Botond Roska

CURRICULUM VITAE

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Education

1995	MD, Semmelweis Medical School, Hungary
2002	PhD, Department of Molecular and Cell Biology, University of California Berkeley, USA

Current Positions

2014–	Professor, Faculty of Medicine, University of Basel, Switzerland
2018–	Founding Director, Institute of Molecular and Clinical Ophthalmology Basel (IOB), Switzerland
2019–	Professor, Faculty of Science, University of Basel, Switzerland

Previous Positions

2002– 2005	Harvard Junior Fellow, Department of Genetics, Harvard Medical School, and Department of Molecular and Cellular Biology, Harvard University, USA
2005–2010	Junior Group Leader, Friedrich Miescher Institute for Biomedical Research (FMI), Switzerland
2010–2018	Senior Group Leader, FMI, Switzerland

Fellowships and Awards

1997	Fulbright Fellow, USA
2001	Bearden Memorial Award for Biophysics, University of California Berkeley, USA
2001	Outstanding Graduate Student Instructor Award, University of California Berkeley, USA
2001	HFSP Short Term Fellow, International
2002	Harvard Junior Fellow, USA
2006	Marie Curie Excellence Grant of the European Union, International
2009	EMBO Young Investigator, International
2010	ERC Starting Grant, International
2010	VIVA Award, Switzerland
2011	Alcon Award, USA
2011	EMBO member, International
2013	Alfred Vogt Award in Ophthalmology, Switzerland
2015	ERC Advanced grant, International
2016	Cogan Award of ARVO, USA
2018	Bressler Prize in Vision Science, USA
2018	Alden W. Spencer Award for Neuroscience, USA
2019	Louis-Jeantet Prize for Medicine, Switzerland
2019	Order of Saint Stephen of Hungary, Hungary

Advisory and Editorial Boards

2009–	Member, Scientific Advisory Board, LASCCO SA, Switzerland
2011–	Chair, Scientific Advisory Board, GenSight Biologics Inc, France
2013–	Member, Scientific Advisory Board, Cell type and connectivity project, Allen Brain Institute, USA
2014–	Member, Scientific Advisory Board, MRC Centre for Developmental Neurobiology, King's College London, UK
2015–	Member, Editorial Committee, Annual Review in Neuroscience, USA
2015–	Member, Scientific Advisory Board, Leenaards Foundation, Switzerland
2016	Member, Scientific Advisory Board, Champalimaud Neuroscience Program, Portugal
2016–	Member, Editorial Board, Current Opinion in Neurobiology, USA
2016–	Chair, Scientific Advisory Board, Cell type and connectivity project, Allen Brain Institute, USA
2016–	Member, Scientific Advisory Board, Inscopix Inc, USA
2016–	Member, FENS Brain Conferences Committee
2016–	Member, Scientific Advisory Board, Max Planck Institute of Neurobiology, Martinsried, Germany
2016–	Associate Editor, Annual Review in Neuroscience, USA
2017	Member, Review Committee, Brain Research Institute, University of Zurich, Switzerland
2017–	Member, Scientific Advisory Board, CRTD, Dresden, Germany
2017–	Co-Editor, Annual Review in Neuroscience, USA
2018–	Member, Editorial Board, Physiological Reviews, UK
2018–	Member, Scientific Advisory Board, Sainsbury Wellcome Centre for Neural Circuits and Behaviour, London, UK

SELECTED PUBLICATIONS

1. Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Duebel J, Oertner TO, Schübeler D, Schratt G, Fehling HJ, Richter J, Bibel M, **Roska B*** and Filipowicz W*. Characterization of microRNAs induced by light adaptation in mouse retina reveals rapid turnover as a common property of neuronal microRNAs. (*shared corresponding authors) ***Cell.*** 2010, 141(4):618–31

2. Busskamp V, Duebel J, Balya D, Fradot M, Viney TJ, Siegert S, Groner AC, Cabuy E, Forster V, Seeliger M, Biel M, Humphries P, Paques M, Mohand-Said S, Trono D, Deisseroth K, Sahel JA, Picaud S, **Roska B.** Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. ***Science.*** 2010, 329(5990):413–7

3. Yonehara K, Balint K, Noda M, Nagel G, Bamberg E, **Roska B.** Spatially asymmetric reorganization of inhibition establishes a motion-sensitive circuit. ***Nature***. 2011, 469(7330):407-10

4. Siegert S, Cabuy E, Gross Scherf B, Kohler H, Panda A, Le YZ, Fehling HJ, Gaidatzis DG, Stadler MB, **Roska B.** Transcriptional code and disease map for adult retinal cell types. ***Nature Neuroscience.*** 2012 22;15(3):487–95

5. Busskamp V, Krol J, Nelidova D, Daum J, Szikra T, Tsuda B, Juettner J, Farrow K, Gross Scherf B, Patino Alvarez CP, Genoud C, Sothilingam V, Tanimoto N, Stadler M, Seeliger M, Stoffel M, Filipowicz W*, **Roska B***. MiRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function (*shared corresponding authors) ***Neuron.*** 2014, 83(3):586–600

6. Wertz A, Trenholm S, Yonehara K, Hillier D, Raics D, Leinweber M, Szalay G, Ghanem A, Keller G, Rózsa B, Conzelmann KK, **Roska B.** Single-cell-initiated Monosynaptic Tracing Reveals Layer-specific Cortical Network Modules. ***Science.*** 2015, 349 (6243):70–4

7. Yonehara K, Fiscella M, Drinnenberg A, Esposti F, Trenholm S, Krol J, Franke F, Scherf BG, Kusnyerik A, Müller J, Szabo A, Jüttner J, Cordoba F, Reddy AP, Németh J, Nagy ZZ, Munier F, Hierlemann A, **Roska B.** Congenital Nystagmus Gene FRMD7 Is Necessary for Establishing a Neuronal Circuit Asymmetry for Direction Selectivity. ***Neuron.*** 2016, 89(1):177–93

8. Schubert R, Trenholm S, Balint K, Kosche G, Cowan CS, Mohr MA, Munz M, Martinez-Martin D, Fläschner G, Newton R, Krol J, Scherf BG, Yonehara K, Wertz A, Ponti A, Ghanem A, Hillier D, Conzelmann KK, Müller DJ*, **Roska B***. Virus stamping for targeted single-cell infection in vitro and in vivo. (*shared corresponding authors) ***Nature Biotechnology*** 2018, 36(1):81–88

9. **Roska B,** Sahel JA. Restoring vision. ***Nature.*** 2018 May;557(7705):359-367.

10. Jüttner J, Szabo A, Gross-Scherf B, Morikawa R, Rompani S, Hantz P, Szikra T, Esposti E, Cowan C, Bharioke A, Patino-Alvarez C, Keles Ö, Kusnyerik A, Azoulay T, Hartl D, Krebs A, Schübeler D, Hajdu R, Lukats A, Nemeth J, Nagy Z, Wu KC, Wu RH, Xiang L, Fang XL, Jin ZB, Goldblum D, Hasler P, Scholl H, Krol J,* **Roska B***. Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates, and humans (*shared corresponding authors) ***Nature Neuroscience*** 2019, 22(8):1345–1356

PATENTS

Inventor of the following 38 international patents and patent applications: WO2008/022772, WO2009/112448, WO2009/127705, WO2011/039161, WO2013/068413, WO2014/033095, WO2014/122605, WO2014/199299, WO2015/044890, WO2015/118507, WO2015/121793, WO2016/174624, WO2017/046084, WO2017/064642, EP3176177, WO2017/093931, WO2017/093934, WO2017/093935, WO2017/093936, EP16169912.9, EP16196818.5, EP16201747.9, EP16201749.5, EP17155193.0, WO 2019/035001, WO 2019/097454, WO 2019/106027, WO 2019/106035, EP18202519.7, EP18202530.4, EP18202534.6, EP18202537.9, EP18202538.7, EP18202508.0, EP19153622.6, EP19153623.4 EP19153626.7, EP19153627.5

UNDERSTANDING AND RESTORING VISION

Botond Roska[1,2]

Vision is of key importance for humans and diseases leading to loss of vision have a major effect on day-to-day life. The process of vision starts in the retina, where images captured by photoreceptors are processed by retinal circuits built from more than hundred cell types. Information from the retina flows via the thalamus to a number of cortical areas. Here I summarize the results of work in my laboratory that is focused on a basic understanding of visual processing in the retina, thalamus and visual cortex at the level of cell types and circuits, and the use of this information to understand disease mechanisms and to develop correcting therapies.

The effects of loss of vision on day-to-day life are ranked higher than loss of memory or loss of a limb[1]. Blindness is ranked as the worst human condition, ahead of Alzheimer disease or cancer. This emphasis on vision diseases is a reflection of modern living, where most forms of information exchange involve the use of visual devices. Given that the proportion of people with visual impairment or blindness is growing exponentially with the increased mean age of the population[2], diseases of vision will continue to be a major problem for society.

Vision starts in the retina, where the visual scene is captured by an array of photoreceptors. Information from the retina flows to a number of subcortical areas, of which the lateral geniculate nucleus is the most important in the formation of a conscious percept. Visual information from the lateral geniculate nucleus reaches the primary visual cortex and then spreads through many higher cortical areas. Although about 30% of the human cortex is devoted to vision[3], and a very large number of cortical neurons are involved, most blinding diseases originate in the retina.

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The retina of vertebrates has a conserved architecture[4] of five major neuronal cell classes organized in three nuclear and two synaptic layers (Figure 1). The neuronal cell classes include excitatory cells, namely photoreceptors, bipolar cells, and ganglion cells. These cell classes are interconnected in an excitatory chain that ends with ganglion cells, which are the output neurons of the retina. There are also two classes of inhibitory cells. Horizontal cells receive and feedback input from photoreceptors and also feed forward to bipolar cells. Amacrine cells receive and feedback input from bipolar cells and also feed forward to ganglion cells. Each of these cell classes is further divided into cell types formed from morphologically and physiologically similar neurons. It is believed that vertebrate retinas contain more than 100 cell types[5] organized in ~30 circuit modules[6]. Each circuit module is made up of one of ~30 ganglion cell types connected to a few types of bipolar and amacrine cells, together with the cell types that connect the bipolar and amacrine types, including rods, cones and horizontal cells. Repetitions of each circuit module cover the retinal surface, thus forming a circuit mosaic that is the elementary unit of retina function. Images arriving via the photoreceptors are

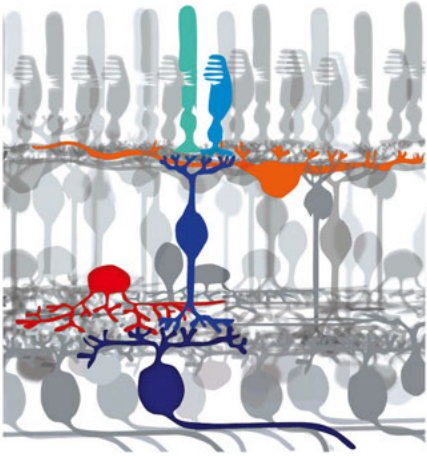

Figure 1. Neuronal cell classes of the vertebrate retina. Shades of blue: rod and cone photoreceptor (top), bipolar cell (middle), ganglion cell (bottom). Orange: horizontal cell. Red: Amacrine cell.

processed by each circuit mosaic according to the connectivity and dynamics of its cell types. This results in a distinct neuronal representation of the image (retinal movie) at its ganglion cell mosaic, which is broadcast to higher brain centers by the ganglion cell axons that form the optic nerve[4]. Since there are ~30 different circuit mosaics, the vertebrate retina forms ~30 different retinal movies that present different features of the visual scene to higher brain centers (Figure 2).

Cell types, circuits and computations

The cell type concept has become a cornerstone of our understanding of visual function. We were intrigued to learn how cell types and circuits of the visual system extract features from the visual scene and started our investigations by creating an atlas of cell-type transcriptomes in the mouse retina[7]. We found that each adult retinal cell type expresses a specific set of genes, including a unique set of transcription factors. We have used this atlas to manipulate retinal cell types using mouse genetics or viruses and to explore the logic by which visual circuits extract information from the visual scene.

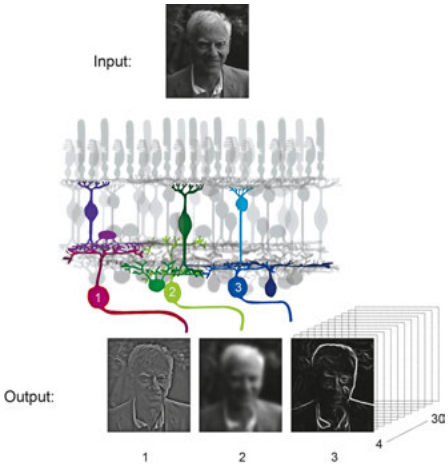

Figure 2. Retinal cell types are organized into ~30 circuit mosaics, each of which receives input from the mosaic of photoreceptors and ends with a mosaic of ganglion cells of a given type that forms the retinal output.

Multifunctional cell types in the retina

Our first insight was that retinal cell types and circuits are multifunctional and can therefore perform radically different functions according to the visual input, or even when the visual input remains the same. We have reported several examples of such circuits.

The first was a retinal circuit that specializes in detecting approaching objects, such as looming predators[8]. Together with Rava Azaredo da Silveira, we identified an approach-sensitive ganglion cell type in the mouse retina (Figure 3), resolved elements of its afferent neural circuit, and described how these confer approach sensitivity to the ganglion cell. The essential building block of the circuit is a rapid inhibitory pathway that selectively suppresses responses to non-approaching objects. This pathway was described previously in the context of night-time vision, but in day-time conditions it conveys signals in the reverse direction. This demonstration of the dual activity of a neural pathway according to varying physiological conditions showed that several functions can be accommodated efficiently in a single circuit.

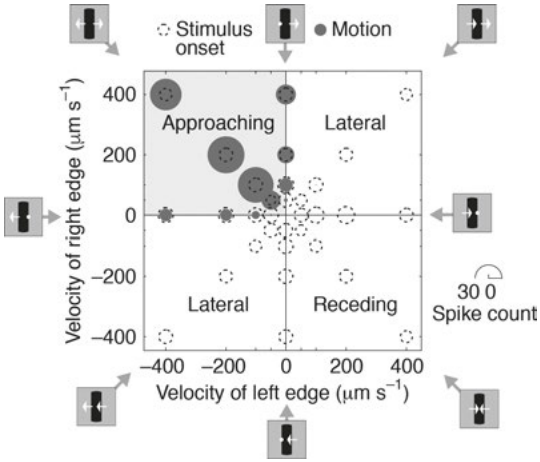

Figure 3. Responses of an approach sensitive neuron. Response is observed at stimulus features which are shown gray. Response amplitude is coded by the diameter of the gray spots.

The second example was a retinal circuit whose function changes with the ambient illumination[9]. By examining a continuous scale of light levels from starlight to daylight, we recorded retinal ganglion cell types that abruptly and reversibly switch the weighting of center and surround interactions in their receptive field around cone threshold (Figure 4). Two-photon-targeted recordings combined with genetic and viral-tracing experiments showed that the circuit element responsible for the switch is a large inhibitory neuron that acts directly on ganglion cells. We found that weak excitatory input via electrical synapses, together with the spiking threshold in inhibitory cells, act as a switch. Thus, circuits in the retina can quickly and reversibly switch between two distinct states, implementing distinct perceptual regimes at different light levels. The work also revealed a switch-like component in the spatial integration properties of human vision at cone threshold.

We discovered in a third example that rods that act as photoreceptors in nightlight, switch their function in daylight[10]. Vision of vertebrates relies on both types of photoreceptors, the rods and the cones, that signal increments in light intensity with graded hyperpolarizations. Rods are

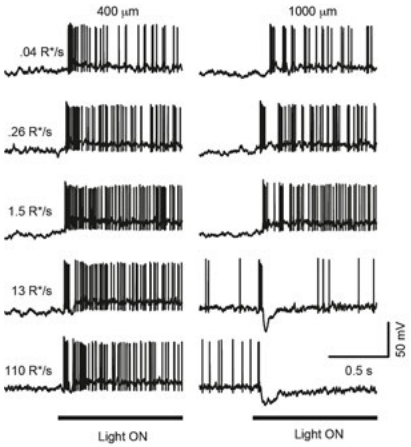

Figure 4. A switch cell. Responses of the cell to a small (left) and a large (right) spot at different intensities (rows). Note the sudden change in the response to a large spot at 13 R/s light intensity.

operational at lower light intensities and cones at brighter intensities. The receptive fields of both photoreceptors exhibit antagonistic center-surround organization. We showed in mice that rods at bright-light levels act as relay cells for cone-driven surround inhibition. Thus, when rods are not directly sensing light, they join the cone circuit.

Circuits can have different functions even when the visual input is the same[11]. In this fourth example, we chemogenetically perturbed horizontal cells, which are an interneuron type providing feedback at the first visual synapse while monitoring light-driven spiking activity in thousands of ganglion cells, namely the retinal output neurons. We discovered six reversible perturbation-induced effects in the response dynamics and ranges of ganglion cells (Figure 5). We assigned specific functions to horizontal cells with respect to different ganglion cell types using a computational model of the retinal circuitry that reproduced all perturbation-induced effects. This combination of experimental and theoretical work showed how a single interneuron type differentially determines the dynamical properties of distinct output channels of the retina.

Computations from retina to cortex

Our second insight was the combination of visual information from several visual channels by cells of the lateral geniculate nucleus and primary visual cortex and the resulting computation of new visual features.

First, we determined the different modes of visual integration in the lateral geniculate nucleus[12]. The thalamus receives sensory input from different circuits in the periphery, but it was not known how these sensory channels are integrated at the level of single thalamic cells. We performed targeted single-cell-initiated transsynaptic tracing to label the retinal ganglion cells (Figure 6) that provide input to individual principal cells in the mouse lateral geniculate nucleus (LGN). We identified three modes of sensory integration by single LGN cells. In the first, a few cells of mostly the same type converged from one eye, indicating a relay mode. In the second, many ganglion cells of different types converged from one eye, revealing a combination mode. In the third, many ganglion cells converged from both eyes, revealing a binocular combination mode in which functionally specialized ipsilateral inputs joined broadly distrib-

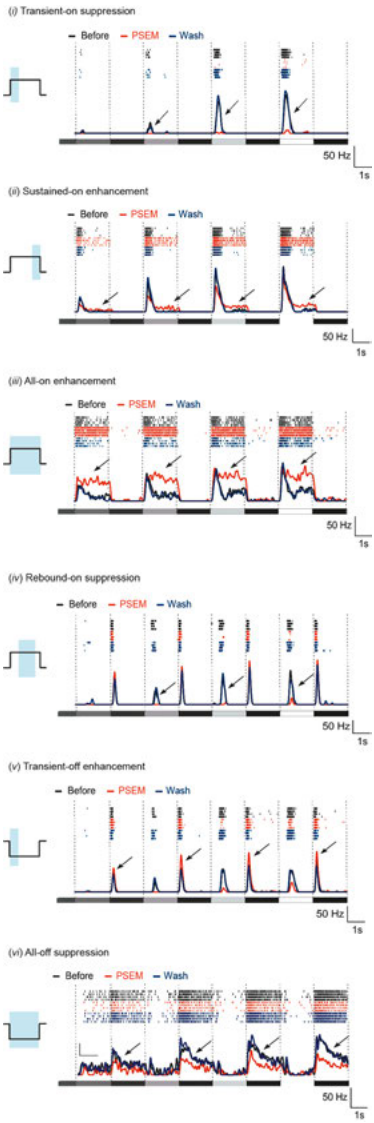

Figure 5. Six reversible horizontal perturbation-induced (PSEM) effects at different ganglion cells.

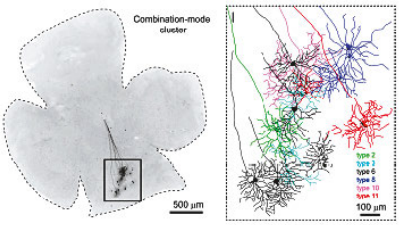

Figure 6. Retinal ganglion cells giving input to a single LGN cell.

uted contralateral inputs. Thus, the LGN is involved in at least three modes of visual input integration, each exhibiting a different degree of specialization.

Second, we provided causal evidence for retina-dependent and -independent visual motion computations in primary visual cortex[13]. How neuronal computations in the sensory periphery contribute to computations in the cortex was not well understood. We examined this question in the con-

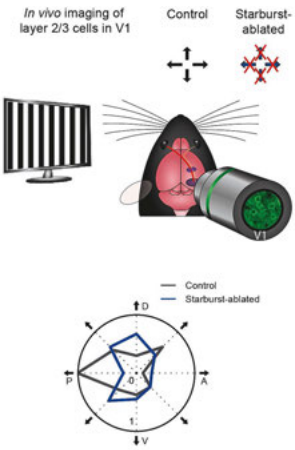

Figure 7. Imaging responses in the primary visual cortex of mice with and without starburst cells (top). The distribution of preferred directions changes after starburst cell ablation.

text of visual motion processing in the retina and primary visual cortex. We genetically disrupted retinal direction selectivity, either along only the horizontal axis using FRMD7 mutant mice or along both cardinal axes using starburst cell-ablated mice, and monitored neuronal activity in layer $^{2}/_{3}$ of primary visual cortex during visual motion. In control mice, we found a strong direction bias for posterior visual motion, which occurs naturally when the animal moves forward. In mice with disrupted retinal direction selectivity, the proportion of posterior motion-preferring cells decreased significantly (Figure 7) and their speed tuning changed. Thus, functionally distinct, retinal direction selectivity-dependent and -independent computation of visual motion occurs in the cortex.

Third, we discovered in mice that the visual cortex is organized in layer-specific cortical network modules[14]. Used single-cell-initiated, monosynaptically restricted retrograde transsynaptic tracing with rabies viruses expressing GCaMP6s (Figure 8), we imaged the visual motion-evoked activity of individual layer $^{2}/_{3}$ pyramidal neurons and their presynaptic networks across layers in mouse primary visual cortex. The neurons within each layer showed similar motion direction preferences and formed layer-specific functional modules. The layer modules in one-third of the networks were locked to the direction preference of the postsynaptic

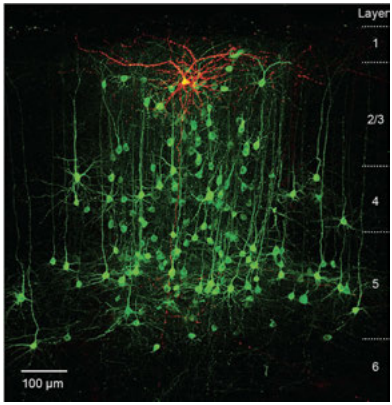

Figure 8. Single-cell-initiated functionalized transsynaptic tracing from a layer $^{2}/_{3}$ cell (red) in the primary visual cortex. The presynaptic cells express GCaMP6s (green).

neuron. For other networks the direction preference varied by layer. Thus, this work identified both feature-locked and feature-variant cortical networks.

A cell-type-specific disease mechanism

To identify the relevant cell types and circuits of genetic diseases of vision with unknown pathology, we designed an experimental logic by first focusing on the function of a specific retinal circuit and then proceeding to study how cell types participate in a given computation. In this way we revealed the gene expression patterns of the relevant cell types and could link cell type-specific genes to human monogenic diseases. Finally, we associated the symptoms of human diseases with the identified cell types and circuits.

Such an approach led to the identification of the circuit mechanism in the common human neurodevelopmental disease, FRMD7 gene-associated congenital nystagmus. We linked the key symptom of loss of the optokinetic response to a single retinal cell type (starburst cells) and a retinal computation, i.e., the computation of motion direction[15].

Following the experimental logic outlined above, we developed further insight into the circuit mechanism of this disease, investigating first the development of the circuit involved in the computation of direction selectivity[16]. We followed the spatial distribution of synaptic strengths between starburst and direction selective ganglion cells (Figure 9) during early postnatal development, before these neurons can respond to a light stimulus. We showed that an asymmetry develops rapidly over a 2-day period through an intermediate state in which random or symmetric synaptic connections are established. This involved the spatially selective reorganization of inhibitory synaptic inputs. These results pointed to a rapid developmental switch from a symmetric to an asymmetric input distribution for inhibition in the neural circuit of a principal cell.

It was not known at which circuit location along the flow of visual information direction selectivity first appears. To obtain insights into the mechanism of direction selectivity, we recorded the concerted activity of the neuronal circuit elements of single direction selective retinal ganglion

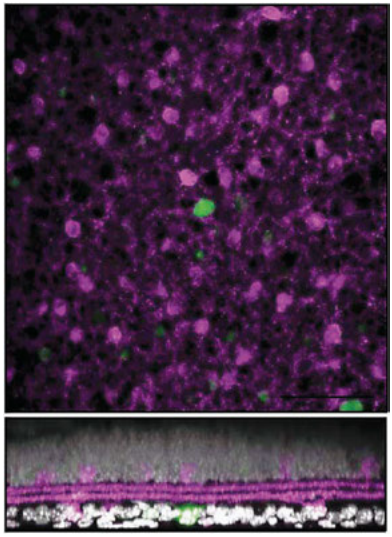

Figure 9. Mapping of connectivity strength between starburst cells expressing an optogenetic sensor (magenta) and a single direction selective ganglion cell expressing GFP (green). Top (top) and side (bottom) view of the retina.

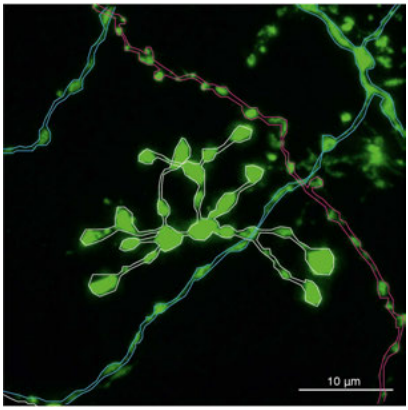

Figure 10. Transsynaptic tracing of the neuronal circuit of a single direction selective ganglion cell in the retina. Processes of bipolar cells (white outline), starburst cells (red outline) and the ganglion cell (blue outline).

cells at subcellular resolution by combining GCaMP3-functionalized transsynaptic viral tracing (Figure 10) and two-photon imaging. While the visually evoked activity of the dendritic segments of the direction selective ganglion cells were direction selective, direction selective activity was absent in the axon terminals of bipolar cells. Furthermore, the glutamate input to direction selective ganglion cells, recorded using a genetically encoded glutamate sensor, also lacked direction selectivity. Thus, we learned that the first stage at which extraction of motion direction occurs is the dendrites of direction selective ganglion cells.

Using the gene expression atlas developed in our lab, we then found that FRMD7 is specifically expressed in starburst cells[15]. We showed that mutation of FRMD7, a gene that is defective in human congenital nystagmus, leads to selective loss of the horizontal optokinetic reflex in mice as well as in humans. Together with our collaborator Andreas Hierlemann, we found that this is accompanied by selective loss of horizontal direction selectivity in retinal ganglion cells (Figure 11) and the transition from asymmetric to symmetric inhibitory input to horizontal direction selective ganglion cells. This work identified FRMD7 as a key regulator in the development of neuronal circuit asymmetry and suggested the involvement of a specific inhibitory neuron type in the pathophysiology of a neurological disease.

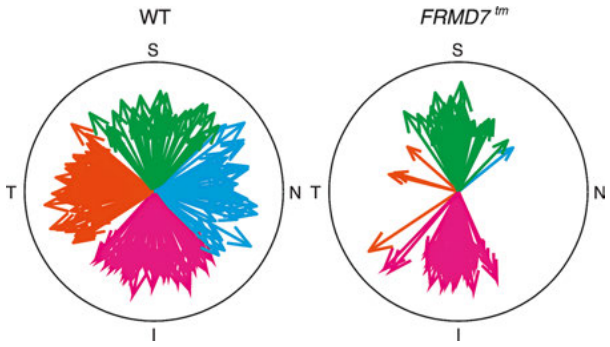

Figure 11. Preferred directions of direction selective cells in wild type (left) and FRMD7 mutant (right) mice.

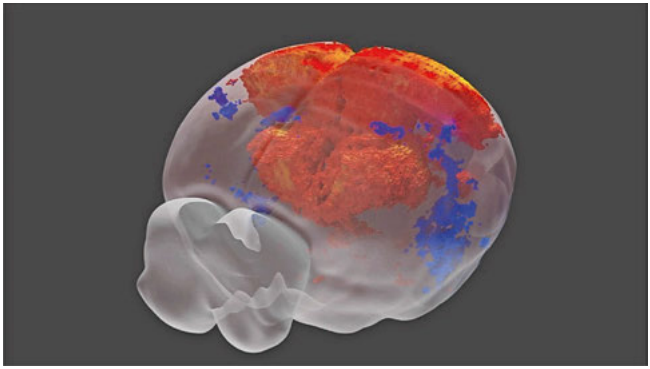

Figure 12. Whole brain functional ultrasound imaging during visual stimulation in mice. Activated regions are red, inhibited regions are blue.

Finally, we developed functional ultrasound imaging to record whole-brain activity in behaving mice at a resolution of ~100 um (Figure 12) and compared activity in healthy and FRMD7 mutant mice[17]. In healthy mice, we detected 87 active brain regions during visual stimulation that evoked the optokinetic reflex. Using FRMD7 mutant mice, we identified a subset of regions whose activity was reflex-dependent. Our work identified the brain regions affected in a mouse model of congenital nystagmus and provided an experimental approach to monitor whole-brain activity of mice in normal and disease states.

Cell type-targeted repair

We used our understanding of the activity, connectivity and gene expression pattern of retinal cell types to design cell type-targeted optogenetic therapies for blinding diseases. We concentrated on a group of genetic diseases, namely retinitis pigmentosa, that affect two million people worldwide and lead to incurable blindness through loss of the light sensitivity of photoreceptors. We showed proof of principle for making key retina cell types light sensitive using cell type-targeted optogenetic approaches[18,19]. We restored visual function first in animal models of retinitis pigmentosa. With our collaborators Jose-Alain Sahel and Serge Picaud, we then provided proof of concept for visual restoration in human

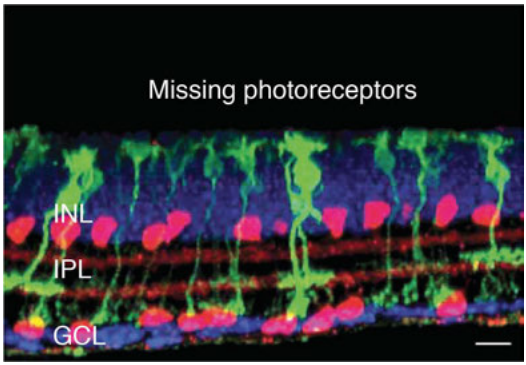

Figure 13. Degenerated retina of rd1 mice expressing ChR2 in ON bipolar cells (green). Starburst cells are labeled red and cell bodies are labeled blue.

retinas ex vivo and identified blind patients who could benefit from the potential therapy. We are currently working on translation of the therapy to patients: a first phase clinical trial run by Gensight Biologics Inc. is ongoing.

First, we genetically targeted a light-activated cation channel, channelrhodopsin-2, to second-order neurons, i.e., ON bipolar cells, of degenerated retinas in vivo in the rd1 mouse model (Figure 13). In the absence of 'classical' photoreceptors, we found that ON bipolar cells that were engineered to be photosensitive induced light-evoked spiking activity in ganglion cells. The rescue of light sensitivity was selective to the ON circuits that would naturally respond to increases in brightness. Despite degeneration of the outer retina, our intervention restored transient responses and center-surround organization of ganglion cells. The resulting signals were relayed to the visual cortex and were sufficient for the animals to successfully perform optomotor behavioral tasks.

Second, rod photoreceptors die early in retinitis pigmentosa, whereas light-insensitive, morphologically altered cone photoreceptors persist longer. It was not known whether these 'dormant' cones are accessible for therapeutic intervention. We showed that expression of archaebacterial halorhodopsin in light-insensitive cones (Figure 14) could substitute for the native phototransduction cascade and restore their light sensitiv-

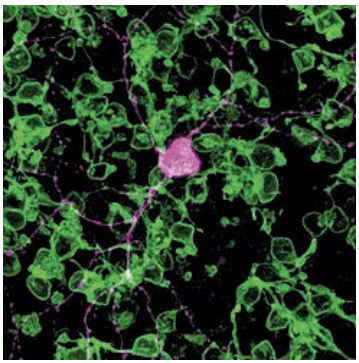

Figure 14. Dormant, light insensitive cones in rd1 mice are reactivated using halorhodopsin (green). A recorded ganglion cell is labeled in magenta.

ity in mouse models of retinitis pigmentosa. Resensitized photoreceptors activated all retinal cone pathways, drove sophisticated retinal circuit functions including directional selectivity, activated cortical circuits, and mediated visually guided behaviors. Using human ex vivo retinas, we showed that halorhodopsin can reactivate light-insensitive human photoreceptors. Finally, we identified blind patients with persisting, light-insensitive cones for potential halorhodopsin-based therapy.

A different therapeutic approach to photoreceptor-based blindness is to prevent loss of photosensitivity. Together with Witold Filipowicz, we identified a key microRNA-based pathway involved in the maintenance of the outer segment of photoreceptors[20]. The outer segments of cones serve as light detectors for daylight color vision, and their dysfunction leads to human blindness conditions. We showed that the cone-specific disruption of DGCR8 in adult mice led to the loss of miRNAs and the loss of outer segments, resulting in photoreceptors with significantly reduced light responses. However, the number of cones remained unchanged. Outer segments were lost gradually over one month, during which time the genetic signature of cones decreased. Re-expression of the sensory-cell-specific miR-182 and miR-183 prevented outer segment loss. These miRNAs were also necessary and sufficient for the formation of inner segments, connecting cilia and short outer segments, as well as

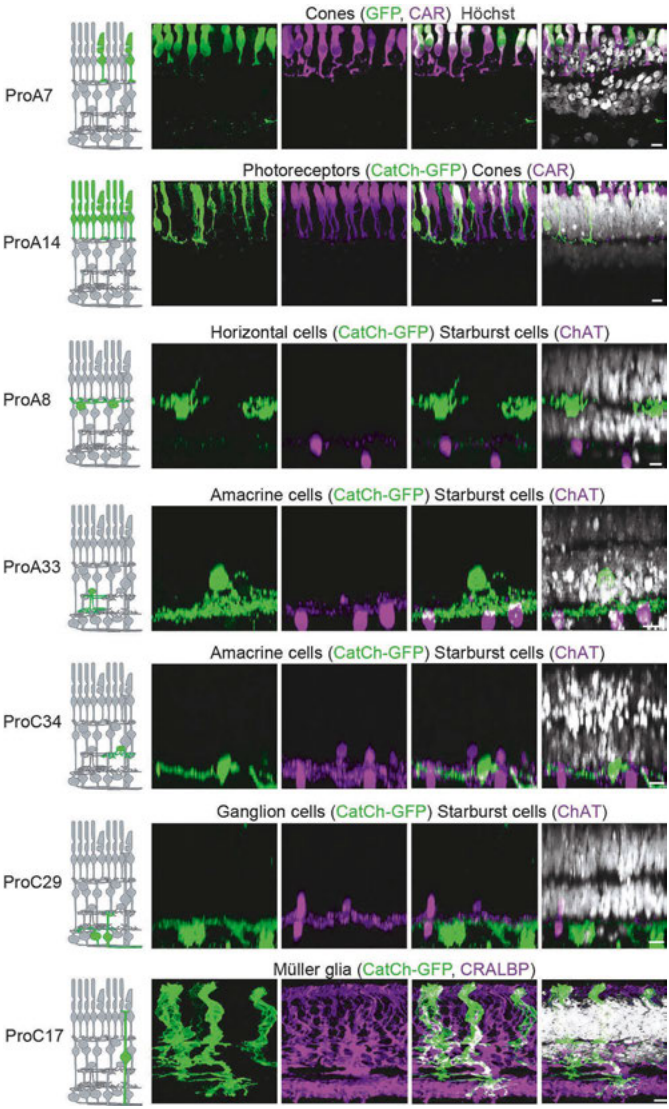

Figure 15. AAVs expressing ChR2-GFP in different cell types of the human retina by different promoters.

light responses in stem-cell-derived retinal cultures. Thus miR-182- and miR-183-regulated pathways are necessary for cone outer segment maintenance in vivo and functional outer segment formation in vitro. It may be possible to manipulate these molecules and prevent loss of photosensitivity in retinitis pigmentosa.

Gene therapy has been held back by the lack of cell type-specific gene delivery vectors. Although Adeno-associated viral vectors (AAVs) are frequently used for gene delivery, targeting expression to specific cell types has been a challenge. We created a library of 230 AAVs, each with a different synthetic promoter designed using four independent strategies[21]. We found that ~11% of these AAVs specifically target expression to neuronal and glial cell types in the mouse retina, the mouse brain, the non-human primate retina in vivo, and in the human retina in vitro (Figure 15). We demonstrated applications for recording, stimulation, and molecular characterization, as well as the intersectional and combinatorial labeling of cell types. These resources and approaches will allow economic, fast, and efficient cell-type targeting in a variety of species, for basic science and for gene therapy.

An additional limitation of current gene therapy approaches is that viral vectors can no longer be controlled after injection into the body. We have begun to address this problem together with our collaborator Daniel Mueller. We have shown that viruses bound to magnetic nanoparticles can be remote controlled in the brain using a magnetic field in a way that leads to infection (Figure 16); we named the process 'virus stamping'[22].

Summary

Traditionally, studies of neuronal circuits and of visual diseases have been pursued separately. Recently, the concept of cell types has brought the two fields together. Cell types are the basic building blocks of neuronal circuits and technologies to deliver genes to cell types are now core components of neuronal circuit studies. Since most retinal diseases are cell-type specific, the notion of cell types and cell type-targeted gene delivery are also at the center of research on disease mechanisms and therapy. As both fields mature, the concepts and tools developed in basic circuit science will help in the development of therapy and, conversely, the con-

cepts and tools developed for therapy will open the door to a more sophisticated understanding of the structure and function of neuronal circuits.

Acknowledgements

I am grateful to Novartis for their extraordinary generous support of my research activities both while I worked at the Friedrich Miescher Institute (FMI) and now that I work at the Institute of Molecular and Clinical Ophthalmology Basel (IOB). I am equally grateful to the University of Basel and the University Hospital of Basel, as the other founders of IOB, and also to the city of Basel for their strong support of IOB. I thank Hendrik Scholl, co-director of IOB as well as Norbert Spirig, the director of operations at IOB, for the exciting journey together to build IOB. I thank my mentors, the members of my lab and my collaborators for their contributions to the work described here. I also thank my colleagues for discussions that shaped my thoughts, at IOB and FMI. Finally, I thank all the grant agencies, foundations and personal donors who supported our work. Thank you also to Elsa Sigle and Patrick King for commenting on the manuscript.

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