., Diamond, J.S., and Briggman, K.L. (2016). Species-specific wiring for direction selectivity in the mammalian retina. Nature 535, 105-110.

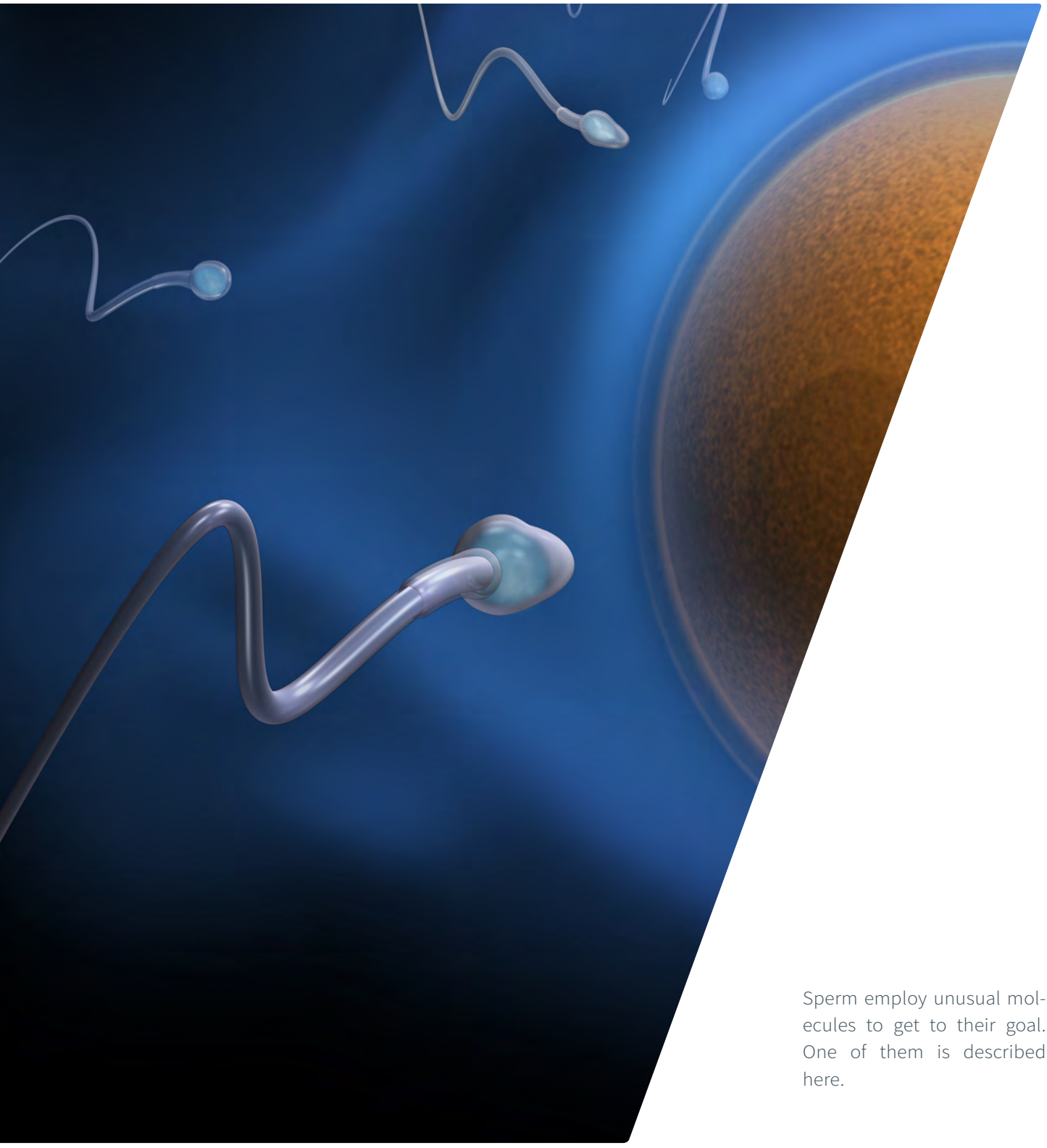
6. Famiglietti, E.V. (1991). Synaptic organization of starburst amacrine cells in rabbit retina: analysis of serial thin sections by electron microscopy and graphic reconstruction. J Comp Neurol 309, 40-70.



About the Department of Computational Neuroethology

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.



Sperm employ unusual molecules to get to their goal. One of them is described here.

An unusual chimera – half ion channel, half transporter

An unusual chimeric transport molecule – half lion, half human – reveals its secret. It allows unexpected insights into the origins of ion transport in cells.

by Prof. U. Benjamin Kaupp | Emeritus group Molecular Sensory Systems

Sperm are unusual. Compared to other cells, such as heart or nerve cells, sperm often use special proteins that do not exist in other cells. With the help of various physical and chemical signposts, sperm swim towards the egg. Navigation is controlled by biochemical signalling pathways in sperm. Only in recent years has it been recognized that molecules familiar to us from normal somatic cells - such as olfactory cells or photoreceptors - do not play a role in sperm cells. More confusing still, the signal molecules that occur in sperm look like familiar faces on the outside – that is, their amino acid sequence is similar. However, it is a “mask” behind which other, unexpected properties and functions are hidden. This masquerade has caused great confusion in reproduction research because some signalling molecules have initially been attributed known properties. Only later did it become apparent that these sperm-specific molecules “tick” quite differently. The example also illustrates how some scientists, instead of being guided by factual hypotheses, are seduced by their preconceived beliefs into drawing false conclusions.

Why does nature go to such great lengths to provide sperm with a unique repertoire of signalling molecules? Normally, cells are permanently embedded in a tissue or organ. Their environment is stable and does not change quickly or dramatically. Sperm are different. From the testicles to the epididymis, seminal fluid, vagina, and fallopian tube, sperm are exposed to very different chemical and cellular environments. In fish, this is obvious: sperm, surrounded by a physiological solution in the testes, are released either into seawater with a salt content of 36% (herring, eel) or into fresh water of a river (salmon). The ion concentrations in sea and river water differ by up to 1000 times. It is therefore assumed that the signalling molecules in sperm

of different animal species have adapted to the respective environment.

One such molecule is the sodium/proton exchanger (NHE) in sperm [1]. NHEs, which regulate the pH value in many cells, belong to a large family of membrane proteins that transport ions and other important nutrients into or out of cells - from simple bacteria to humans. The inward or outward directed mass transport is coupled: The Na^+ transport into the cell is connected to H^+ outflow. This exchange is reversible: Na^+ and H^+ can be transported in both directions. The mechanism by which molecules are passed through the cell membrane in a *pas de deux* always seems to be the same: a rocking motion (Fig. 1). A Na^+ ion first binds to the outside of the exchanger; almost simultaneously, a proton binds to the inside. With to-and-from movements, as in a rocking-chair, the ions are continuously transported inwards and outwards. This rocking-chair mechanism seems to have been preserved for the transport of ions, amino acids, sugars, or fats in all transporters. However, the NHE molecule in sperm deviates from this rule. Many transport molecules are switched on. In ion channels, this is called gating, because a gate is opened through which ions can flow. In ion channels, a physical gate actually blocks the pore through which ions can flow. The gate can

> On their way to the egg sperm must pass different milieus. They use unique molecules to cope with these challenges.

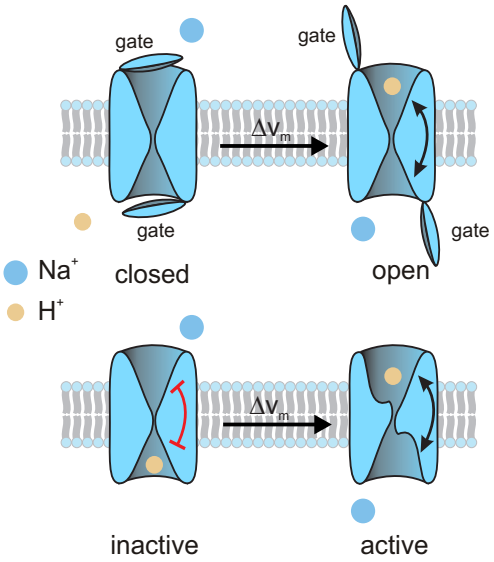


Figure 1: Proposal for two different gating mechanisms of the Na^+/H^+ exchanger. A lid blocks the ions' access to the respective binding sites. A voltage jump opens the lid; the rocking chair mechanism is now enabled (top). The ion-binding sites are accessible, but the rocking chair movement is locked (red bar). The voltage jump unlocks the mechanism (bottom).

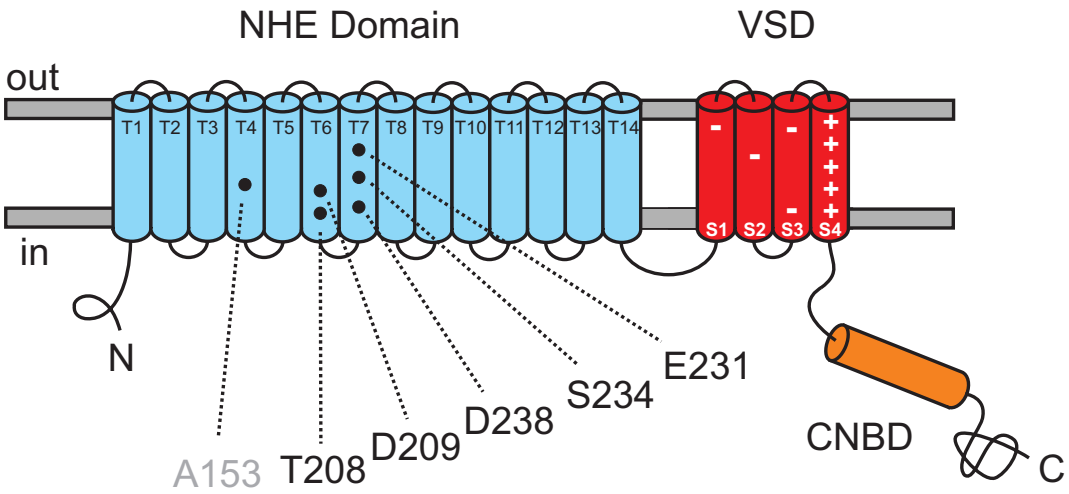


Figure 2: Schematic representation of the NHE topology in the cell membrane. The exchange domain (blue) shows the amino acids (black) of the Na^+ binding site, which are conserved between NHEs of archaea and sperm; transmembrane segments are marked as T1-T14. Voltage sensor (VSD; red) with positively and negatively charged amino acids. Cyclic nucleotide-binding domain (CNBD; orange).

be opened by membrane voltage or chemical ligands. Which mechanism switches transporters on and off and sets the rocking movement in motion? It's the ions themselves! When they bind to the transport molecule, they simultaneously change its structure: the binding site moves up and down and carries the ions along with it as if in an elevator.

This gating mechanism is different in the Na^+/H^+ exchanger of sperm. The exchanger has two other domains known from ion channels: a voltage-sensing domain (VSD) and a cyclic nucleotide-binding domain (CNBD) (Fig. 2). This observation was very surprising and indicated that the additional domains had something to do with the gating of the NHE.

Although the NHEs from mammalian sperm cells had already been discovered in 2004, their properties remained unknown because the corresponding mammalian genes could not be expressed in cell lines and the characterization in sperm cells was technically difficult. We have, therefore, tested NHE molecules from other animal species to see if they could be expressed functionally. We succeeded with the exchanger from sea urchin sperm.

We were able to show that the NHE molecule actually exchanges Na^+- for H^+ ions and that the exchange activity is switched on and off by changes in membrane voltage. The most exciting finding, however, was that we – *en passant* – could reveal the role of the

cellular messenger cAMP in sea urchin sperm. Although it has been known for almost 40 years that cAMP is somehow involved in the signalling pathway, the exact function could not be deciphered. Our work shows that cAMP makes the exchanger more sensitive and that ion transport can be switched on by smaller voltage jumps. We had already discovered a similar mechanism years ago in the pacemaker channel of the heart [2]. Thus, also here a *déjà vu*. In essence, the exchanger is a chimera between transporters and channels. One of the most exciting questions is: How can one and the same voltage sensor and a cAMP-binding domain control two such different mechanisms of ion transport. The elucidation of the 3-dimensional architecture by biological structural research will hopefully provide the answer as to how the mechanisms of ion transport have differentiated during evolution.

References

1. Windler, F., Bönigk, W., Körschen, H.G., Grahn, E., Strünker, T., Seifert, R., Kaupp, U.B. (2018). The solute carrier SLC9C1 is a Na^+/H^+-exchanger gated by an S4-type voltage-sensor and cyclic-nucleotide binding. Nat. Commun., in press.

2. Gauss, R., Seifert, R. and Kaupp, U.B. (1998). Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. Nature 393, 583-587.

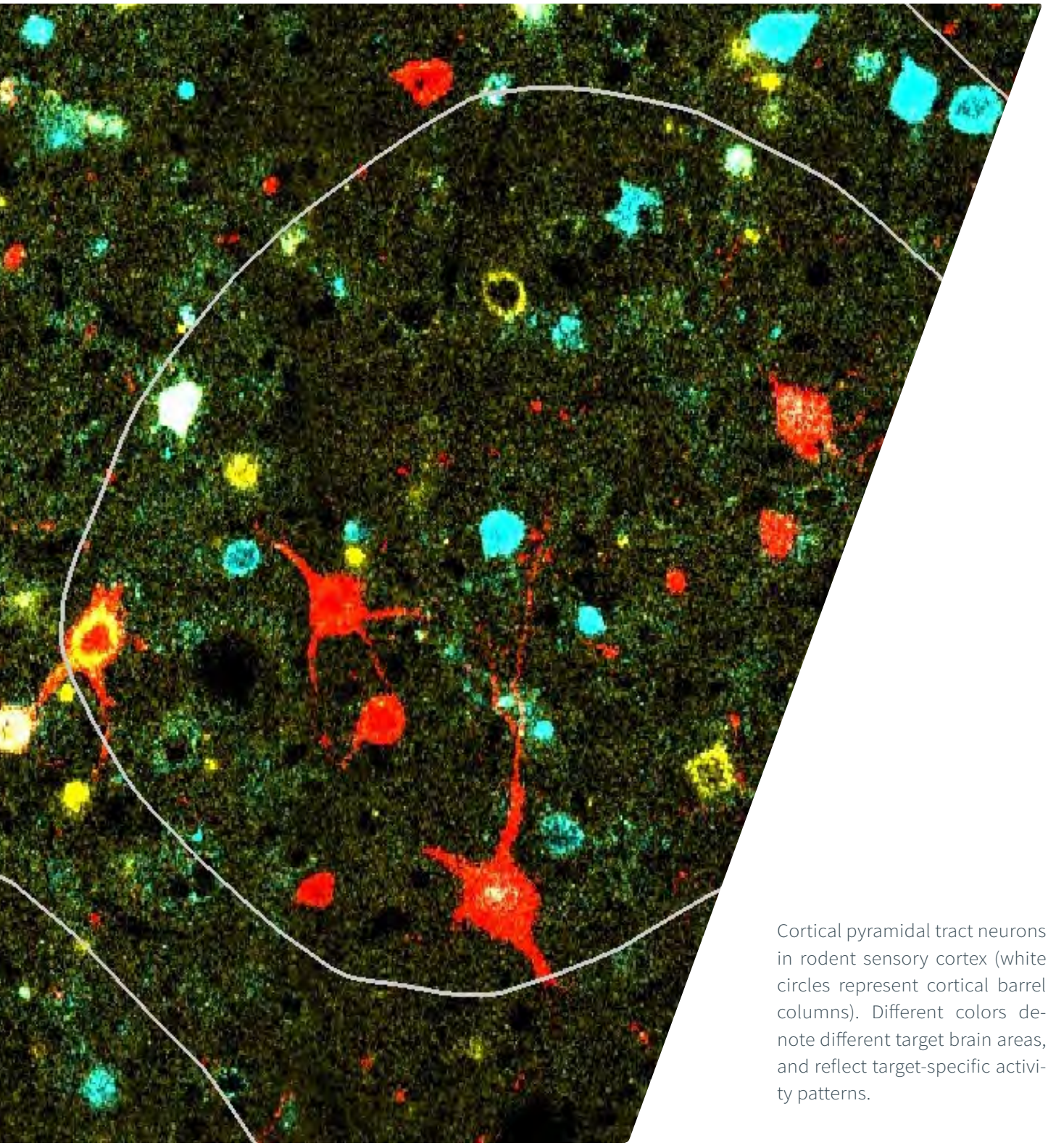
About the Emeritus group: Molecular Sensory Systems

We study signal processing in cells. We want to understand how sensory stimuli are detected by cells and converted into a cellular response. The in-depth understanding of this complex process requires the use of biological, chemical, and physical techniques. Therefore, biologists, chemists, and physicists work closely together in the department.

Our research aims to reveal the molecular mechanisms underlying sensory transduction. To this end, we study the structure, the function, and the interaction of proteins involved in these processes. In particular, we study ion channels that are involved in the generation of electrical signals.

The success of fertilization depends on the ability of motile sperm to locate the egg. We study the signaling pathways underlying chemosensation in sperm of sea urchins and humans. In particular, we are interested in the receptors, cellular messengers and ion channels that endow sperm with such exquisite sensitivity.

We develop optical switches that are used in the photonic control of receptors and ion channels. With the help of compound “Trojan horses” cellular signal pathways can be resolved with great precision, both temporally and spatially. The so-called caged compounds are used to track conformational changes of proteins in space and time.



Cortical pyramidal tract neurons in rodent sensory cortex (white circles represent cortical barrel columns). Different colors denote different target brain areas, and reflect target-specific activity patterns.

How the neocortex can transform sensory input into motor output

Pyramidal tract neurons (PTs) – the major output cell type of the neocortex – integrate feed-forward thalamic with recurrent cortical inputs, and thereby transform sensory information into activity patterns that reflect the PTs' target brain regions.

By Dr. Marcel Oberlaender | Max Planck Research Group: In Silico Brain Sciences

The neocortex is the part of the mammalian brain involved in higher-order functions such as sensory perception, cognition and generation of motor commands. In 2017, we reported basic principles by which cortical circuits integrate sensory information from different stimuli, transform those inputs into activity patterns that are related to the respective target brain areas, and identified which of those output pathways can be involved in motor control.

Background

Rodents actively move their facial whiskers (vibrissae) to explore their environment. The vibrissal system is thus a common model system to investigate principles of sensory information processing, motor control, and interactions between sensory and motor pathways. Recent studies have provided detailed insight into the central pattern generator pathways that drive rhythmic whisker movements at the level of the brainstem (BS). Furthermore, accumulating evidence suggests that several cortical pathways to a variety of brainstem structures may play a crucial role in sensory-motor feedback during exploratory behaviors.

In a series of recent studies, the "In Silico Brain Sciences" group thus investigated the structural and functional basis of cortical sensory-motor feedback in the rodent whisker system. To do so, we focused our research on the major output cell type of the neocortex: pyramidal tract neurons (PTs). PTs integrate sensory-evoked feed-forward

excitation from thalamocortical (TC) circuits, with recurrent and top-down inputs from local and long-range intracortical (IC) circuits, and broadcast the results of this integration process to specific ensembles of downstream targets.

How sensory input drives activity in PTs

The origin of the PTs' sensory-evoked responses remains unknown within the present concepts of cortical circuit organization. To investigate how PTs integrate and transform stimulus-specific (i.e., here from nine different whiskers) synaptic input patterns into cortical output patterns, we developed a multi-scale model, which can be regarded as the most comprehensive digital representation of the cortical circuitry to date. We show that the model allows performing simulations that mimic *in vivo* conditions, and which predict activity patterns *in silico* that are consistent with experimental observations that range from synaptic to cellular and network levels. The simulations revealed that a stimulus-specific combination of deep layer TC and IC synaptic input is driving cortical output. The populations that were hypothesized previously to generate sensory-evoked activity in PTs were shown to have structural and/or functional properties that – in principle – prevent them from driving PTs. Our findings give rise to a novel theory that unifies and augments the present concepts of 'columnar' and 'canonical circuit' cortex organization [1].

> The data revealed that a subset of PTs – which provide cortical output specifically to neurons in the trigeminal complex of the brainstem – have the shortest synaptic distance to the whisker muscles, compared to all other neurons throughout the neocortex.

Sensory-evoked activity patterns of PTs are target area-specific

Recent studies suggested that cortical neurons may have different functional roles when processing the same sensory stimulus, depending on the brain area into which they project their respective long-range axons. Therefore, we investigated whether long-range target-specific segregation into multiple functional channels applies to PTs. The lack of genetic or molecular markers that correlate with the specific long-range targets of PTs, as well as the large distances to and between the various subcortical target areas, so far prevented from identifying or reconstructing the long-range targets of PTs after electrophysiological measurements *in vivo*.

To overcome these limitations, we injected retrograde tracer agents into multiple subcortical areas, performed *in vivo* cell-attached recordings of the retrogradely-labeled PTs and filled them to reconstruct their mor-

phologies. We found that soma depth location and layer-specific dendrite distributions allow predicting the respective subcortical target area of PTs, and that activity patterns during both, periods of ongoing activity and during whisker stimulation are target-related. These findings indicate that stimulus features may be differentially extracted by PTs via long-range target-specific subnetworks, which could be reflected by the target-specific embedding of somata and dendrites into the cortical circuitry [2].

Revealing the structural basis of sensory-motor feedback by PTs

To reveal whether PTs may indeed be embedded into long-range target-specific subnetworks – and to dissect which of those subnetworks may be involved in whisker motor control – we investigated the structural organization of whisker muscle-related neuronal networks throughout the brain.

To do so, we injected retrograde tracer agents into the intrinsic muscles that move a single whisker. This allowed labeling a sparse population of whisker-specific motoneurons (vMNs), and to trans-synaptically label brainstem and cortical neurons that provide input to these vMNs. The data revealed that a subset of PTs in vS1 – which provide cortical output specifically to neurons in the trigeminal complex of the brainstem – have the shortest synaptic distance to the whisker muscles, compared to all other neurons throughout

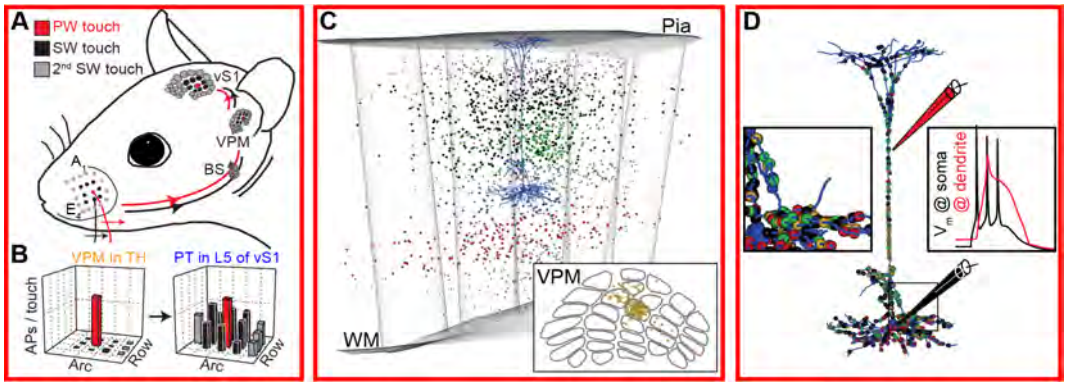


Figure 1: a. Schematic of sensory pathways that underlie whisker-evoked responses of PTs in vS1. b. PTs in vS1 respond to touch of several whiskers, whereas their TC input neurons respond only to a single whisker. c. Multi-scale model of TC and IC networks. TC and IC neurons (colored by cell type) that provide input to the PT used for simulations (blue). d. Cell type-specific synapses that drive whisker-evoked responses during simulations (right panel). Images were adopted and modified from [1].

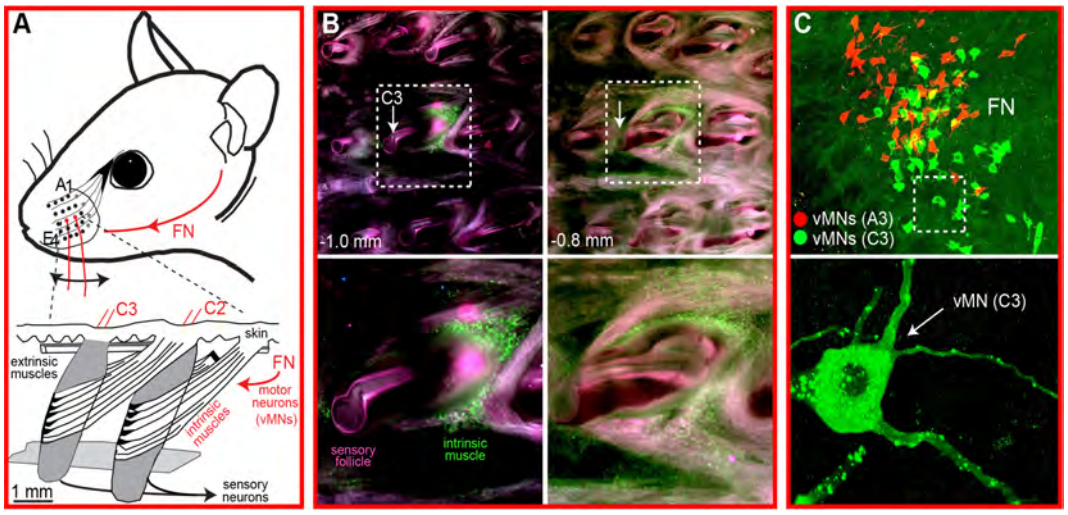


Figure 2: a. Schematic illustration of the musculature underlying whisker motor control. b. Injections of retrograde tracer agents were targeted to the intrinsic muscles of individual whiskers. Top panels show the center locations of the injection (white arrow) of the targeted C3 whisker. Bottom panels: Zoom-ins illustrate that the tracer spreads only within the injected muscle. c. Top panel: The retrograde tracers label specific sets of vMNs, which control the respectively injected whisker muscles. Bottom panel: Zoom-in to one retrogradely labeled vMNs. Images were adopted and modified from [2].

the neocortex. These observations set the stage to extend our digital cortex model to the subcortical networks of whisker motor control, which will be a necessary prerequisite for revealing basic principles of how cortex (e.g. PTs in vS1) is able to provide sensory-motor feedback, and thereby orchestrates sensory-guided behaviors [3].

References

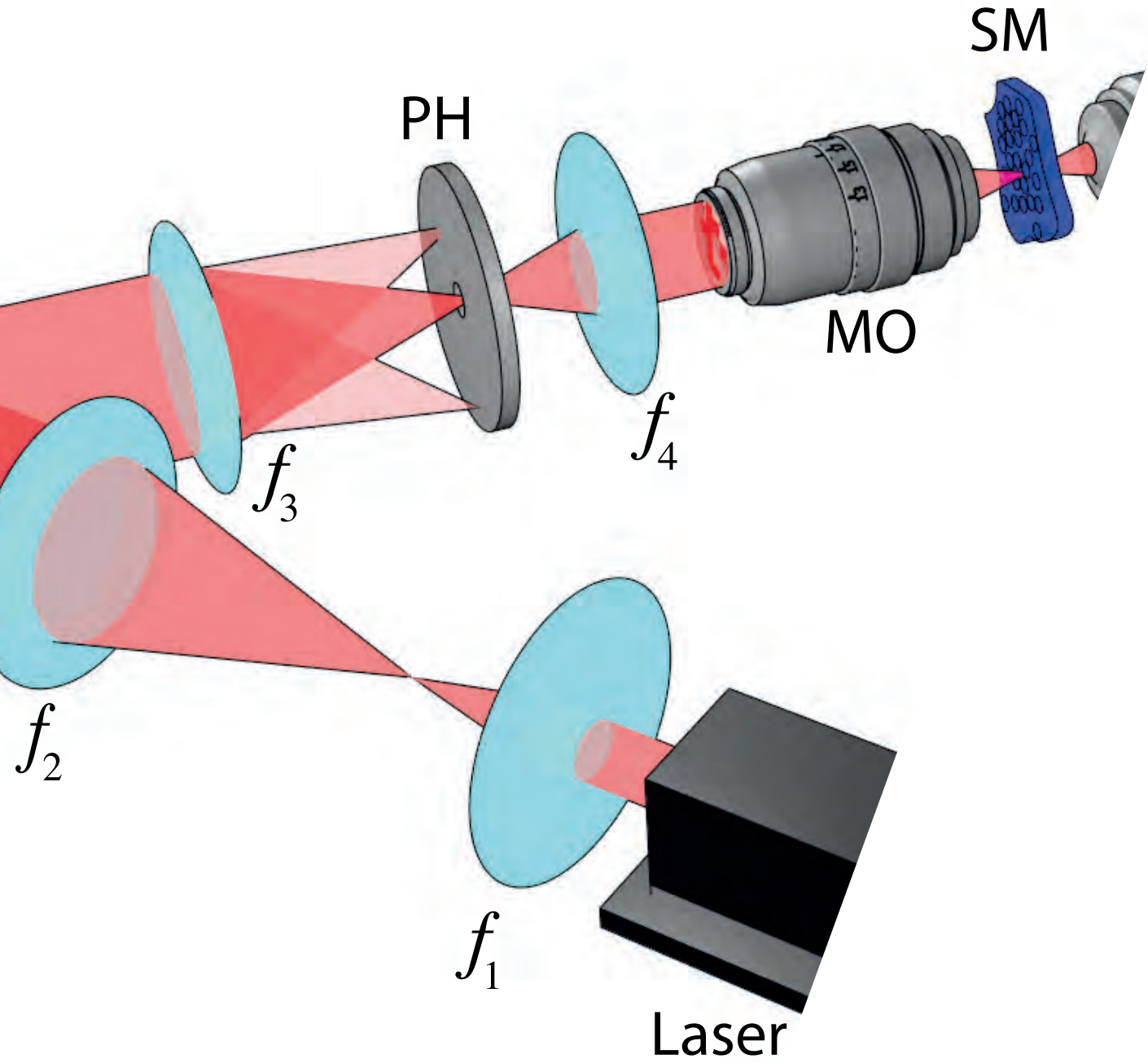
1. Egger, R., Narayanan, R. T., Bast, A., Udvary, D., De Kock, C.P., and Oberlaender, M. (2018). Cortical output is driven by transcolumnar pathways in deep layers. under review.

2. Rojas-Piloni, G., Guest, J.M., Egger, R., Johnson, A.S., Sakmann, B., and Oberlaender, M. (2017). Relationships between structure, in vivo function and long-range axonal target of cortical pyramidal tract neurons. Nat Commun 8, 870.

3. Guest, J.M., Seetharama, M.M., Wendel, E.S., Strick, P.L., and Oberlaender, M. (2018). 3D reconstruction and standardization of the rat facial nucleus for precise mapping of vibrissal motor networks. Neuroscience 368, 171-186.

About the Max Planck Research Group: In Silico Brain Sciences

One of the key challenges in neuroscience research is to elucidate mechanistic principles of how the brain integrates sensory information from its environment to generate behavior. At present, experimental methods to directly monitor sensory-evoked streams of excitation throughout the brain, at cellular and millisecond resolution are lacking. To overcome these limitations, the 'Max Planck Research Group: In Silico Brain Sciences' seeks to develop an alternative reverse engineering approach. This approach combines electrophysiological measurements in the intact brain (*in vivo*) with reconstructions of the detailed 3D anatomy of local and long-range neuronal networks. These measurements provide the structural and functional basis for data-driven digital models of brain-wide neuronal circuits. For the example of the rodent whisker system, the reverse engineering approach is currently applied to generate anatomically and physiologically realistic models of the whisker-related primary areas in the brain stem, thalamus and cortex. The models allow performing computer simulations (*in silico*) that mimic the *in vivo* stream of touch-evoked signal flow, and thereby provide novel insights into basic principles of sensory information processing.



Setup for a light scattering experiment. A digital micro-mirror device (DMD) modifies the wavefront of a laser beam such that it can penetrate through a scattering material.

Optical microscopy in the fruit fly

Light scattering is limiting for many applications that rely on the controlled propagation of light, for example in optical microscopy, but also in other areas that need to detect or deliver light for imaging, sensing or manipulation. We have developed a machine learning approach to improve the control of light under scattering conditions.

by Dr. Johannes Seelig | Max Planck Research Group: Neural Circuits

We use optical microscopy techniques, such as two-photon calcium imaging, as well as patch-clamp recordings to understand how neural networks in the brain change over multiple timescales, from the millisecond range of single neuron dynamics to the orders of magnitude slower behavioral adaptations that are linked to the rhythms of day and night. Our research focuses on the model organism *Drosophila melanogaster* where genetically identified, comprehensive neural networks underlying adaptive behaviors can be studied. The fly has a multitude of sophisticated behaviors, such as visual pattern recognition - it can for example learn to distinguish and upright 'T' from an inverted 'T' - or visually guided place learning, where it remembers a place with respect to visual landmarks in its surroundings. Molecular, structural, and functional similarities of neural circuits across species suggest that insights into the computations that underlie such behavior gained in the fly will also contribute to our understanding of the mammalian brain [1].

Tools for non-invasive imaging in tissue

However, monitoring structural or functional dynamical changes that occur throughout the fly's nervous system at diffraction limited resolution *in vivo*, and in particular over long timescales, poses a variety of technical challenges. This is due to the fact that the fly's nervous system is embedded in multiple tissue layers and shielded by a cuticle. Together, these layers scatter light strongly and prevent direct optical access to the brain or other parts of the nervous system. Optical microscopy in the fly therefore typically relies on removing parts of the

cuticle and underlying tissue to gain optical access, which however is invasive and shortens the lifespan of the animal.

The problem of limited non-invasive access into tissue is encountered generally with optical microscopy, which is limited in its imaging depth by light scattering. As light propagates in tissue (or any other non-homogenous material), slight variations in the optical properties of the material, for example between different cells, lead to scattering of light and a distortion of the propagating wavefront (a familiar example would be the reduced visibility in fog). As light penetrates deeper into tissue, these distortions ultimately prevent the formation of an image.

Adaptive optics correct for light scattering

Multiple techniques have been developed to overcome such scattering of light in brain tissue [2, 3]. One approach, which for example contributes to the success of multi-photon microscopy, relies on using light of longer wavelengths that therefore scatters less. An alternative and complementary set of techniques that can be applied for light of any wavelength is summarized under the name of adaptive optics. These techniques originated in astronomy - where scattering in the atmosphere degrades the image in terrestrial telescopes - and were developed with the aim of compensating for such distortions. This is achieved by shaping the wavefront in such a way that it compensates for the distortions encountered on its path through the scattering material.

> We found that efficient models for light scattering can be established with a variety of network architectures.

Adaptive optics techniques can be differentiated by the severity of the scattering that they aim to compensate. One can broadly distinguish two different regimes, weak and strong scattering, that typically require different approaches for determining the appropriate wavefront correction. While for weakly scattering samples a focus is essentially preserved and light from the focal volume can be collected, strong scattering results in a distributed granular interference pattern, a so called speckle pattern, that does not reflect a spatially or temporally localized light distribution suitable for imaging applications. Nevertheless, such scattered light distributions encode information about the underlying scattering process. The main challenge in adaptive optics lies in determining the appropriate shape of the wavefront for scattering correction, an issue that we are addressing in our current research.

Neural networks for light control in scattering media

We investigated whether tools from machine learning, and in particular neural network techniques that in recent years have

emerged as efficient tools for image processing in many different situations, could be used for the analysis of speckle patterns. For this neural network approach we illuminate the scattering sample with a large number of randomly generated illumination patterns and record the resulting speckle images (see Fig. 1, adapted from [4]). Feeding these data sets into neural networks to extract a functional relationship between the original illumination pattern and the scattered light distribution, we found that indeed efficient models for light scattering can be established with a variety of network architectures [4]. In our proof-of-principle experiments we used two common networks, single layer neural networks (SLNNs) as well as multilayer convolutional neural networks (CNNs). The training of these SLNN models required a few tens of seconds, while CNNs in addition required a longer pretraining phase of about 20 minutes. While linear networks turned out to be ideally suited for scattering control, since they take advantage of the linearity of scattering in the samples we used (see Fig. 2, adapted from [4]), CNNs are less constrained

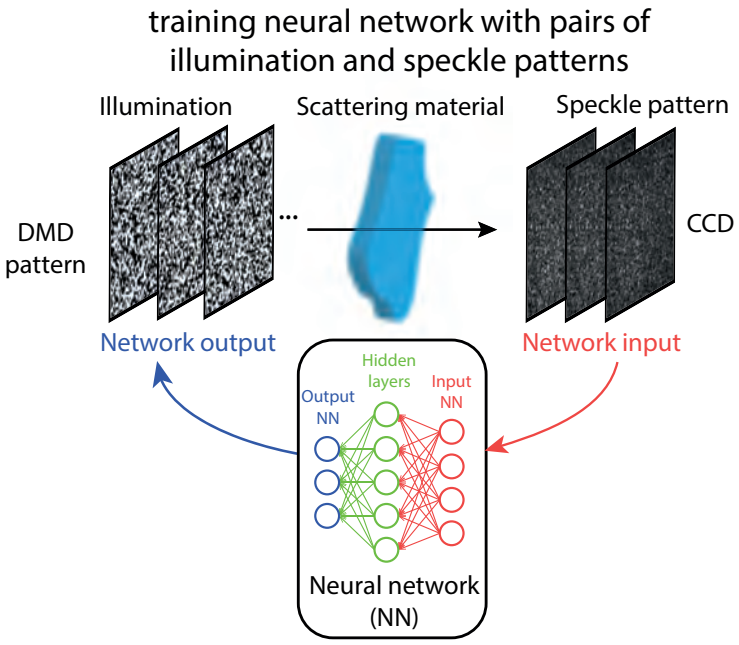


Figure 1: A neural network is trained with pairs of illumination and speckle patterns, using the speckle as input of the network and the illumination as output. Once the NN is trained, it is used to predict the illumination necessary to generate a target pattern after the scattering material (adapted from [4]).

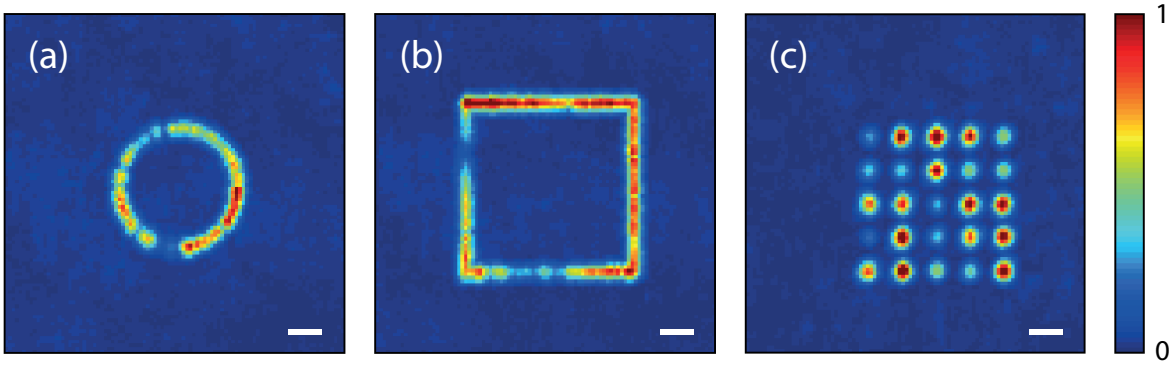


Figure 2: Maximum intensity projections of a single focus scanned through a scattering glass diffuser following (a) a circle, (b) a square, and (c) a grid of 5 × 5 points with a trained SLNN.

by assumptions about the nature of the underlying physical process, which might proof advantageous in applications. These experiments demonstrate the versatility of neural networks for scattering control and we now continue to further improve these techniques with the goal of applying them for imaging in the brain.

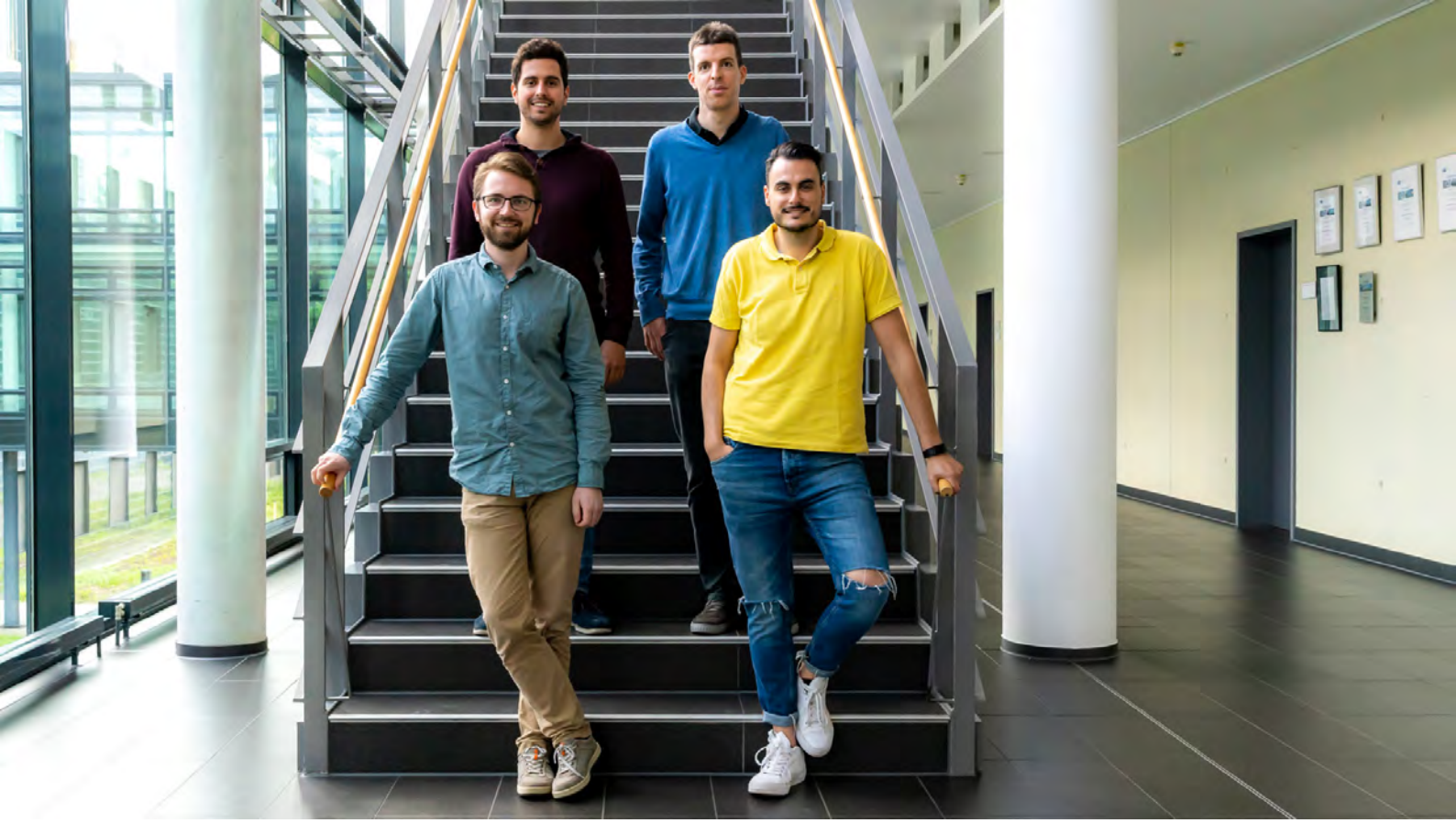
References

1. Seelig, J.D., and Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. Nature 521, 186-191.

2. Ji, N. (2017). Adaptive optical fluorescence microscopy. Nat Methods 14, 374-380.

3. Rotter, S., and Gigan, S. (2017). Light fields in complex media: Mesoscopic scattering meets wave control. Rev Mod Phys 89.

4. Turpin, A., Vishniakou, I., and Seelig, J.D. (2018). Light control through scattering media with neural networks. under review.



About the Max Planck Research Group: Neural Circuits

We use optical microscopy techniques, such as two-photon calcium imaging, as well as patch-clamp recordings to understand how neural networks in the brain change over time, from single synapses to entire circuits, and how these changes relate to behavior. Our research focuses on the model organism *Drosophila melanogaster* that can be used to study genetically identified, comprehensive neural networks underlying adaptive behaviors. We interpret data from our experiments using computational modeling of the dynamics of single cells as well as neural networks. Molecular, structural, and functional similarities of neural circuits across species suggest that insights gained in the fly brain will also contribute to our understanding of the mammalian brain.

In a second line of research we apply and develop novel optical microscopy methods with the aim of optimizing the temporal as well as spatial resolution of functional imaging in animals engaged in virtual reality behavior.

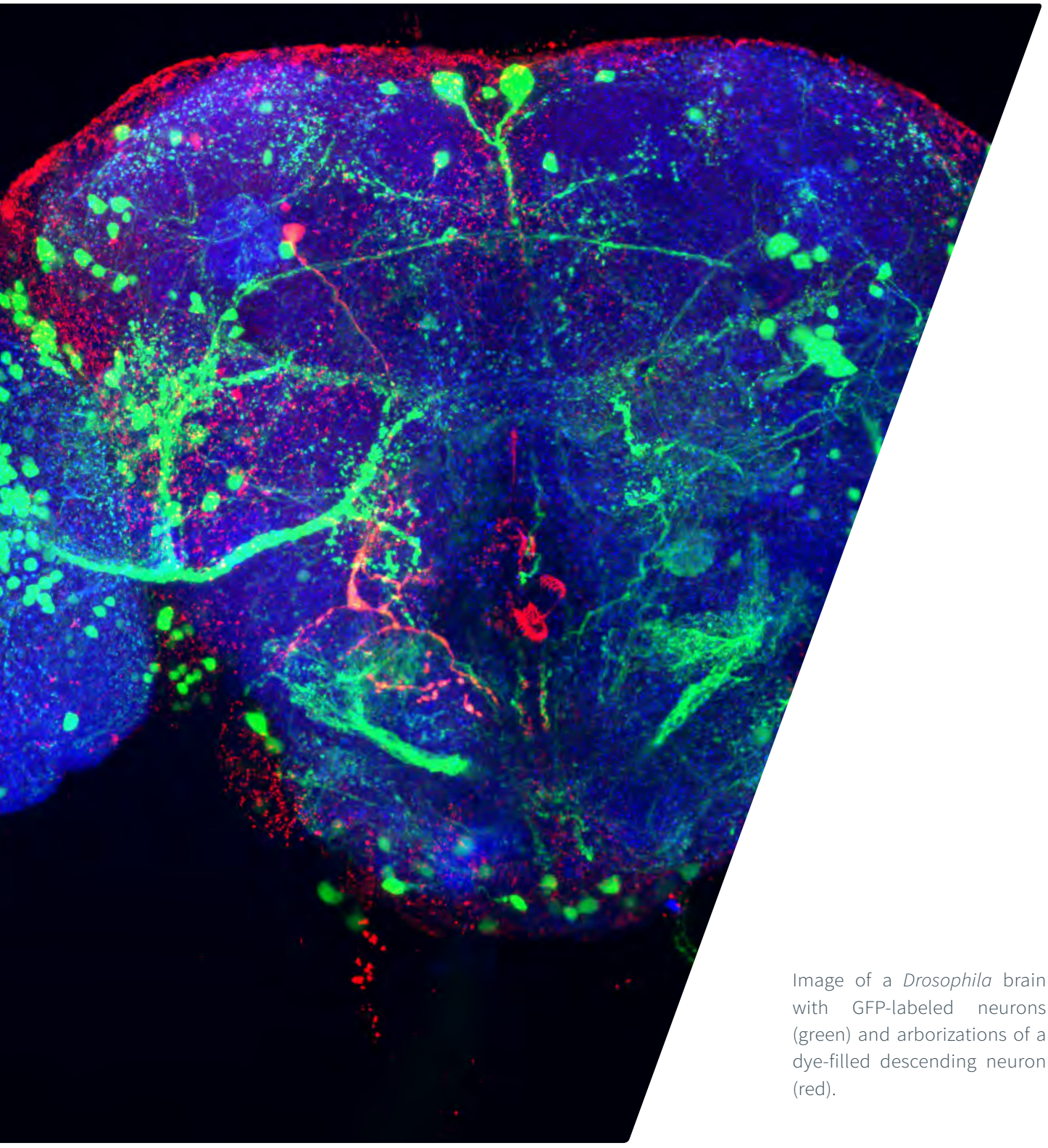


Image of a *Drosophila* brain with GFP-labeled neurons (green) and arborizations of a dye-filled descending neuron (red).

To turn or not to turn, that is the question

Understanding how a brain controls behavior is one of the central goals in neuroscience. We study that question in the fruit fly *Drosophila* by analyzing the neurons involved in the control of steering maneuvers during flight.

by Dr. Bettina Schnell | Emmy-Noether-Group: Neurobiology of Flight Control

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 the research group "Neurobiology of Flight Control" 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.

Visually elicited flight maneuvers

Flies have a particular flight style, in which stretches of straight flight are interspersed with rapid changes in direction termed saccades. To stabilize a straight path even in the face of disturbances (e.g. wind), flies rely heavily on the visual information that is caused by their own motion, the so-called optic flow. With the optomotor response flies compensate for deviations from a straight flight path by turning with the direction of wide-field visual motion. Turns around the vertical body axis in response to horizontal motion can be measured in tethered flight by monitoring the difference in wing stroke amplitude between the left and right wing (L-R WSA).

Changes in heading can also be elicited by visual stimuli. Expanding bars or circles for example mimic the approach of a potential predator or an impending collision and elicit evasive turns. However, flies also perform spontaneous saccades that are not directly triggered by sensory stimuli.

The visual system of the fly

Large parts of the fly brain, the optic lobes, are dedicated to the processing of visual

information [1]. Tangential cells within the optic lobes of the fly, the so called lobula plate tangential cells (LPTCs), have been well-studied. They have large receptive fields and respond to wide-field motion in a directional-selective manner. Therefore, they are thought to underlie compensatory optomotor responses. Among them, the horizontal system (HS) cells are responsive to horizontal motion. Recent research revealed the influence of an efference copy on the membrane potential of HS cells during voluntary turns [2,3]. This effect has the right sign to suppress responses to the visual motion stimulus caused by the fly's intended behavior. LPTCs make synapses with descending neurons, which relay information from the brain to the motor system within the ventral nerve cord [4]. However, only few descending neurons have been functionally described in the fly, such as the DNOVS cells that are responsive to vertical motion and the giant fiber, which mediates escape maneuvers. As descending neurons are the output neurons of the brain, their characteristics will be highly informative about the computations performed by presynaptic circuits.

Neural circuits underlying flight maneuvers

My group wants to study the neural circuits involved in course control in *Drosophila melanogaster* using simultaneous recordings of neuronal and behavioral activity during tethered flight as well as genetic manipulations. The main focus will be on descending neurons that are involved in the control of steering maneuvers during flight, which we will characterize by performing whole-cell patch-clamp recordings. During recording, we will present a set of sensory stimuli including moving visual patterns to describe their response properties in detail. Descending neurons could either encode sensory stimulus information (e.g. rotation of the visual scene around a specific axis), intended behavioral output (e.g. a turn around a specific body axis), or specific motor commands (e.g. an increase in the stroke amplitude of one wing). We will be able to discriminate between these possibilities by comparing neuronal activity and motor output during presentation of different sensory stimuli and by using genetic techniques to perturb the function of specific neurons during behavior.

My group wants to study the neural circuits involved in course control in *Drosophila melanogaster* using simultaneous recordings of neuronal and behavioral activity during tethered flight as well as genetic manipulations.

A descending neuron controlling saccades

During my postdoctoral work in the laboratory of Michael Dickinson, I started working on identifying descending neurons involved in flight control. Using 2-photon calcium imaging and electrophysiological recordings in tethered flying *Drosophila*, I have identified a previously undescribed descending neuron whose activity is correlated with fictive turns

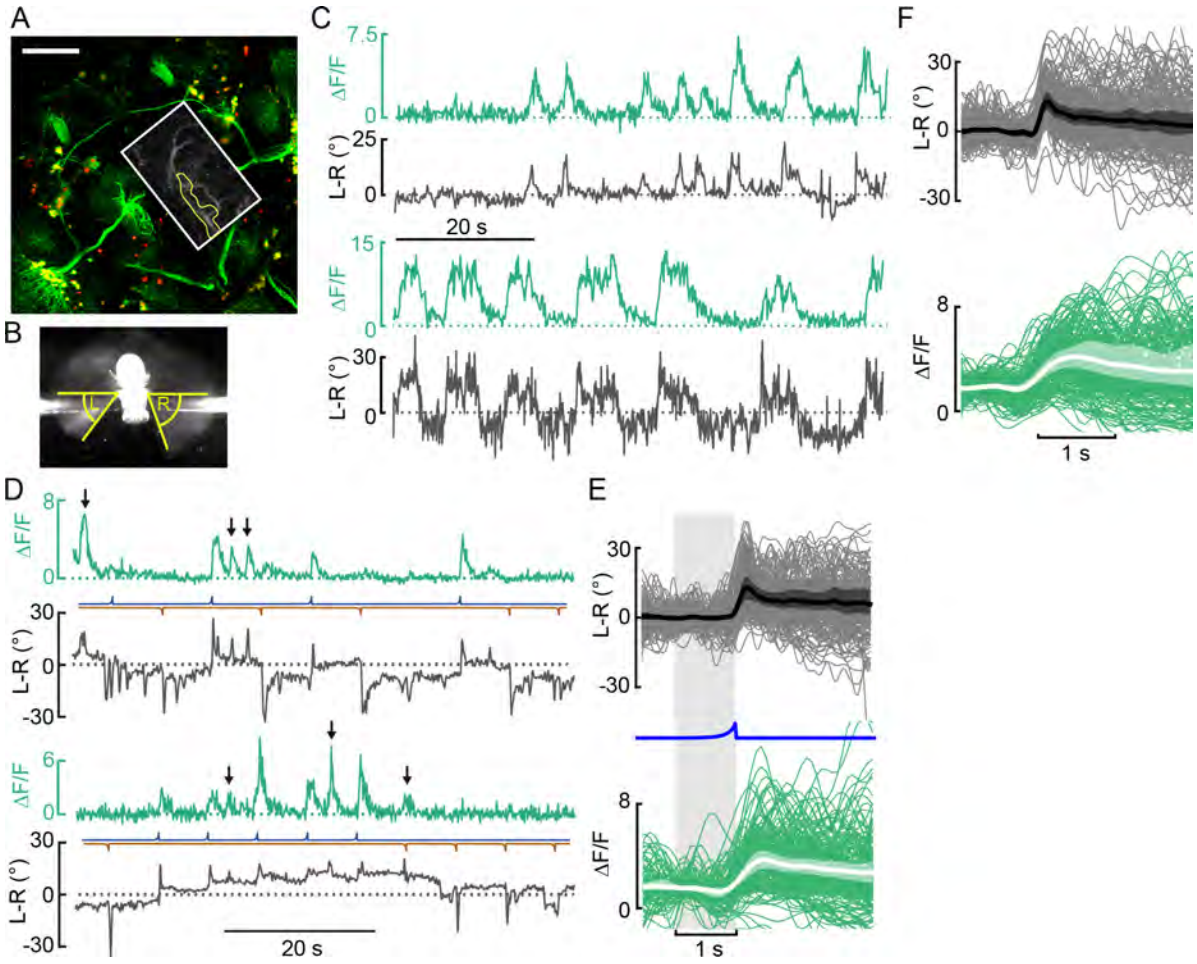


Figure 1: a. Maximum-intensity projection of GFP expression in the whole brain driven by the line R56G08-Gal4. (scale bar, 50 µm). The approximate imaging area is depicted with a white box. An example image with the region of interest highlighted in yellow is shown as inset. b. Image of a fly taken from below illustrating the measurement of left (L) and right (R) wing stroke amplitude (WSA). c. Representative traces of spontaneous changes in L-R WSA (L-R) and GCaMP6f fluorescence in the descending neuron (ΔF/F) in the absence of visual stimulation. d. Two example traces of changes in L-R and ΔF/F during presentation of looming stimuli on either left (blue) or right (brown). Several spontaneous saccades (black arrows) occur in between looming stimuli. e. Mean L-R and ΔF/F (thick line) and individual responses (thin lines) to looming stimuli on the left (blue line). N = 13. (F) Same as (E), with L-R and ΔF/F for spontaneous saccades. Modified from [5].

during tethered flight (Fig. 1) [5]. The cell's activity suggests that it does not underlie stabilizing optomotor responses, but rather controls saccadic turns away from a straight path. It is both active during spontaneous as well as visually elicited saccades, but never in cases, where the fly does not perform a turn. The activity of this neuron can explain some of the behavioral variability observed in the optomotor response and appears sufficient for eliciting turns when artificially activated. This work provides an entry point into studying the circuits underlying behavioral decision making in the fly brain.

Controlling locomotion is a central task of any brain and requires a complex interaction of compensatory reflexes for path stabilization with commands leading to a change in direction. I aim to identify how this interaction is achieved by analyzing how steering commands are encoded at the level of descending neurons in *Drosophila*, and how sensory information is integrated with the initiation of spontaneous behaviors. Altogether, the work will provide insight into the circuits and mechanisms underlying this process and has the potential to reveal general mechanisms underlying the control of complex behaviors.

References

1. Borst, A. (2014). Fly visual course control: behaviour, algorithms and circuits. Nat Rev Neurosci 15, 590-599.

2. Schnell, B., Weir, P.T., Roth, E., Fairhall, A.L., and Dickinson, M.H. (2014). Cellular mechanisms for integral feedback in visually guided behavior. Proc Natl Acad Sci U S A 111, 5700-5705.

3. Kim, A.J., Fitzgerald, J.K., and Maimon, G. (2015). Cellular evidence for efference copy in *Drosophila* visuomotor processing. Nat Neurosci 18, 1247-1255.

4. Hsu, C.T., and Bhandawat, V. (2016). Organization of descending neurons in *Drosophila* melanogaster. Sci Rep 6, 20259.

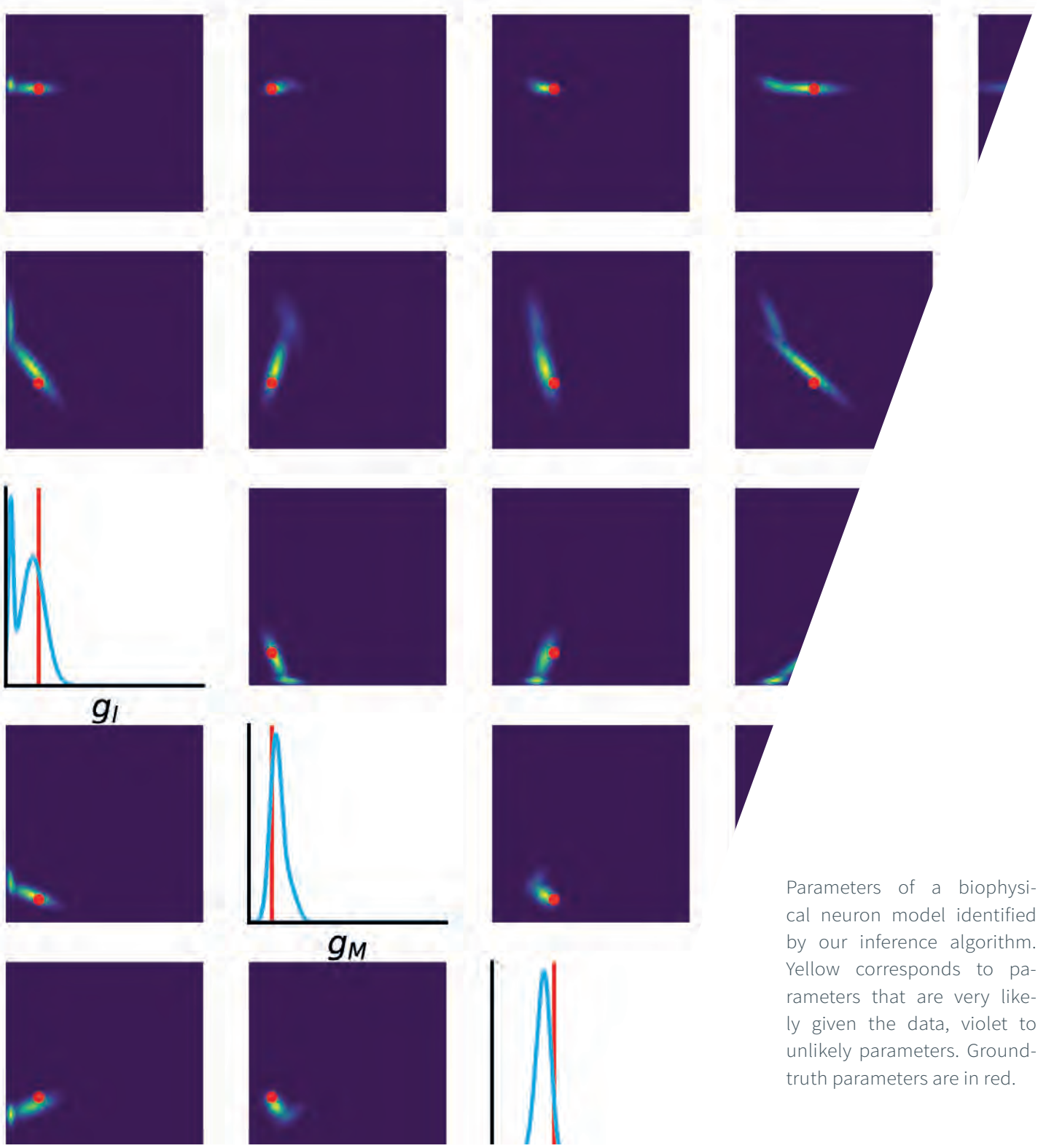
5. Schnell, B., Ros, I.G., and Dickinson, M.H. (2017). A Descending Neuron Correlated with the Rapid Steering Maneuvers of Flying *Drosophila*. Curr Biol 27, 1200-1205.

About the Emmy-Noether-Group: Neurobiology of Flight Control

The research group “Neurobiology of Flight Control” is interested in how neural circuits integrate information into motor commands that control behavioral output. We use flight maneuvers of the fruit fly *Drosophila* as a model system, because of the numerical simplicity of the fly brain and its genetic accessibility.

To study the circuits underlying flight control, we make use of recent technological advances, which allow us to measure the activity of single neurons in behaving animals. We can monitor intended steering maneuvers by tracking the motion of the wings in head-fixed flies. In addition, we use the elaborate genetic tool kit available in *Drosophila* to manipulate the function of specific neurons.

Combining 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 decision-making.



Parameters of a biophysical neuron model identified by our inference algorithm. Yellow corresponds to parameters that are very likely given the data, violet to unlikely parameters. Ground-truth parameters are in red.

Neural Systems Analysis

How can we make sense of complex experimental data in neuroscience? We build statistical methods and machine-learning algorithms for analyzing high-dimensional measurements of neural activity and behavior, and for linking them with theoretical models of neural dynamics and computation.

by Prof. Dr. Jakob Macke | Max Planck Research Group: Neural Systems Analysis

Advances in experimental techniques make it possible to measure the activity of many neurons simultaneously, and to map the connectivity of neural circuits at unprecedented scale and resolution. However, interpreting the complex data generated by these approaches has proven to be a difficult challenge. At the same time, the field of machine learning is being revolutionized by 'deep learning' approaches: Powerful algorithms for extracting structure from data can be built using artificial neural networks, providing practical tools and conceptual frameworks for analyzing neural data. In addition, the success of neural networks poses the question of how principles learned from biological computing devices can inform the design of artificial ones.

We here report on two central questions which we are pursuing in collaboration with experimental laboratories: First, how can we link models of neural dynamics with electrophysiological recordings and imaging data [1]? Second, how can we interpret large-scale recordings of neural activity [2]?. In addition, we are also interested in understanding perceptual strategies used by animals by quantitatively analyzing their behavior [3].

How can we link mechanistic models of neural dynamics with experimental observations?

Computational neuroscientists have developed a cornucopia of models that aim to capture neural dynamics, and give insights into the underlying mechanisms. However, a common challenge in working with these models is the fact that it is typically difficult to quantitatively link them to data. Identifying which mechanistic models are consistent with empirical data can be very challenging – we use artificial neural networks to find them for us. In particular, we are developing a method for statistical inference on simulation-based models which can be applied in a 'black

box' manner to a wide range of models in neuroscience. The key idea is to first generate simulations from a model, and then to train an artificial neural network to identify parameters that are consistent with the data (Fig. 1 a). Having characterized these parameters, we can then refine models, or estimate which additional data will be most useful for constraining or comparing models. Our approach is applicable to a wide range of models in neuroscience. For example, it can be used to rapidly consider estimating the receptive field of a neuron: Given observed data (here, the so-called spike-triggered average, i.e. the average stimulus that elicits a spike), we can infer the location and orientation of a receptive field (Fig. 1 b). Using our approach, we identify consistent model parameters (Fig. 1 c). In simulations, we can verify that the ground-truth parameters (red) have high probability (Fig. 1 c). These approaches lead to efficient inference: Once the neural network is trained, parameters for new data can be identified at negligible computational cost. This efficiency is in contrast to most conventional statistical inference approaches, which require expensive computations for each new data set. We are also developing similar approaches for statistical inference on mechanistic models to identify underlying events from imaging data [4]. In collaboration with the group of Marcel Oberlaender, we have started to apply our statistical inference to search for connectivity rules that can explain the statistical structure of neural connectivity in cortical networks.

> Identifying which mechanistic models are consistent with empirical data can be very challenging - we use artificial neural networks to find them for us.

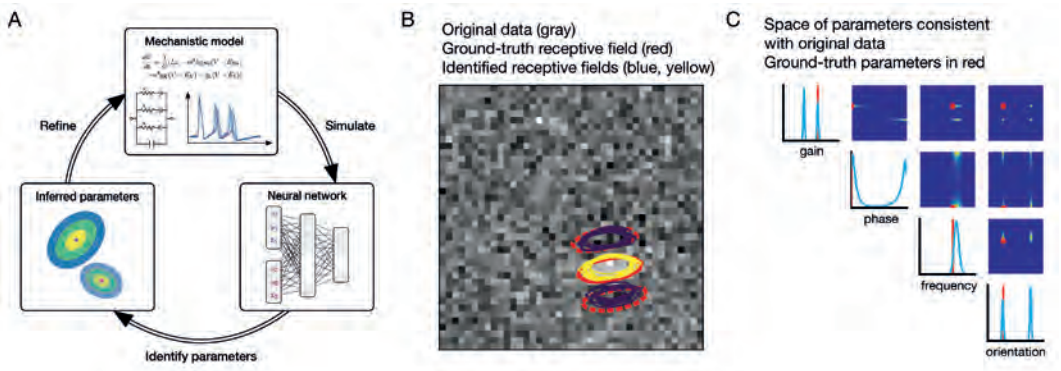


Figure 1: a. Inference approach: A neural network is trained to perform inference on simulated data. b. Original data (spike-triggered average), ground-truth receptive field, and identified receptive fields. c. Space of parameters consistent with original data. Ground-truth parameters (red) have high posterior probability, illustrating the success of our approach.

How can we describe the collective activity of large neuronal populations?

Large-scale recording methods make it possible to measure the activity of large neural populations. A promising approach to analyzing these data has been to use concepts from other fields, e.g. statistical physics, in search for underlying principles. One hypothesis that has emerged from this approach is that neural populations are optimized to be poised at a so-called `thermodynamic critical point' (e.g. [5]). Physical systems exhibit interesting behaviour near critical points, and therefore characterizing their critical points can yield valuable insights. For example, zero degree Celsius is a critical point for water, as lowering the temperature below that point causes it to freeze, i.e. to dramatically change its qualitative properties. The hypothesis that neural systems might exhibit similar transitions is therefore intriguing, and raises the question of what mechanisms keep the system at the critical point.

Support for criticality in neural systems has come from a recent series of studies which identified so-called 'signatures of criticality' in activity recorded from populations of retinal ganglion cells (Fig. 2 a). But are these previous results really conclusive about neural coding principles? What mechanisms can explain these signatures? We showed through numerical simulations and analytical calculations that previously identified signatures of criticality can be found in simple neuron population models (Fig. 2 b), even ones that do in fact not correspond to critical systems. We showed that signatures of criticality spuriously arise from common data-analysis techniques (Fig. 2 c), and can also be reproduced and studied in analytically tractable models (Fig. 2 d). Thus, previous reports about criticality in neural systems are not conclusive, and likely point to short-

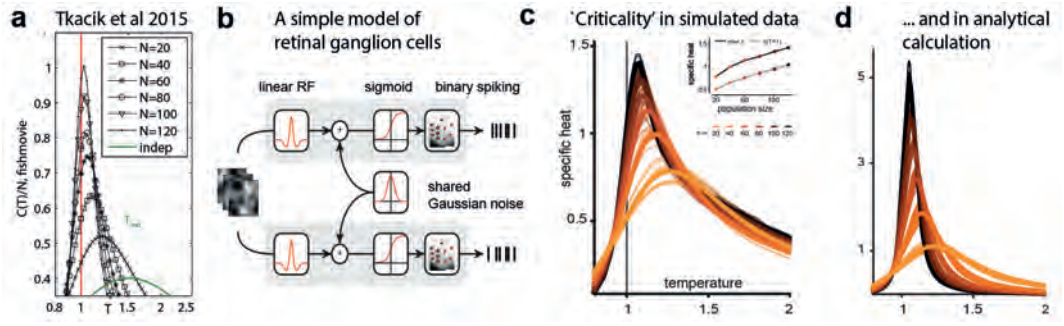


Figure 2: a. Signatures of criticality in neural populations reported in the literature: the specific heat capacity (a quantity describing the variance of fluctuations in activity) grows with population size (reproduced from [5]). b. Simulations of simple population models reproduce these signatures. c.,d. Simulations of simple population models reproduce these signatures.

comings of analysis approaches rather than biological phenomena.

To provide more useful analysis approaches, we are developing algorithms for mechanistic neural population models, extending our previous work on statistical inference for dynamical systems [6]. These models explicitly capture the measurement and data-analysis process for neural population recordings, and thereby overcome the limitations of commonly used analysis methods.

References

1. Lueckmann, J.M., Goncalves, P., Bassetto, G., Öcal, K., Nonnenmacher, M., and Macke, J. H. (2017). Flexible statistical inference for mechanistic models of neural dynamics. Adv Neural Inf Process Syst 30, 1289–1299.

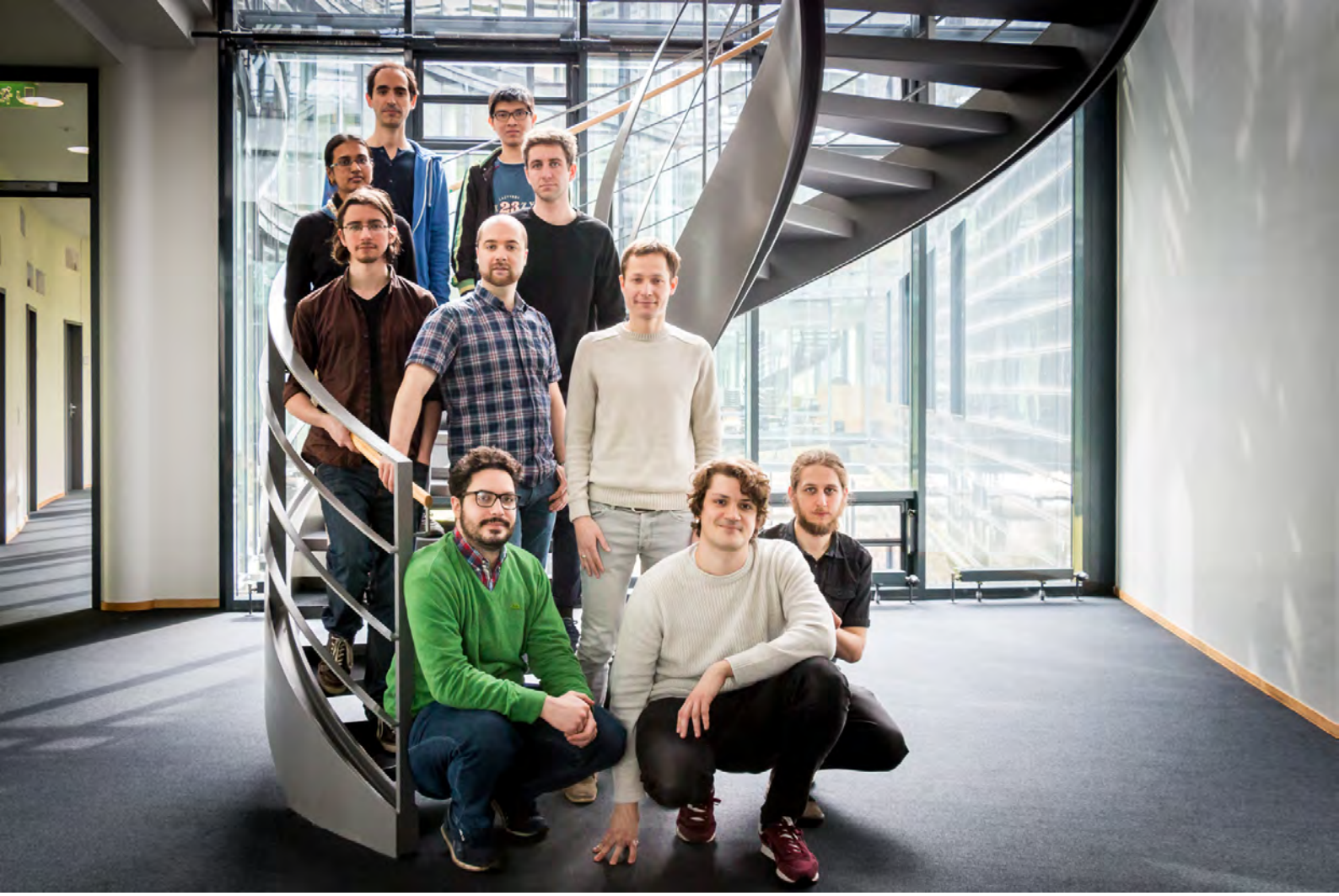
2. Nonnenmacher, M., Behrens, C., Berens, P., Bethge, M., and Macke, J.H. (2017). Signatures of criticality arise from random subsampling in simple population models. PLoS Comput Biol 13, e1005718.

3. Lueckmann, J.M., Macke, J.H., and Nienborg, H. (2018). Can Serial Dependencies in Choices and Neural Activity Explain Choice Probabilities? J Neurosci 38, 3495-3506.

4. Speiser, A., Jinyao, Y., Archer, E., Buesing, L., Turaga, S.C., and Macke, J.H. (2017). Fast amortized inference of neural activity from calcium imaging data with variational autoencoders. Adv Neural Inf Process Syst 30, 4024–4034.

5. Tkacik, G., Mora, T., Marre, O., Amodei, D., Palmer, S.E., Berry, M.J., 2nd, and Bialek, W. (2015). Thermodynamics and signatures of criticality in a network of neurons. Proc Natl Acad Sci U S A 112, 11508-11513.

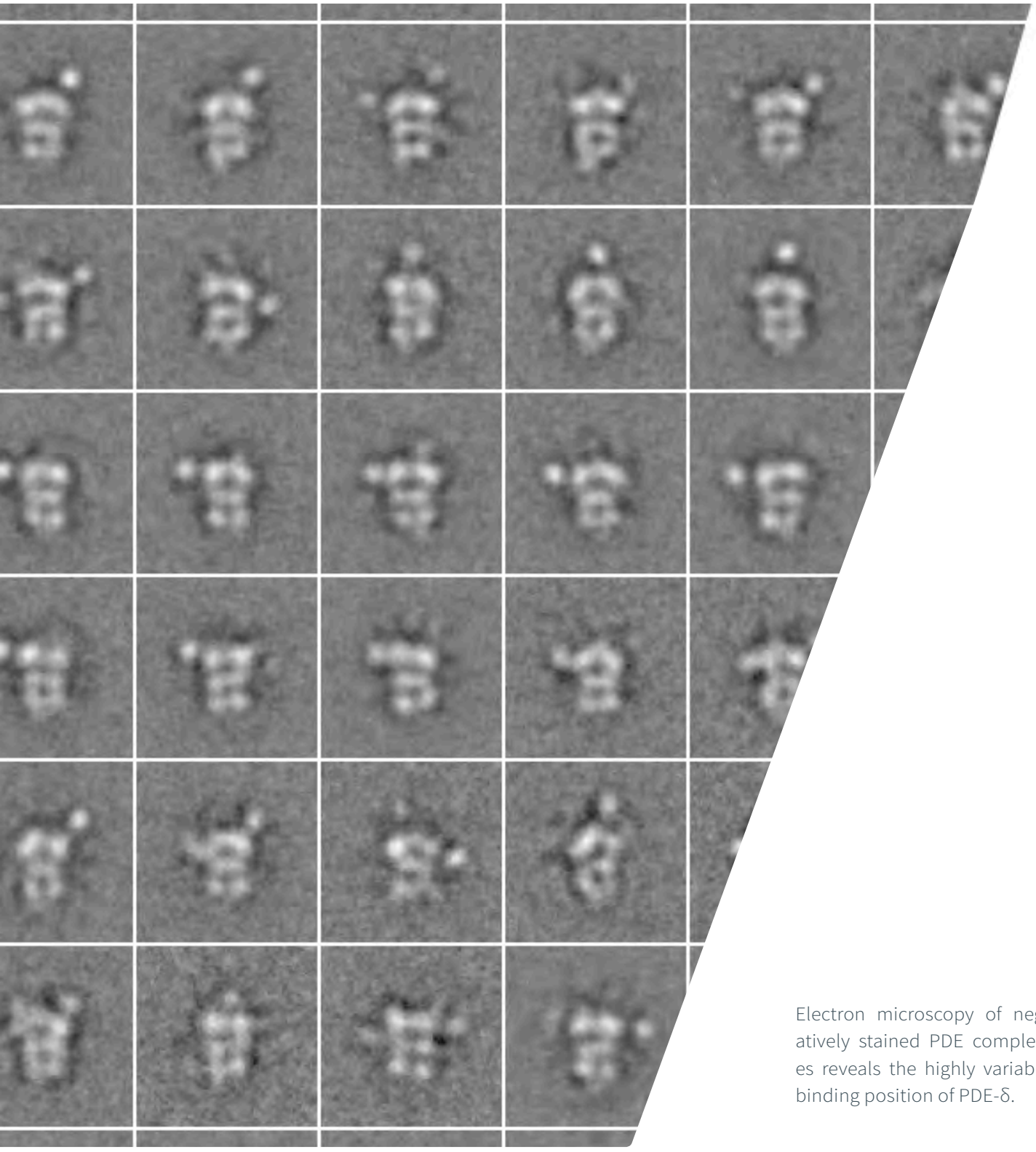
6. Nonnenmacher, M., Srinivas, T. C., and Macke, J. H. (2017). Extracting low-dimensional dynamics from multiple large-scale neural population recordings by learning to predict correlations. Adv Neural Inf Process Syst 30, 5702–5712.



About the Max Planck Research Group: Neural Systems Analysis

The central goal of the Neural Systems Analysis group is to understand how populations of neurons collectively process sensory input, perform computations and control behaviour. To this end, we develop statistical tools and machine learning algorithms for interpreting neural and behavioral data. Our goal is to provide tools which make it possible to distill interpretable descriptions from high-dimensional, complex data, and to link these data to theoretical models of neural dynamics and computation. We build on recent advances in Bayesian inference and deep learning to design statistical models and efficient algorithms for inferring their parameters.

These methods allows us to characterize which models are consistent with existing measurements, and which additional measurements would be most informative in constraining them. We extensively collaborate with experimental groups at caesar Bonn as well as at other research institutes.



Electron microscopy of negatively stained PDE complexes reveals the highly variable binding position of PDE-δ.

Once does not count: Suppressing noise in the eye

Humans are able to detect and potentially sense single photons. We recently identified a molecular mechanism helping to suppress thermal noise at a key step of signal amplification.

by Prof. Dr. Elmar Behrmann | Max Planck Research Group: Structural Dynamics of Proteins

Vision is one of our key senses. Consequently, evolution has created a biological 'camera' that compares favorably to our most advanced technological cameras – especially when considering versatility. For example, we are able to perceive light over a vast range of intensities: from bright day-light down to dim star-light, when only few, or even single, photons hit the retina [1,2]. The ability to sense individual photons is a remarkable feat, especially considering that the thermal background energy – that is our body heat - is only 100 times less than the energy in a single photon. Therefore, as with any high-gain amplification system, noise suppression is as important as signal amplification.

The parts that make the camera

The molecular components required to capture and amplify a single-photon event are localized at intracellular membranous structures in our photosensitive cells (Fig. 1). The antenna protein, rhodopsin, contains a light-sensitive chemical that can be excited by absorbing an incoming photon. This in turn leads to conformational changes in rhodopsin, allowing it to exchange a small molecule, namely GDP for GTP, in an associated protein, the G-protein transducin. GTP-bound transducin splits into its subunits, of which the subunit T-α in turn activates a membrane-localized phosphodiesterase, PDE6. PDE6 then degrades a chemical messenger molecule, cGMP, which results in the closure of ion channels, so-called CNG channels, at the plasma membrane, and initiates a hyperpolarization event recognized by associated nerve cells.

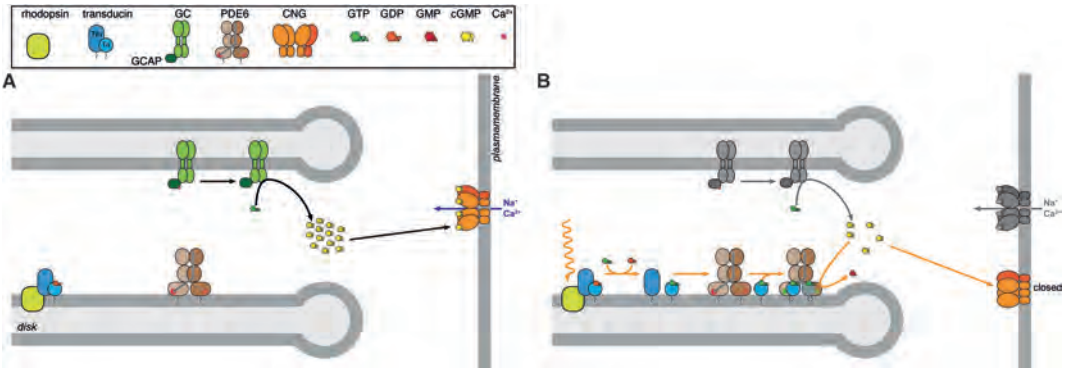


Figure 1: The visual signalling cascade. a. In the resting state, membrane-bound guanylate cyclases (GC) maintain a steady pool of cyclic GMP (cGMP). This basal concentration of cGMP maintains constant opening of the ion channel CNG at the plasma membrane. This generates a constant inflow of sodium and calcium, which keeps the plasma membrane in a depolarized state. b. Signalling is induced by the photon-induced activation of rhodopsin, which in turn activates its associated G-protein transducin by stimulating exchange of GDP with GTP. The GTP-bound transducin subunit T-α in turn can displace PDE-γ from the PDE6 complex and thus activate the hydrolytic center of this phosphodiesterase. Active PDE6 rapidly reduces the concentration of available cGMP, resulting in the closure of CNG channels, and thus induce a hyperpolarization of the cell membrane.

Fate of the chemical messenger

The chemical messenger cGMP plays a key role in the signalling cascade, as it is highly mobile and thus allows relaying the signal from the intracellular membranous structures to the plasma membrane. While cGMP is more or less constantly produced by guanylate cyclases (GC) from the precursor molecule GTP, the regulation of PDE6 activity is much more complex. PDE6 is a dimeric complex comprising an α and a β subunit, each consisting of two GAF domains and a catalytic domain responsible for degrading the secondary messenger cGMP (Fig. 2 a). Different from other PDEs, PDE6 is believed to exist in an always active 'open' conformation, but being inactivated by binding of an additional subunit, PDE-γ, onto the active sites. Only when PDE-γ is displaced by T-α, the active site becomes accessible to cGMP and hydrolysis can occur.

We recently investigated an additional layer of control, namely the localization of PDE6 to the intracellular membranous structures by its solubilization factor, PDE-δ [3]. Among other findings, we observed by negative-stain electron microscopy that the position of PDE-δ on the PDE complex is highly variable (see front image). Because we know that

PDE-δ binds to a defined position on PDE6, namely a lipid anchor situated at the catalytic domain, this implies that the position of the catalytic domain itself, with relation to the remaining complex, must be variable. Interestingly, we also observed asymmetric binding of PDE-δ to the complex, implying that the catalytic domains do not always adopt identical positions. This was somewhat unexpected, as both catalytic domains had previously been assumed to be equal and show indistinguishable activity, at least when studied in solution in a test tube.

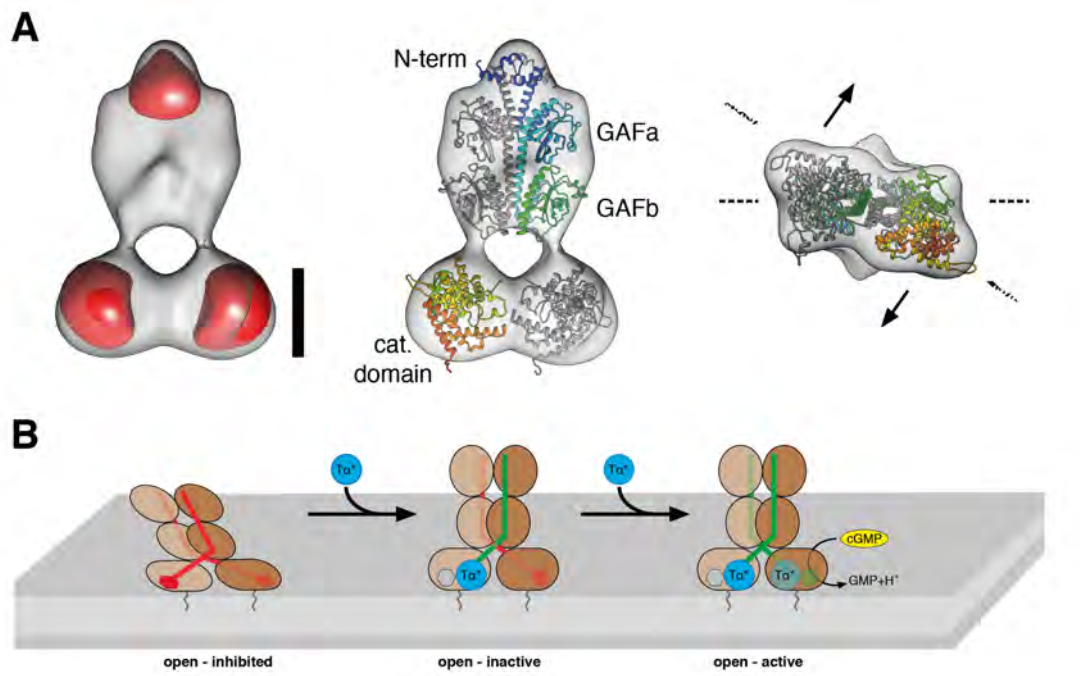


Figure 2: Structural reasons for asymmetric activation. a. Cryo-EM analysis of the PDE6 complex confirm variability (shown in red) of the catalytic domain (cat. domain). While the complex is indeed symmetric, both active sites face towards different faces of the complex (arrows in the top view). Scale bar is 5 nm. b. Asymmetric activation of PDE6 in the presence of membranes could be explained if PDE6 in the resting states adopts a flat orientation on the membrane that prevents access to one of the catalytic domains. Only after binding of the first T-α, PDE6 assumes an upright position. While this does not yet activate hydrolysis, it makes the second T-α binding site available – albeit at reduced affinity. Only in the presence of a high, local concentration of T-α PDE6 will thus be fully activated.

Symmetric but not identical

We therefore decided to followed up on this observation with further functional experiments and structural characterization by cryo-electron microscopy [4]. Indeed, we directly observed structural variability in the catalytic domain using cryo-EM (Fig. 2 a) confirming our initial hypothesis based on the observed flexibility of PDE-δ. Additionally, the cryo-EM structure shows that, while symmetric, the two catalytic sites face to opposing sites of the PDE6 complex. Functional assays moreover clearly showed that these two catalytic sites, while indistinguishable in solution, become unequal in the presence of membranes: While a first T-α is bound to the complex with high affinity, this elicits virtually no activation of the PDE6 complex. Only when a second T-α binds, now with lower affinity, the full activation of the PDE6 complex is induced.

How can membranes induce such an functional asymmetry? Potentially, PDE6 at resting conditions is adopting a flat orientation on the membrane, making only one of the active sites accessible to T-α (Fig. 2 b). T-α binding could trigger PDE6 to adopt an upright orientation that now allows access to the second site, which would be in agreement with earlier light-scattering experiments [5]. Given that such a reorientation of the complex would directly affect the catalytic domains, where the membrane anchor is localized, a change in both affinity and activity for the second T-α to bind would not be surprising.

Asymmetric noise filtering

Using computer simulations, we explored functional implications of such an asymmetric, two-stage activation mechanism. Intriguingly, these simulations uncovered that this mechanism would show properties of a noise filter: thermally activated T-α, corresponding to background-noise, would be locally sparse and could only lead to singly-occupied T-α-PDE6 complexes that remain largely inactive. Only when an activated rhodopsin molecule is producing a high local concentration of T-α, the threshold required for formation PDE6 complexes occupied by two copies of T-α, and thus with full catalytic activity, would be overcome.

References

1. Hecht, S., Shlaer, S., and Pirenne, M.H. (1942). Energy, Quanta, and Vision. J Gen Physiol 25, 819-840.

2. Tinsley, J.N., Molodtsov, M.I., Prevedel, R., Wartmann, D., Espigule-Pons, J., Lauwers, M., and Vaziri, A. (2016). Direct detection of a single photon by humans. Nat CBCommun 7, 12172.

3. Qureshi, B.M., Schmidt, A., Behrmann, E., Burger, J., Mielke, T., Spahn, C.M.T., Heck, M., and Scheerer, P. (2018). Mechanistic insights into the role of prenyl-binding protein PrBP/delta in membrane dissociation of phosphodiesterase 6. Nat Commun 9, 90.

4. Qureshi, B. M., Behrmann, E., Schöneberg, J., Loerke, J., Bürger, J., Mielke, T., ... & Spahn, C. M. (2018). It takes two transducins to activate the cGMP-phosphodiesterase 6 in retinal rods. Open Biology 8, 180075.

5. Heck, M., and Hofmann, K.P. (1993). G-protein-effector coupling: a real-time light-scattering assay for transducin-phosphodiesterase interaction. Biochemistry 32, 8220-8227.

About the Max Planck Research Group: Structural Dynamics of Proteins

Life is not static and neither are the majority of proteins crucial to the function of our cells. Akin to tiny machines, proteins have to adopt many different structural arrangements – so called conformations – to carry out their cellular function. We focus on the application of electron microscopy to investigate these structural dynamics of proteins. Electron microscopy is ideally suited for this as it allows us to trap ongoing reactions within the fraction of a millisecond. In combination with sophisticated image processing techniques this allows us to determine the major conformations present in the reaction, as well as to pinpoint flexible regions in the protein structure. Our main interest is in those proteins that are associated with or embedded into the lipid membranes of our cells. Membranes are paramount for the identity of a cell, as they shield the interior from the surrounding environment, but must not be static as controlled passage of molecules and information over these biological barriers is necessary for life. The structural basis for how proteins can give diverse functions to membranes is still largely lacking, especially with regard to the dynamic interplay between lipids and proteins. However, studying dynamic proteins in membranous environments is a challenging task posing a wide range of experimental issues. To address these issues, we are developing specialized sample preparation strategies that range from novel lipid bilayer mimics to functionalized sample grid carriers.

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).

Nanostructures: From aptamer-based nano-capsules to bio-hybrid motors

Our research focuses on nucleic acid-based nanostructures. We have assembled aptamer-based nano-capsules for the targeted delivery of molecular cargo to cells, and have constructed a bio-hybrid rotational DNA motor that moves along predefined tracks.

by Prof. Dr. Michael Famulok, Dr. Deepak K. Prusty, Dr. Stephan Irsen, Dr. Julian Valero | Max Planck Fellow: Chemical Biology

The Max Planck fellow group "Chemical Biology" at the caesar research center is focusing on nanostructures that are constructed of nucleic acids. In one of the projects, we constructed supramolecular nano-capsules from DNA-aptamers that are equipped with lipid groups. The capsules were used for attacking cancer cells by detecting a cell surface receptor. They are constructed to release a chemotherapeutic substance into the cancer cells induced by light, hindering cell growth, and discriminate between cancerous and benign cells. In our second project, we produced bio-hybrid supramolecular DNA nano-structures featuring a stator unit that sets a mechanically linked DNA ring into unidirectional rotational motion. The result is a synthetic rotation motor, whose continuous rotation depends on the consumption of chemical energy. Long RNA chains are produced as a by-product and remain firmly attached to the rotation motor. This allows for the RNA to be used as an anchor, to create a walking nanomachine that performs a specified journey.

Supramolecular aptamer nano-capsules for a targeted attack on cancer cells via light induced release of chemotherapeutic substances

Aptamers are specific ligand binding nucleic acids, which - similar to antibodies - are used for recognizing specific cells that feature a receptor molecule on their surface that the aptamer detects. These characteristics make aptamers interesting components for a targeted transport of cargo to such cells. Prerequisites for this process are sufficient stability in the blood serum, a targeted and efficient uptake by the target cells, and an inducible release of the cargo inside the cell. For this project we integrated multiple structural motifs of lipidated aptamers in a nano-capsule, which were designed to detect the hepatocyte growth factor receptor cMet on the surface of cancer cells.

The nano-capsules consist of a lipidated anti-cMet aptamer and a DNA hairpin motif, loaded with the toxic chemotherapeutic substance doxorubicin. This motif contains photosensitive molecules that allow for the doxorubicin to be released through photoisomerization. The aptamer units featuring lipid groups organize themselves into spherical nano-capsules that specifically detect cMet on the surface of cancer cells, and are therefore absorbed by these cells in a targeted manner (Fig. 1). This self-organization into nano-capsules leads to increased resistance to nucleases and more efficient absorption into the cells. A selective release of the doxorubicin is triggered by brief exposure to light, thus causing mortality of the attacked cells

> The simplicity of the design can be easily adjusted to other types of biological nano-architecture. This should facilitate the construction of complex NTP-powered bio-hybrid nano-machines.

[1]. This modular approach opens up new possibilities for aptamer-based therapeutics, as well as for innovative research tools.

We have examined the appearance of the nano-capsules by electron microscopy in cooperation with Dr. Stephan Irsen at caesar. Most of the work was carried out by Dr. Deepak K. Prusty, during his employment at caesar. Further principles for light-induced switches of biomolecules have also been examined [2].

A bio-hybrid DNA nanomotor that moves along specified routes

Biological motors are highly complex protein arrangements that cause linear or rotational motion, powered by chemical energy. Synthetic motors, based on DNA nanostructures, bio-hybrid constructs, or synthetic organic chemistry have been described successfully in the past [3]. However, unidirectionally rotating biomimetic motors with rotor / stator units, powered by the consumption of chemical energy, were previously unknown.

For this project, we constructed a bio-hybrid nanomachine, consisting of a catalytic stator and an interlocked DNA wheel, whose rota-

tion is powered by the hydrolysis of nucleotide triphosphates (NTPs). The nanomachine contains a modified T7 RNA polymerase, which is firmly connected to the DNA ring of the stator, which interlocks with the rotor DNA ring to form a catenane. The rotating wheel produces long, recurring RNA transcripts that are connected firmly to the nanomachine. We use this RNA to achieve a targeted directional movement along a specified track on a DNA nanotube. As a result it became possible to transform rotational movement into directional movement [4]. The simplicity of the design can be easily adjusted to other types of biological nano-architectures. This should facilitate the construction of complex NTP-powered bio-hybrid nanomachines.

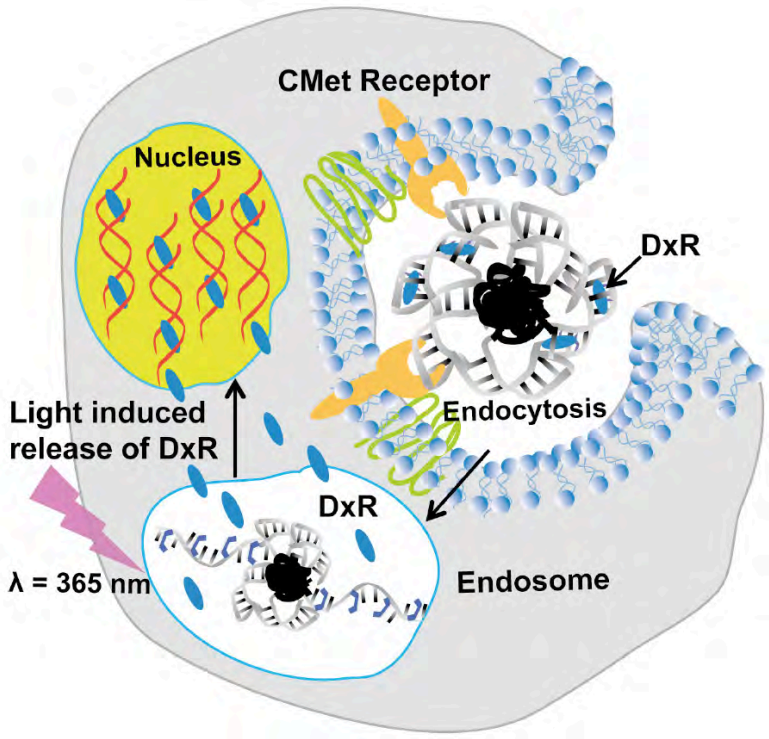


Figure 1: Schematic of the assembly and the mechanism of action of the aptamer nano-capsules. The lipidated anti-cMet and doxorubicin (DxR) binding aptamers form micellar nano-capsules. These are internalized via cMet receptor mediated endocytosis. A light-induced structural change of the DxR aptamer at 365 nm releases the DxR inside the cell, causing the attacked cells to die.

For this project, we collaborated with Dr. Elmar Behrmann's EM group within caesar. We succeeded in visualizing the DNA nanotubes, but not the nanomachine located on them. We achieved this by means of scanning probe microscopy. Most of the work was carried out by Dr. Julian Valero, who was employed at caesar.

References

1. Prusty, D.K., Adam, V., Zadegan, R.M., Irsen, S., and Famulok, M. (2018). Supramolecular aptamer nano-constructs for receptor-mediated targeting and light-triggered release of chemotherapeutics into cancer cells. Nat. Commun. 9, 535.

2. Adam, V., Prusty, D.K., Centola, M., Škugor, M., Hannam, J.S., Valero, J., Klöckner, B., and Famulok, M. (2018). Expanding the Toolbox of Photoswitches for DNA Nanotechnology Using Arylazopyrazoles. Chemistry 24, 1062-1066.

3. von Delius, M. and Leigh, D.A. (2011). Walking molecules. Chem. Soc. Rev. 40, 3656-3676.

4. Valero, J., Pal, N., Dhakal, S., Walter, N.G., and Famulok, M. (2018). A bio-hybrid DNA rotor-stator nanoengine that moves along predefined tracks. Nat Nanotechnol. 13, 496-503.

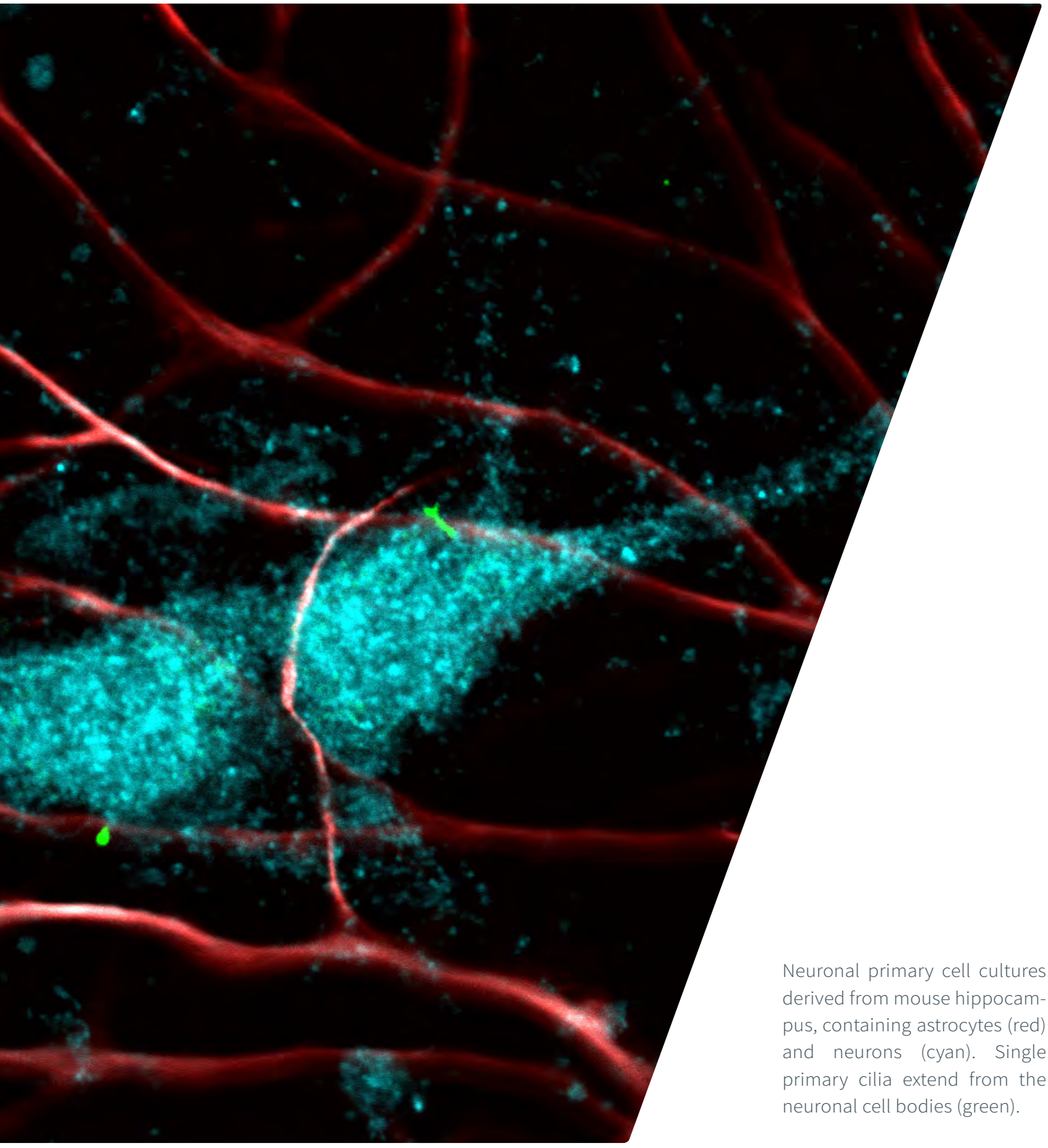
About the Max Planck Fellow Group: Chemical Biology

We focus on the structural analysis of biomolecules and on photo-activatable substances. Synthetic chemistry is often used in chemical biology to generate tools for investigating mechanisms in biology or biomedicine. For example, proteins can be chemically modified or the effects of biomolecules can be altered. Cytohesins are proteins that regulate cellular signaling pathways but their exact mode of action is largely unknown.

A better understanding could lead to insights into disease mechanism and to development of treatment. We study the cellular function of cytohesins by combining chemical methods and structural biology.

We are interested in how cytohesins interact with receptor tyrosin kinases (RTKs), e.g. during insulin receptor (IR) signaling, and in their mechanism of RTK activation.

Furthermore, we develop DNA nanomachines that can exert defined functions, and aptameric nanocarriers for intracellular delivery of diverse molecular cargo.



Neuronal primary cell cultures derived from mouse hippocampus, containing astrocytes (red) and neurons (cyan). Single primary cilia extend from the neuronal cell bodies (green).

How to shed light on ciliary function

Primary cilia were first discovered 150 years ago and were thought to be of no use. Recent advances in science technology revealed that primary cilia function as signaling hubs that play a pivotal role in many signaling processes.

by Prof. Dr. Dagmar Wachten | Minerva-Group: Molecular Physiology

Cilia – what they are and what they do

Cilia are membrane protrusions that come in two different flavors - they are either motile or immotile. Motile cilia are used to move fluid flows along the cellular surface, e.g. on epithelial cells in the lung, or to propel cells forward, e.g. sperm. Immotile cilia are called primary cilia and almost every single cell in our body contains a primary cilium. Still, we do not really understand what they do. They have been proposed to act as a cellular antenna that receives extracellular cues and transmits this information into a cellular response. Loss of ciliary function seems to be detrimental, causing a variety of pathologies commonly referred to as ciliopathies. They comprise polycystic kidney disease, blindness, mental retardation, or obesity [1].

Primary cilia constitute a unique cellular compartment – also in neurons

To function as a cellular antenna, primary cilia contain a) receptors that receive the signal from the environment and transduce the information into the inside of the cell, and b) an amplifier, which usually is an enzyme that produces a messenger that transmits the information into an intracellular response [2]. However, every cell type in our body seems to be different in the composition of the molecular components that form this cellular antenna, allowing different cells to respond and transduce different stimuli. We had a closer look at the primary cilia in the brain, in particular the hypothalamus (Fig. 1). Hypothalamic neurons display a primary cilium at the cell body and express specific receptors (NPY2R) and amplifiers of the signal (AC3) (Fig. 1).

How to analyze primary cilia signaling and function?

The primary cilium only makes up a tiny fraction of the total volume of the cell (ca. 1/2000). It is 5-10 µm in length, 400 nm in width, and contains a volume of less than 1 fl. A major challenge is to analyze signaling in this tiny subcellular compartment independent from the rest of the cell. Our approach to tackle this problem is to use light for manipulating and analyzing signaling pathways for the following reason: It is necessary to manipulate cellular signaling with high temporal and spatial precision. Light is particularly well suited to this task as it can be switched on and off quickly, can be focused on very small spots within a cell, and does not affect other cellular processes. Cells are genetically modified to produce light-sensitive proteins and, thereby, can be controlled by light. This biological technique is called optogenetics. We apply a combination of optogenetics with genetically-encoded biosensors to manipulate and measure ciliary signaling with spatial and temporal resolution *in vitro* and *in vivo* [3, 4, 5]. To bring our tools specifically into the primary cilium, we need to high-jack the transport machinery that transports proteins in and out of the cilium. This approach is shown as an example in Figure 2, where we localized a biosensor to primary cilia.

cAMP – a crucial messenger for ciliary signaling

The second messenger cAMP allows to transduce a signal from the outside into a cellular response. Dysfunction of cAMP signaling has been correlated with the occurrence of ciliopathies. It has been proposed that cAMP is crucial for transducing external stimuli in the primary cilium into an intracellular response. In this model, the primary cilium creates a unique cAMP signaling domain, which controls cellular functions. Using our approach, we are aiming to unravel the role of ciliary cAMP signaling in controlling cellular functions and how dysfunction of ciliary cAMP signaling might lead to the development of ciliopathies.

References

1. Reiter, J. F. and Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. Nat. Rev. Mol. Cell Biol. 18, 533-547.

2. Pazour, G. J. and Witman, G. B. (2003). The vertebrate primary cilium is a sensory organelle. Curr. Opin. Cell. Biol. 15, 105-110.

3. Jansen, V., Alvarez, L., Balbach, M., Strünker, T., Hegemann, P., Kaupp, U. B., and Wachten, D. (2015). Controlling fertilization and cAMP signaling in sperm by optogenetics. ELife 4, e05161.

4. Jansen, V., Jikeli, J. F., and Wachten, D. (2017). How to control cyclic nucleotide signaling by light. Curr. Opin. Biotechnol. 48, 15-20.

5. Mukherjee, S., Jansen, V., Jikeli, J. F., Hamzeh, H., Alvarez, L., Dombrowski, M., Balbach, M., Strünker, T., Seifert, R., Kaupp, U. B., and Wachten, D. (2016). A novel biosensor to study cAMP dynamics in cilia and flagella. ELife 5, e1405.

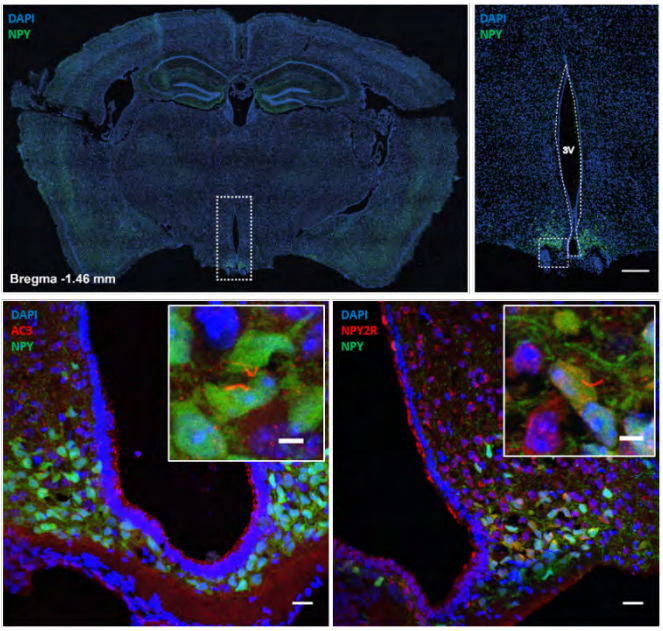
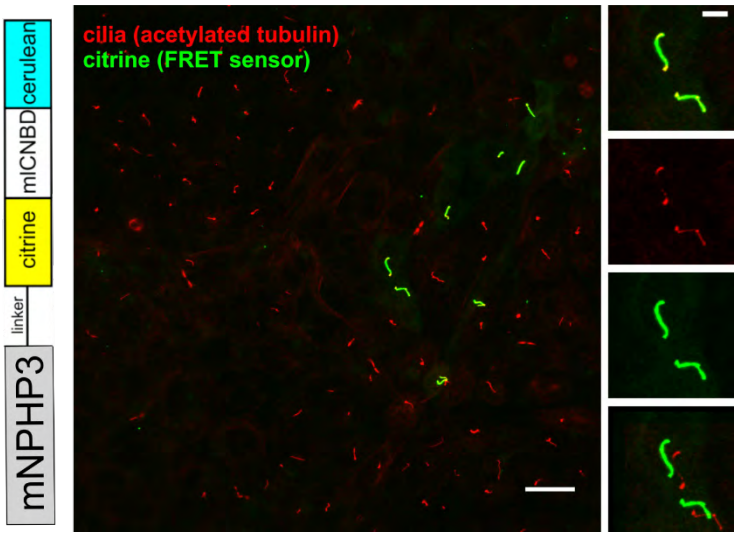


Figure 1: Primary cilia in the brain. One region in the hypothalamus, the arcuate nucleus, is labeled in green (NPY). In this region, neurons display primary cilia, expressing specific receptors (right: red, NPY2R) and enzymes (left: red, AC3).

Scale bars: top: 200 pxs; bottom: 20 µm; inset: 5 µm.

Figure 2: Expression of a biosensor in primary cilia of mouse kidney (IMCD-3) cells. Cilia are labeled in red, the sensor is labeled in green. Almost every cell displays a primary cilium and the sensor is localized exclusively to primary cilia.

Scale bar: 20 µm, inset: 5 µm.



About the Minerva-Group: Molecular Physiology

We aim to understand how cilia work and what their function is. Cilia are subcellular compartments that protrude from the surface of almost every mammalian cell. Cilia can be grouped into two major classes: a) primary cilia, which are immotile and b) motile cilia, which are also called flagella. A prominent example for the latter are sperm flagella. Ciliary dysfunction leads to severe diseases commonly referred to as ciliopathies. They comprise e.g. polycystic kidney disease, obesity, blindness, and infertility. However, the signaling pathways controlling ciliary function are ill-defined. To study ciliary signaling with high spatial and temporal precision, we combine optogenetics and genetically-encoded biosensors with high-resolution microscopy, mouse genetics, and biochemistry. This multidisciplinary approach allows us not only to investigate ciliary signaling, but can be applied to any subcellular compartment to study its function with spatial and temporal resolution.

PUBLICATIONS

Publications

Publications with caesar affiliation in 2017

- Alvarez, L. (2017). The tailored sperm cell. J Plant Res 130, 455-464.
- Ambadipudi, S., Biernat, J., Riedel, D., Mandelkow, E., and Zweckstetter, M. (2017). Liquid-liquid phase separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. Nat Commun 8, 275.
- Baker, J.D., Shelton, L.B., Zheng, D., Favretto, F., Nordhues, B.A., Darling, A., Sullivan, L.E., Sun, Z., Solanki, P.K., Martin, M.D., et al. (2017). Human cyclophilin 40 unravels neurotoxic amyloids. PLoS Biol 15, e2001336.
- Balbach, M., Beckert, V., Hansen, J.N., and Wachten, D. (2017). Shedding light on the role of cAMP in mammalian sperm physiology. Mol Cell Endocrinol., published online 2017-11-13
- Berger, T.K., Fusshöller, D.M., Goodwin, N., Bönigk, W., Müller, A., Dokani Khesroshahi, N., Brenker, C., Wachten, D., Krause, E., Kaupp, U.B., et al. (2017). Post-translational cleavage of Hv1 in human sperm tunes pH- and voltage-dependent gating. J Physiol 595, 1533-1546.
- Centola, M., Valero, J., and Famulok, M. (2017). Allosteric Control of Oxidative Catalysis by a DNA Rotaxane Nanostructure. J Am Chem Soc 139, 16044-16047.
- Collu, G., Farci, D., Esposito, F., Pintus, F., Kirkpatrick, J., and Piano, D. (2017). New insights into the operative network of FaEO, an enone oxidoreductase from Fragaria x ananassa Duch. Plant Mol Biol 94, 125-136.
- Domenyuk, V., Zhong, Z., Stark, A., Xiao, N., O'Neill, H.A., Wei, X., Wang, J., Tinder, T.T., Tonapi, S., Duncan, J., et al. (2017). Plasma Exosome Profiling of Cancer Patients by a Next Generation Systems Biology Approach. Sci Rep 7, 42741.
- Farci, D., Esposito, F., El Alaoui, S., and Piano, D. (2017a). S-layer proteins as a source of carotenoids: Isolation of the carotenoid cofactor deinoxanthin from its S-layer protein DR_2577. Food Res Int 99, 868-876.
- Farci, D., Kirkpatrick, J., and Piano, D. (2017b). A new procedure for fast soft staining of BN-PAGEs on photosynthetic complexes. Electrophoresis 38, 441-446.
- Fontela, Y.C., Kadavath, H., Biernat, J., Riedel, D., Mandelkow, E., and Zweckstetter, M. (2017). Multivalent cross-linking of actin filaments and microtubules through the microtubule-associated protein Tau. Nat. Commun. 8, 1981.
- Handa, T., Unno, S., and Mikami, A. (2017). Temporal property of single-cell activity in response to motion-defined shapes in monkey dorsal and ventral cortical areas. Neuroreport 28, 793-799.
- Hernández-Garcia, C., Turpin, A., San Román, J., Picón, A., Drevinskas, R., Cerkauskaite, A., Kazansky, P.G., Durfee, C.G., and Sola, I.J. (2017). Extreme ultraviolet vector beams driven by infrared lasers. Optica 4, 520-526.

- Jansen, V., Jikeli, J.F., and Wachten, D. (2017). How to control cyclic nucleotide signaling by light. Curr Opin Biotechnol 48, 15-20.
- Joseph, M., Anglada-Huguet, M., Paesler, K., Mandelkow, E., and Mandelkow, E.M. (2017). Anti-aggregant tau mutant promotes neurogenesis. Mol Neurodegener 12, 88.
- Kaniyappan, S., Chandupatla, R.R., Mandelkow, E.M., and Mandelkow, E. (2017). Extracellular low-n oligomers of tau cause selective synaptotoxicity without affecting cell viability. Alzheimers Dement 13, 1270-1291.
- Kaupp, U.B., and Strünker, T. (2017). Signaling in Sperm: More Different than Similar. Trends Cell Biol 27, 101-109.
- Kim, E.S., Ackermann, C., Toth, I., Dierks, P., Eberhard, J.M., Wroblewski, R., Scherg, F., Geyer, M., Schmidt, R.E., Beisel, C., et al. (2017). Down-regulation of CD73 on B cells of patients with viremic HIV correlates with B cell activation and disease progression. J Leukoc Biol 101, 1263-1271.
- Kornfeld, J., Benezra, S.E., Narayanan, R.T., Svara, F., Egger, R., Oberlaender, M., Denk, W., and Long, M.A. (2017). EM connectomics reveals axonal target variation in a sequence-generating network. Elife 6, e24364.
- Körschgen, H., Kuske, M., Karmilin, K., Yiallouros, I., Balbach, M., Floehr, J., Wachten, D., Jahnen-Dechent, W., and Stöcker, W. (2017). Intracellular activation of ovastacin mediates pre-fertilization hardening of the zona pellucida. Mol Hum Reprod 23, 607-616.
- Lawatscheck, C., Pickhardt, M., Grafl, A., Linkert, K., Polster, F., Mandelkow, E., and Borner, H.G. (2017). Gaining Insights into Specific Drug Formulation Additives for Solubilizing a Potential Anti-Alzheimer Disease Drug B4A1. Macromol Biosci 17, 1700109.
- Lim, E., Johnson, S.F., Geyer, M., Serra, V., and Shapiro, G.I. (2017). Sensitizing HR-proficient cancers to PARP inhibitors. Mol Cell Oncol 4, e1299272.
- Lu, R., Sun, W., Liang, Y., Kerlin, A., Bierfeld, J., Seelig, J.D., Wilson, D.E., Scholl, B., Mohar, B., Tanimoto, M., et al. (2017). Video-rate volumetric functional imaging of the brain at synaptic resolution. Nat Neurosci 20, 620-628.
- Lueckmann, J.M., Goncalves, P., Bassetto, G., Öcal, K., Nonnenmacher, M. and Macke, J.H. (2017). Flexible statistical inference for mechanistic models of neural dynamics. Adv Neural Inf Process Syst 30, 1289-1299.
- Manrique, S., Sauter, D., Horenkamp, F.A., Lulf, S., Yu, H., Hotter, D., Anand, K., Kirchhoff, F., and Geyer, M. (2017). Endocytic sorting motif interactions involved in Nef-mediated down-modulation of CD4 and CD3. Nat Commun 8, 442.
- Mudher, A., Colin, M., Dujardin, S., Medina, M., Dewachter, I., Alavi Naini, S.M., Mandelkow, E.M., Mandelkow, E., Buee, L., Goedert, M., et al. (2017). What is the evidence that tau pathology spreads through prion-like propagation? Acta Neuropathol Commun 5, 99.
- Narayanan, R.T., Udvary, D., and Oberlaender, M. (2017). Cell Type-Specific Structural Organization of the Six Layers in Rat Barrel Cortex. Front Neuroanat 11, 91.
- Nonnenmacher, M., Behrens, C., Berens, P., Bethge, M., and Macke, J.H. (2017). Signatures of criticality arise from random subsampling in simple population models. PLoS Comput Biol 13, e1005718.
- Nonnenmacher, M., Srinivas, T.C., and Macke, J.H. (2017). Extracting low-dimensional dynamics from multiple large-scale neural population recordings by learning to predict

correlations. Adv Neural Inf Process Syst 30, 5702-5712.
- Pickhardt, M., Biernat, J., Hubschmann, S., Dennissen, F.J.A., Timm, T., Aho, A., Mandelkow, E.M., and Mandelkow, E. (2017a). Time course of Tau toxicity and pharmacologic prevention in a cell model of Tauopathy. Neurobiol Aging 57, 47-63.
- Pickhardt, M., Lawatscheck, C., Borner, H.G., and Mandelkow, E. (2017b). Inhibition of Tau Protein Aggregation by Rhodanine-based Compounds Solubilized Via Specific Formulation Additives to Improve Bioavailability and Cell Viability. Curr Alzheimer Res 14, 742-752.
- Pir, G.J., Choudhary, B., and Mandelkow, E. (2017). Caenorhabditis elegans models of tauopathy. FASEB J 31, 5137-5148.
- Rennhack, A., Grahn, E., Kaupp, U.B., and Berger, T.K. (2017). Photocontrol of the Hv1 Proton Channel. ACS Chem Biol 12, 2952-2957.
- Rojas-Piloni, G., Guest, J.M., Egger, R., Johnson, A.S., Sakmann, B., and Oberlaender, M. (2017). Relationships between structure, in vivo function and long-range axonal target of cortical pyramidal tract neurons. Nat Commun 8, 870.
- Saggiorato, G., Alvarez, L., Jikeli, J.F., Kaupp, U.B., Gompper, G., and Elgeti, J. (2017). Human sperm steer with second harmonics of the flagellar beat. Nat Commun 8, 1415.
- Said, N., Krupp, F., Anedchenko, E., Santos, K.F., Dybkov, O., Huang, Y.H., Lee, C.T., Behrmann, E., Bürger, J., Mielke, T., Loerke, J., Urlaub, H., Spahn, C.M.T., Weber, G., and Wahl, M.C. (2017). Structural basis for λN-dependent processive transcription antitermination. Nat. Microbiol. 2, 17062.
- Schelle, J., Häsler, L.M., Göpfert, J.C., Joos, T.O., Vanderstichele, H., Stoops, E., Mandelkow, E.M., Neumann, U., Shimshek, D.R., Staufenbiel, M., et al. (2017). Prevention of tau increase in cerebrospinal fluid of APP transgenic mice suggests downstream effect of BACE1 inhibition. Alzheimers Dement 13, 701-709.
- Schonauer, S., Körschen, H.G., Penno, A., Rennhack, A., Breiden, B., Sandhoff, K., Gutbrod, K., Dörmann, P., Raju, D.N., Haberkant, P., et al. (2017). Identification of a feedback loop involving beta-glucosidase 2 and its product sphingosine sheds light on the molecular mechanisms in Gaucher disease. J Biol Chem 292, 6177-6189.
- Sethuramanujam, S., Yao, X., deRosenroll, G., Briggman, K.L., Field, G.D., and Awatramani, G.B. (2017). “Silent” NMDA Synapses Enhance Motion Sensitivity in a Mature Retinal Circuit. Neuron 96, 1099-1111 e3.
- Sotiropoulos, I., Galas, M.C., Silva, J.M., Skoulakis, E., Wegmann, S., Maina, M.B., Blum, D., Sayas, C.L., Mandelkow, E.M., Mandelkow, E., et al. (2017). Atypical, non-standard functions of the microtubule associated Tau protein. Acta Neuropathol Commun 5, 91.
- Speiser, A., Jinyao, Y., Archer, E., Buesing, L., Turaga, S.C., and Macke, J.H. (2017). Fast amortized inference of neural activity from calcium imaging data with variational autoencoders. Adv Neural Inf Process Syst 30, 4024-4034.
- Stutz, A., Kolbe, C.C., Stahl, R., Horvath, G.L., Franklin, B.S., van Ray, O., Brinkschulte, R., Geyer, M., Meissner, F., and Latz, E. (2017). NLRP3 inflammasome assembly is regulated by phosphorylation of the pyrin domain. J Exp Med 214, 1725-1736.
- Tafazoli, S., Safaai, H., De Franceschi, G., Rosselli, F. B., Vanzella, W., Riggi, M., et al. (2017). Emergence of transformation-tolerant representations of visual objects in rat lateral extras-

triate cortex. eLife, 6: e22794.
- Turner-Evans, D., Wegener, S., Rouault, H., Franconville, R., Wolff, T., Seelig, J.D., Druckmann, S., and Jayaraman, V. (2017). Angular velocity integration in a fly heading circuit. Elife 6, e23496.
- Turpin, A., Rego, L., Picon, A., San Roman, J., and Hérnandez-Garcia, C. (2017). Extreme Ultraviolet Fractional Orbital Angular Momentum Beams from High Harmonic Generation. Sci Rep 7, 43888.
- Valero, J., Lohman, F., and Famulok, M. (2017) Interlocked DNA topologies for nanotechnology. Curr Opin Biotechnol 48, 159-167.
- von Gerichten, J., Schlosser, K., Lamprecht, D., Morace, I., Eckhardt, M., Wachten, D., Jennemann, R., Gröne, H.J., Mack, M., and Sandhoff, R. (2017). Diastereomer-specific quantification of bioactive hexosylceramides from bacteria and mammals. J Lipid Res 58, 1247-1258.
- Wachten, D., Jikeli, J.F., and Kaupp, U.B. (2017). Sperm sensory signaling. Cold Spring Harb Perspect Biol 9, a028225.
- Wang, Y., Balaji, V., Kaniyappan, S., Krüger, L., Irsen, S., Tepper, K., Chandupatla, R., Maetzler, W., Schneider, A., Mandelkow, E., et al. (2017). The release and trans-synaptic transmission of Tau via exosomes. Mol Neurodegener 12, 5.
- Weber, T., Kiel, T., Irsen, S., Busch, K., and Linden, S. (2017) Near-field study on the transition from localized to propagating plasmons on 2D nano-triangles. Opt Express 25, 16947-16956.
- Wischhof, L., Maida, S., Piazzesi, A., Gioran, A., Barragan Sanz, K., Irsen, S., Beyer, M., Schultze, J.L., Dyer, M.J., Salomoni, P., et al. (2017). The SWI/SNF subunit Bcl7a contributes to motor coordination and Purkinje cell function. Sci Rep 7, 17055.
- Woeste, M.A., and Wachten, D. (2017). The enigmatic role of GBA2 in controlling locomotor function. Front Mol Neurosci 10, 386.
- Xiang, S., Kulminskaya, N., Habenstein, B., Biernat, J., Tepper, K., Paulat, M., Griesinger, C., Becker, S., Lange, A., Mandelkow, E., et al. (2017). A two-component adhesive: Tau fibrils arise from a combination of a well-defined motif and conformationally flexible interactions. J Am Chem Soc 139, 2639-2646.
- Yin, D.M., Hannam, J.S., Schmitz, A., Schiemann, O., Hagelueken, G., and Famulok, M. (2017). studying the conformation of a receptor tyrosine kinase in solution by inhibitor-based spin labeling. Angew Chem Int Ed Engl 56, 8417-8421.
- Zanello, M., Poulon, F., Pallud, J., Varlet, P., Hamzeh, H., Abi Lahoud, G., Andreiuolo, F., Ibrahim, A., Pages, M., Chretien, F., et al. (2017). Multimodal optical analysis discriminates freshly extracted human sample of gliomas, metastases and meningiomas from their appropriate controls. Sci Rep 7, 41724.
- Zempel, H., Dennissen, F.J.A., Kumar, Y., Luedtke, J., Biernat, J., Mandelkow, E.M., and Mandelkow, E. (2017a). Axodendritic sorting and pathological missorting of Tau are isoform-specific and determined by axon initial segment architecture. J Biol Chem 292, 12192-12207.
- Zempel, H., Luedtke, J., and Mandelkow, E.M. (2017b). Tracking tau in neurons: How to transfect and track exogenous tau into primary neurons. Methods Mol Biol 1523, 335-340.
- Zempel, H., and Mandelkow, E.M. (2017). Tracking Tau in Neurons: How to grow, fix, and stain primary neurons for the investigation of tau in all developmental stages. Methods Mol Biol

1523, 327-334.
- Zhong, W., Ciatipis, M., Wolfenstetter, T., Jessberger, J., Müller, C., Ponsel, S., Yanovsky, Y., Brankack, J., Tort, A.B.L., and Draguhn, A. (2017). Selective entrainment of gamma subbands by different slow network oscillations. Proc Natl Acad Sci U S A 114, 4519-4524.

Dissertations

- Balbach, M. (2017): Action of zona pellucida glycoproteins in mouse and human sperm, Dissertation: Mathematisch-Naturwissenschaftliche Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn
- Farci, D. (2017): Spatial organization of a chemoreceptor guanylate cyclase in the flagellum of Arbacia punctulata sperm, Dissertation: Mathematisch-Naturwissenschaftliche Fakultät der Universität zu Köln
- Fridman, D. (2017): Temperaturwahrnehmung in menschlichen Spermien, Dissertation: Mathematisch-Naturwissenschaftliche Fakultät der Universität zu Köln
- Hamzeh, H. (2017): Organization and function of signaling molecules in sperm, Dissertation: Mathematisch-Naturwissenschaftliche Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

CAESAR INSIGHTS

News from IMPRS

The International Max Planck Research School (IMPRS) for Brain and Behavior completed its second full year of operation in 2017. 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.

by Denise Butler | IMPRS coordination office

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) and two universities (the University of Bonn and Florida Atlantic University). The program is funded predominantly by a Max Planck Society IMPRS grant with additional support for student positions, research costs and overhead by the participating institutions, and grants to individual investigators. caesar hosts the coordination office for the school in Bonn.

The Steering Committee of the IMPRS advises the Speaker and Co-Speakers on all items relevant to the successful running of the IMPRS. All policy change proposals including the addition of new IMPRS faculty and committee appointments are approved by a majority vote. In 2017 Dr. Kevin Briggman (caesar) and Dr. Robert Stackman (FAU) joined while Prof. Dr. U. Benjamin Kaupp (caesar) and Dr. Elizabeth Matthews (Uni Bonn) left the committee. Significant committee decisions in 2017 were: increase the number of IMPRS funded PhD fellowships from 3 to 4, establish 4 master student fellowships with the Uni Bonn MSc Neuroscience program, and institute a Travel Grant component. Further, the committee added two new faculty, Prof. Dr. Jens Brüning (MPI for Metabolism Research) in March and Dr. Kevin Briggman (caesar) in September.

Research Scope

One of the central questions of biology is the understanding of how the brains of animals encode the outside world and how this enables them to interact with their environment. Although this subject has been investigated for decades, it is only now that the necessary tools are becoming available to finally address this topic. These tools are based on optical, electrophysiological, and genetic techniques that, when combined, provide

unparalleled insight into neuronal circuits in the whole brain and are truly advancing neuroscience. Thus the research focus of the IMPRS for Brain and Behavior is to combine knowledge from the traditional disciplines of biology, mathematics and physics to address this question at multiple levels; from defining the role of molecules involved in signaling at the synapse to understanding how animals use sensory information to make decisions.

Applications and Selection Symposium

The program's Selection Committee utilizes a two-tier online system to review and rate applications. This process has proven to be successful and the committee voted to continue this procedure.

The 2017 applicant pool was reduced to 15 invitees, of which 12 attended the Selection Symposium at caesar March 13-14, 2017. Due to feedback, the format of the symposium was changed to include a poster session and then interview sessions with each of three committee panels. Six of the twelve candidates were offered admission to IMPRS. The top three students were awarded 3-year fellowships. The second day was devoted to finding a mentor who matched research interests with the candidates from the 27 available funded positions. The symposium concluded with dinner and an international exchange/social event organized by current IMPRS students.

Students

In 2017 we admitted our second class consisting of 4 students. In total, there are 19 students coming to us from 10 different countries: 21% from US and 26% from Germany; 11 males, 8 females; 7 work in the US and 12 in Germany. Students are accepted annually and our application to acceptance ratio was 17:1 in 2017. The anticipated class size at steady state is 12 students. Therefore for a 3-year program, we expect approximately 36 students in the program. The Selection Committee decided that maintaining excellence takes priority over maintaining constant yearly class size. For 2017, it was clarified that excellent students from other disciplines, such as physics and computer science, are an asset to the program and will not be excluded based on lack of neurobiology background. Remedial training in biology and neuroscience will be offered at our partner universities.

In March, student representatives briefed the Steering Committee on student concerns. Suggestions included travel grants to attend workshops or courses at the partner institutes and international meetings, and inclusion of faculty from Florida and/or Bonn on student's Thesis Advisory Committee (TAC). In September, the committee approved a new Travel Grant program. Provisions for fostering more exchange between Bonn and Florida have been explored and at least one student has TAC members in both Bonn and Florida.

Developments

In the last two years IMPRS students have successfully integrated into the local scientific community. We now have an elected member on the BIGS Neuroscience Student Council in Bonn and several IMPRS students joined the BIGS retreat in October. During the BonnBrain[3] held at caesar in March, a majority of the students presented posters of ongoing work. In September, IMPRS held a well-attended Master Student Info Session at University of Bonn which attracted students from the neurosciences and other relevant master disciplines from area universities. Speakers Dr. Jason Kerr and Dr. Kevin Briggman presented the IMPRS program and research topics to the audience. In November, at the annual meeting of the Society for Neuroscience (SfN) in Washington, we had an information booth at the Graduate School Fair and were also represented at the NeuroSchools-Germany and the DFG booths. Co-Speaker, Dr. McLean Bolton (MPFI) seized the opportunity to profile our IMPRS in an interview conducted at SfN for an article to be published in the SfN member journal Neuroline. The interview focused on how programs are developing curricula that keep up with the evolving field, and to also gather advice for prospective and current graduate students. Importantly, the first student of the IMPRS, Daniel Wilson (MPFI), defended his thesis in December 2017.

Prof. Hidde Ploegh's lecture "Den Kampf zwischen Immun- und Krebszellen sichtbar machen", about the battle between the immune system and cancer, was well received among the caesarium audience.

Events at caesar

caesarium

Our public lecture series "caesarium" continued with great success in 2017. Internationally recognized scientists presented a broad range of topics, generated for a lay audience. The chosen topics were of current social relevance and thereby led to inspiring public discussions. Thus, the "caesarium" continues to provide further insights to the excitement and fascination of science and research.

The following speakers presented their work at the "caesarium" in 2017:

- **Prof. Dr. Jeffrey Friedman**
  Rockefeller University, New York, USA
  “Leptin and the Biologic Basis of Obesity“
  16.02.2017

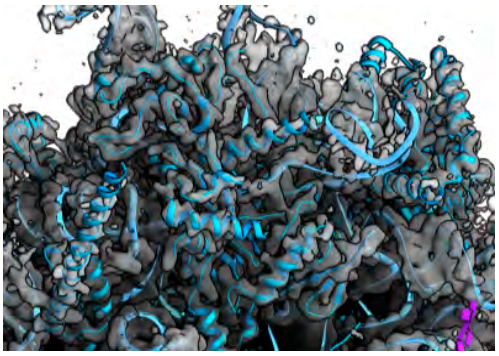
- **Prof. em. Dr. Dr. h.c. mult. Wolf Singer**
  Emeritus at Max Planck Institute for Brain Research and Senior-Fellow at the Ernst-Strüngmann-Institut, Frankfurt
  “Wissen wollen, eine ethische Verpflichtung?”
  06.04.2017

- **Prof. Dr. Philippe Bastiaens**
  Max Planck Institute for Molecular Physiology, Dortmund
  "Können unsere Zellen denken und dürfen sie das?“
  05.10.2017

- **Prof. Dr. Hidde Ploegh**
  Harvard Medical School, Boston, USA
  "Den Kampf zwischen Immun- und Krebszellen sichtbar machen”
  09.11.2017

caesar conference (September 12-15, 2017)

The 6th International caesar conference "Overcoming barriers: Atomic resolution and beyond" brought together the leaders in molecular cryo-electron microscopy to talk about the latest developments in this rapidly evolving field. Discussions focused on recent technological as well as methodological development and their applications to fundamental biological questions. The conference was opened by a keynote lecture from Richard Henderson (who won the Nobel prize for his work just a few weeks later) and attended by around 150 scientists, old and young alike. Prior to the conference we also had two practical workshops showcasing new methodology with 30 participants.



Science slam (May 11, 2017)

A fantastic way to communicate science in an understandable and entertaining manner. In a series of short presentations, passionate scientists explained their work to a non-specialised public audience. In a competition format the audience voted for their favourite science slammer. It is a real challenge to get on a stage, getting some serious research across and yet make the audience laugh. It was a truly engaging and interactive event, demonstrating the excitement of science and inspiring the audience.

Art exhibition ARTenVielfalt (April 10, - June 9, 2017)

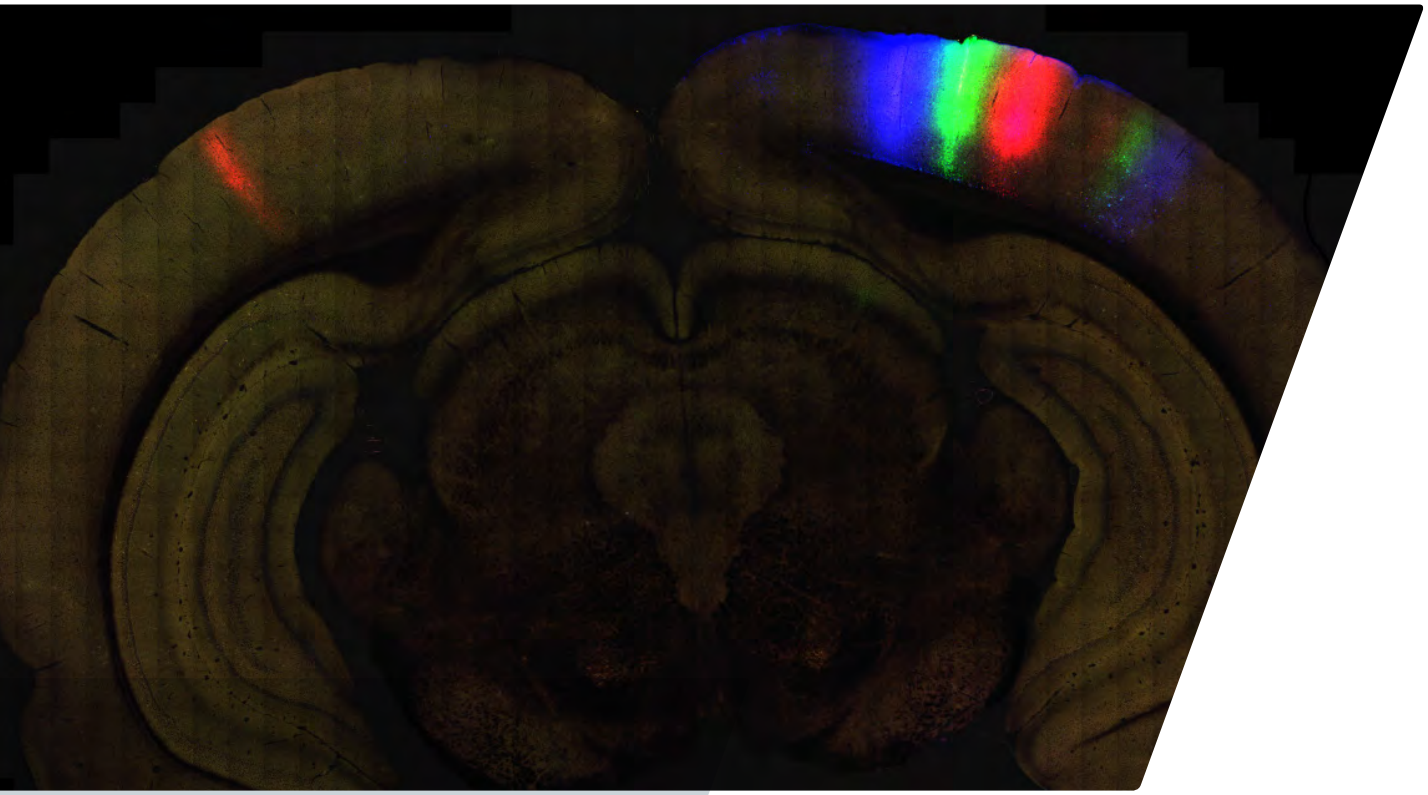
Twelve artists, mainly regional, were exhibiting at the "ARTenVielfalt" from April 10 to June 9. The exhibition opened up to various artistic disciplines and styles: painting, sculpture, photography, installation, object art, media, tape art and neo-pop art. The approaches of the artists were as diverse as the artistic genres and creative processes.

Company run (September 14, 2017)

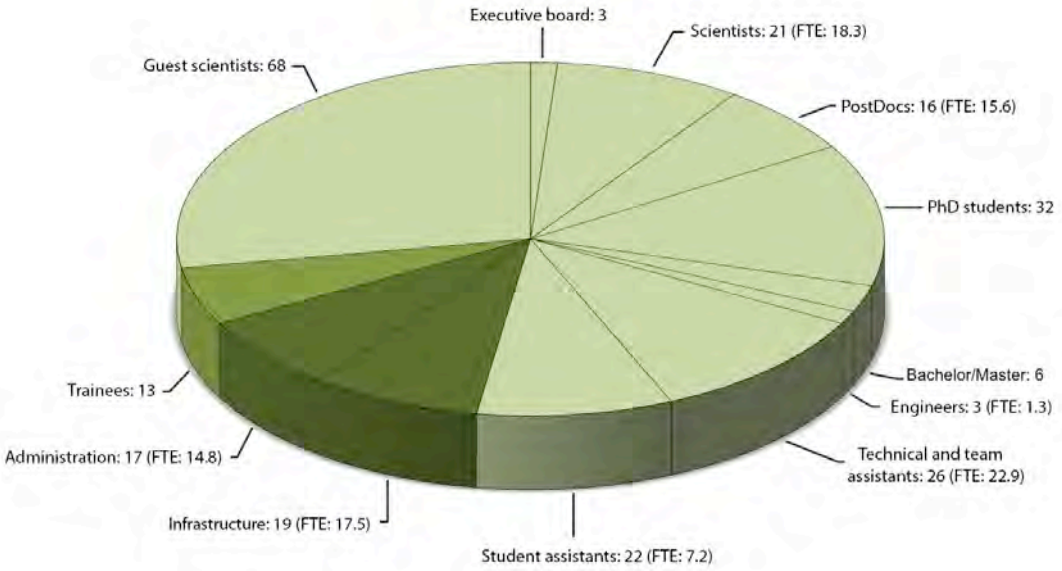
Once again Bonn transformed into a race track, along the Rhine, for the 11th company run on September 14, 2017. Together with 12.400 runners from over 450 different companies and institutes, caesar members defied the weather and successfully crossed the finish line of the 5.7 km long distance.

FACTS & FIGURES

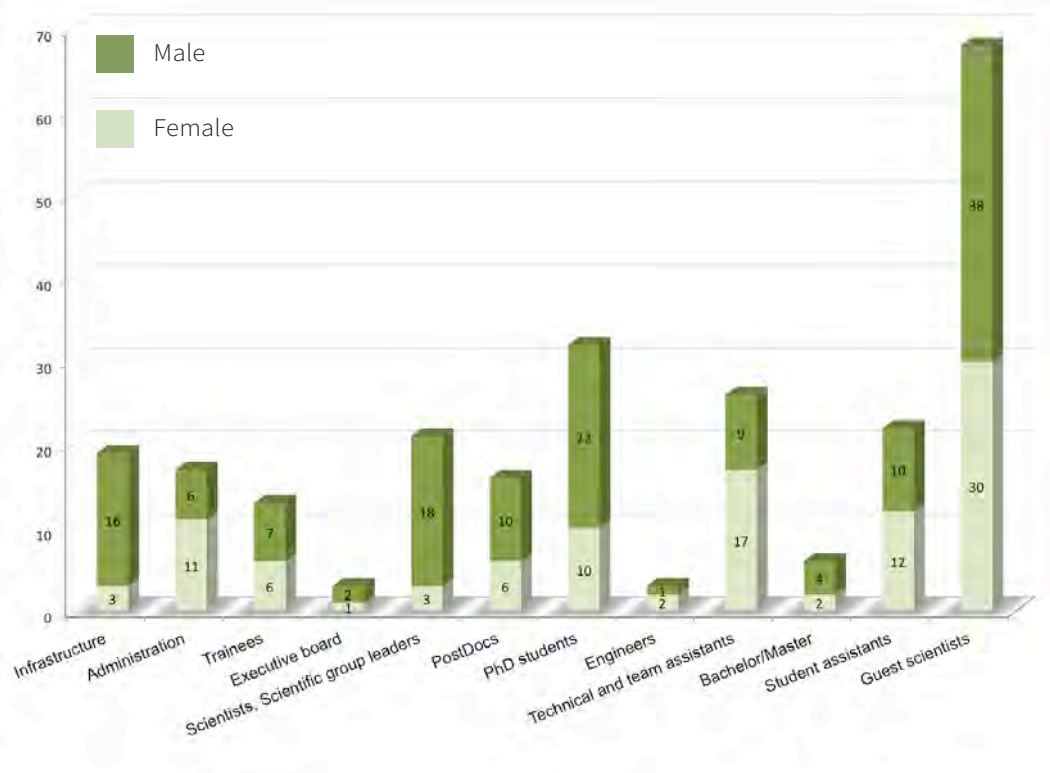
Facts and figures



As of January 1st, 2017 the research center caesar employed 175 in total and hosted 68 guest scientists. The distribution of the employees within the departments and functions is deductible from the following graph. Converted into full-time positions there are 14.8 people working in the administration and 17.5 in the infrastructure. Within the science departments and its corresponding section of scientific infrastructure 194 employees are associated. At the turn of the year, 68 guest scientists were reasearching at caesar in the context of scientific cooperations. Furthermore, caesar instructed 13 trainees in 6 departments: tool mechanic, laboratory assistant in biology and chemistry, administration and animal care.



The ratio of female coworkers in the scientific departments amounts to 42%. The distribution of female and male employees, regarding the individual functional areas, is represented by the following graph.



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 among others the research of caesar.

ASSETS	
Tangible assets	84.511.122 €
Financial assets	301.736.604 €
Miscellaneous	23.171.446 €
Total assets	409.419.172 €

INCOME 2017	
Income from sales revenues and promotions	5.130.675 €
Income form securities and interest	13.628.916 €
Total income	18.759.591 €

EXPENSES 2017	
Personnel expenses	8.235.874 €
Material expenses	5.065.418 €
Amortisations	3.594.483 €
Total expense	16.895.775 €

Balance sheet

Final balance as of December 31st, 2017

ASSETS	31.12.2017	31.12.2016
A. Capital assets		
I. Intangible assets	139.286 €	229.160 €
II. Tangible assets		
Property and construction	69.969.487 €	71.163.312 €
Various investments	13.289.248 €	10.070.155 €
Advance payment & investment in construction	712.387 €	681.875 €
Total of II.	84.511.122 €	81.915.342 €
III. Financial assets		
Assets	330.000 €	330.000 €
Securities of capital assets for investment of foundation capital	290.575.021 €	290.575.021 €
Securities of capital assets for reacquisition of property	10.831.583 €	10.831.583 €
Total of III.	301.736.604 €	301.736.604 €
Total of A.	386.387.012 €	383.881.106 €

Final balance as of December 31st, 2017

B. CURRENT ASSETS	31.12.2017	31.12.2016
I. Inventory stock	319.950 €	273.946 €
II. Accounts and other assets		
Trade accounts receivables	599.357 €	936.514 €
Receivables from affilliated companies	0 €	0 €
Miscellaneous assets	2.033.002 €	1.061.860 €
Total of II.	2.632.359 €	1.998.374 €
III. Securities	0 €	1.000.000 €
IV. Cash assets, Federal bank balances, credit balances and checks	19.797.225 €	20.788.813 €
Total of B.	22.746.534 €	24.061.133 €
C. Deferred income	285.626 €	317.941 €
Total capital	409.419.172 €	408.260.181 €

Final balance as of December 31st, 2017

LIABILITIES	31.12.2017	31.12.2016
A. Capital assets		
I. Foundation assets		
Financing capital	286.323.453 €	286.323.453 €
Investing capital	97.145.457 €	97.145.457 €
Support City of Bonn	6.681.051 €	6.681.051 €
Apropriation reserves	1.283.957 €	1.283.957 €
Total of I.	391.433.918 €	391.433.918 €
II. Reserves		
Free reserves in accordance with § 58 Nr. 7a AO	8.371.133 €	6.507.317 €
Maintenance reserves	4.943.789 €	4.943.788
Total of II.	13.314.922 €	11.451.105 €
III. Result		
Annual surplus / deficit	0 €	0 €
Total of III.	0 €	0 €
Total of A.	404.748.840 €	402.885.023 €
B. Exceptional items for investment subsidies	3.207.721 €	3.646.087 €
C. Provisions	511.007 €	€608.030 €
D. Payables		
Trade account payables	793.619 €	929.315 €
Other payables	157.985 €	190.675 €
Total of D.	951.604 €	1.119.990 €
E. Deferred income	0 €	1.051 €
Entire assets	409.419.172 €	408.260.181 €

Foundation bodies

Foundation board

As of Dec 31st, 2017, 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

- MinDir'in Bärbel Brumme-Bothe
  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 for Education and Research,
  Bonn

- Karl Schultheis
  Member of the Landtag NRW,
  Düsseldorf

- Ashok-Alexander Sridharan
  Head major 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, 2017, the caesar executive board consisted of:

- Dr. Jason Kerr
  Managing director
  Director of the Department of "Behavior and Brain Organization“

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

- Gertrud Bilski
  Administrative director

IMPRINT

Published by

Forschungszentrum caesar
Ludwig-Erhard-Allee 2
D-53175 Bonn

Editor-in-chief

Dr. Jason Kerr
Dr. Kevin Briggman
Getrud Bilski

Editors

Sebastian Scherrer
Dr. Mia von Scheven
Dr. Christoph Geisen

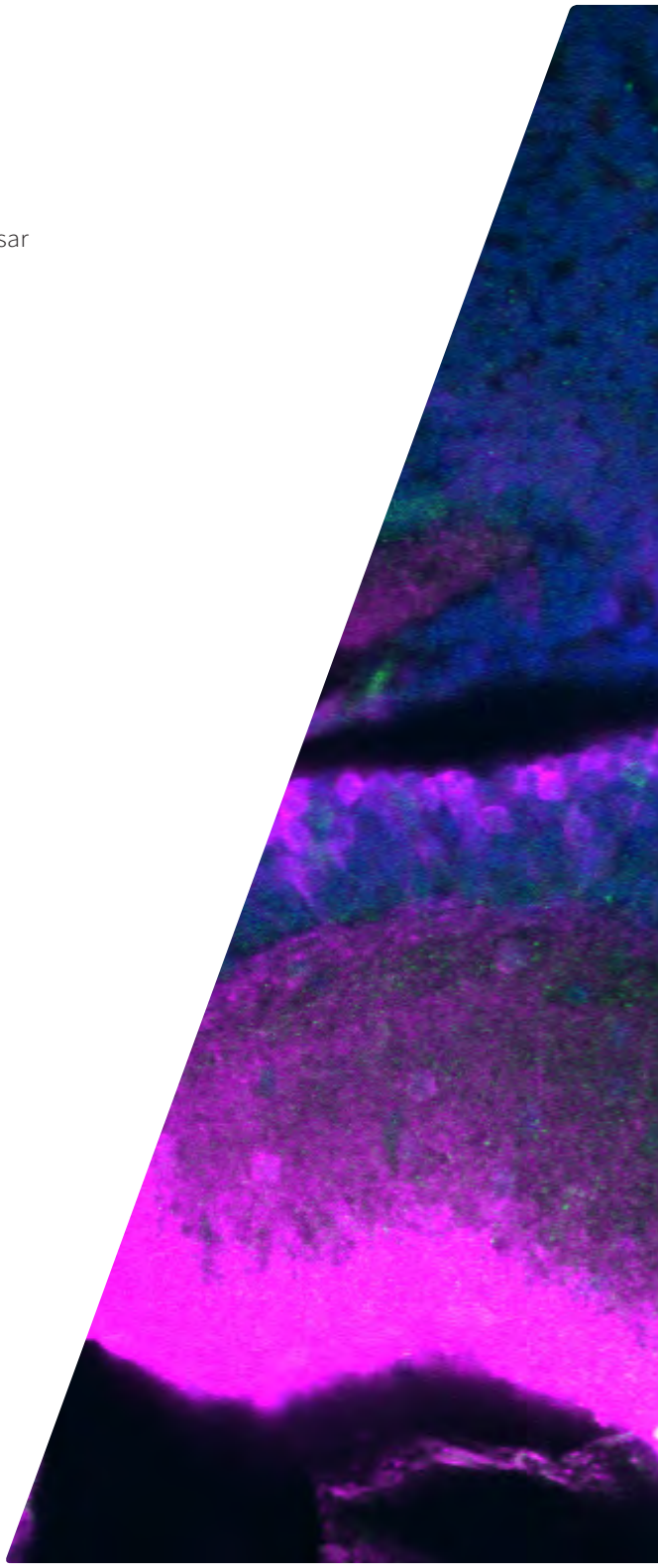
Contact information

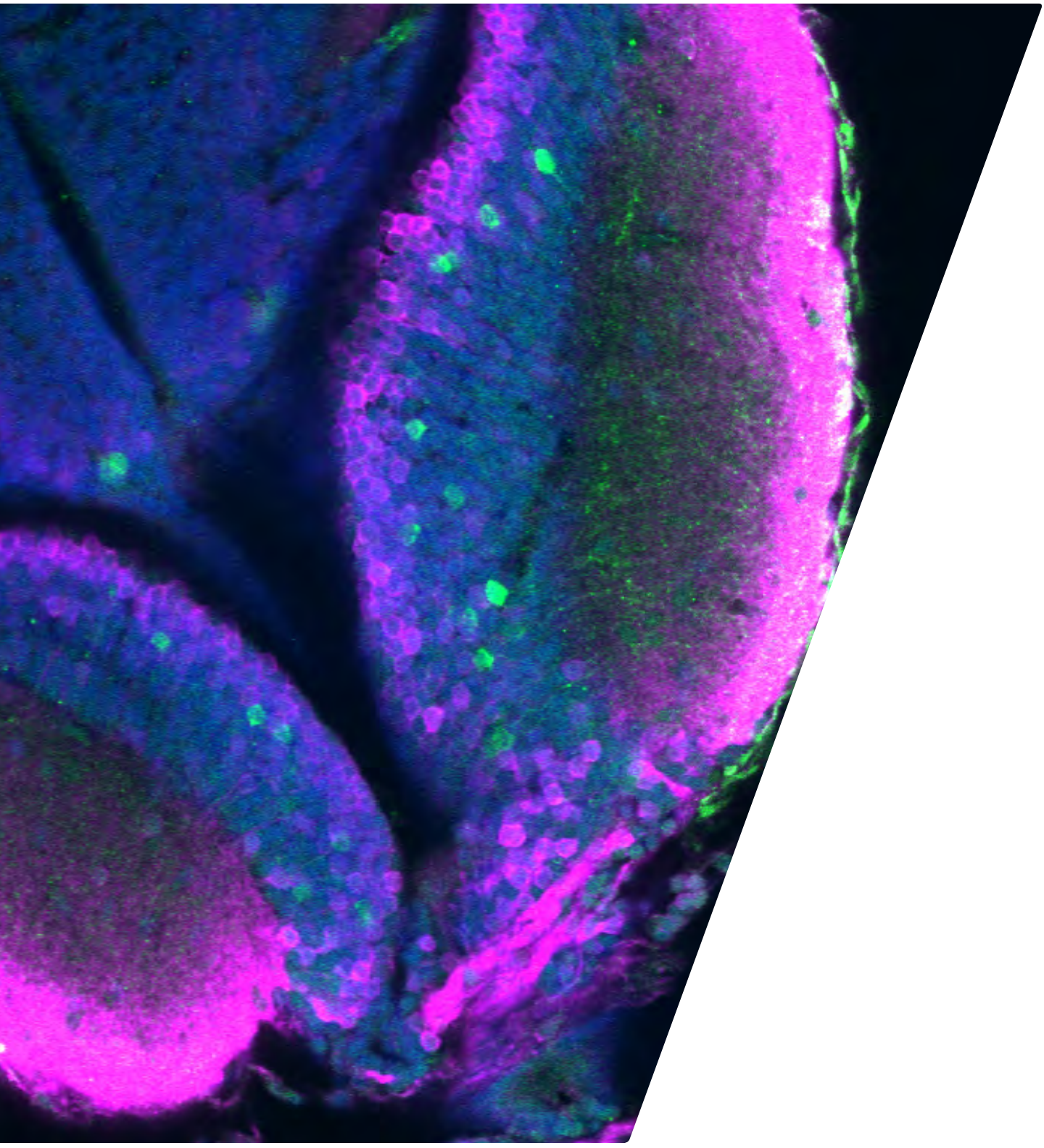
Stiftung caesar
Ludwig-Erhard-Allee 2
D-53175 Bonn

Tel.: +49 (0)228 9656-0
Fax: +49 (0)228 9656-111

E-Mail: office@caesar.de

http://www.caesar.de





www.caesar.de