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FACULTY OF MEDICINE

Molecular Genetic Studies into the Development, Function, Pathology and Repair of Retinal Ganglion Cell Types

HABILITATION THESIS

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A

Abstract

I am presenting in this habilitation thesis a summary of my scientific investigations and my professional and academic progression since obtaining my PhD at Johns Hopkins University School of Medicine. I also outline some of my future projects as part of establishing a molecular genetics and neuroscience research program at Transilvania University.

My career progression (section B.III.1) is somewhat unusual. I obtained an M.D. from Iuliu Hatieganu University in Cluj, then pursued a pathology fellowship at University of Maryland. I then switched careers towards basic science, and obtained a Master of Arts in Biological sciences from Columbia University in New York, and Ph.D. in Biochemistry, Cell and Molecular Biology from Johns Hopkins. I then completed my postdoctoral fellowship in Molecular Biology and Genetics and Johns Hopkins School of Medicine. I then joined the USA National Institutes of Health in Bethesda M.D. as an investigator of the National Eye Institute and lead a group of undergraduates, Ph.D. and postdoctoral students for 11 years. I recently joined the Research and Development Institute at Transilvania University of Brasov in the Faculty of Medicine, where I am in the process of establishing a research group focused on molecular genetics and neuroscience.

My research resulted in 59 peer reviewed manuscripts, all published in WOS/ISI indexed journals, with a total of 3109 citations and an H-index of 27 (WOS, 28 December 2021). Of these, 48 papers were published since my graduation, including some in Nature, Cell, Neuron, PNAS and other high impact prestigious journals. Several manuscripts are under review/in press or deposited on BioRxiv. I am an editor for PLOSOne and Frontiers in Neuroscience, have reviewed manuscripts for more 20 WOS/ISI indexed journals, and served as grant reviewer for many funding bodies in Europe and the USA. I have mentored 23 students at Postdoctoral, PhD, M.Sc. and post-baccalaureate level, and served on many committees for recruitment, tenure or resource management at the NIH or elsewhere.

I am first briefly discussing the current state of the art in the field of neuronal cell type studies (section B.I), by reviewing anatomic, physiological, molecular and functional criteria for cell type definitions, and the lack of comprehensive surveys that combine all these criteria into a unitary concept. My major focus for the last 16 years have been Retinal Ganglion Cells (RGCs), the cells that carry visual information from the eye to the brain. Using RGCs as a test case, I illustrate how neuronal cell types can be defined and how their function within the system and their development can be studied. I then briefly describe the transcriptional mechanisms regulating RGC type formation, with a particular focus on Brn3/Pou4f transcription factor family, the major focus of my research in the last 15 years.

Section B.II lays out my contributions to these fields. Subchapter B.II.1.1 briefly describes my doctoral work on sparse random recombination as a tool to study neuronal cell types. This is necessary, as my postdoctoral work and the work of the laboratory I have headed at the NEI is based methodologically and conceptually on some of the tools and concepts I have pioneered

during my PhD. Sections B.II.2 through B.II.8 describe my major contributions organized by topic and/or methodology.

A significant aspect of my work has consisted in developing new genetic strategies for cell and gene manipulation (B.II.1), by generating conditional knock-in alleles, and Cre recombinase drivers. Through the intersection of these genetically modified mouse lines, specific cell types of interest can be labelled and/or manipulated. More recently we have also employed a second recombinase, Dre, in our genetic manipulations. Most of these genetic manipulations have helped me and my collaborators understand how transcription factors control RGC type specification. We have discovered cell autonomous mechanisms, transcriptional combinatorial codes, and interactions with neurotrophic signals (B.II.2). As a consequence, I have addressed potential molecular mechanisms for RGC type specification, by analyzing, in our group or through collaborations the transcriptional targets of Brn3 transcription factors in RGC type specification (B.II.2 and B.II.3). One particularly productive direction has been the study of ipRGCs (B.II.4), a specific class of RGCs that are intrinsically responsive to light by virtue of expressing the photopigment Opn4/Melanopsin. Using genetic manipulations employing some of the lines I have developed, I collaborated with colleagues at Hopkins, U. of Maryland and NIH and helped discover that ipRGCs can be subdivided in two subpopulations, responsible for circadian photo-entrainment and pupillary light reflex, respectively.

Similar transcriptional and signaling cascades regulate the development of multiple classes of projection sensory neurons (e.g. RGC, dorsal root ganglion and trigeminal ganglion somatosensory neurons, auditory and vestibular ganglion neurons). Thus, using our conditional knock-in alleles, we were able to achieve significant progress in the classification and anatomic description of these classes of neurons (B.II.5).

In order to analyze the consequences of our genetic manipulations on RGC electrophysiology and mouse visual function, we led a vigurous program of technique development, by building ex vivo and in vivo functional analysis apparatus and software (B.II.6). We were therefore in position to make significant contribution to the characterization of a series of retinal developmental disorders, genetic defects, and disease models.

My initial background and education in biomedical research was centered on immunopathological mechanisms of autoimmune or degenerative disorders. More specifically, I began my research career in the USA as a Pathology fellow, studying the effects of complement on cell signaling and transcription in somatic cells, in University of Maryland, under the guidance of Horea Rus and Florin Niculescu. These studies, carried out before my PhD (B.II.7.1), resulted in the cloning and functional characterization of RGC-32 (more recently renamed to Rgcc), a gene involved in cell cycle regulation and phenotypic changes of a variety of somatic cells and immune system components. Subsequently, during my PhD, Postdoctoral and Independent Investigator years, I continued my collaboration with the Rus lab, and, together we generated a RGC-32 knockout mouse, which we employed to demonstrate RGC-32 involvement in several animal models for autoimmune disease and tissue fibrosis (Section B.II.7). I am particularly interested in the role of RGC-32 in pathology, since recent work from our group and others discovered the involvement of RGC-32 in epithelial-mesenchimal transition, and phenotypic

programming of olygodendrocytes and astrocytes as a result of inflammatory cues. These changes occur in several neurological disorders including some that are important in visual pathology (e.g. Glaucoma, Multiple Sclerosis or Age-related Macular Degeneration).

Looking forward, I plan to develop a molecular genetics and neuroscience center at Transilvania University, progress to Senior Scientist I (Cercetator Stiintific I) or Professor, and develop a multidisciplinary research program involving students at all levels, from undergraduate to postdoctoral fellow. I hope to do this by interacting with a very diverse range of experts at Transilvania University, in other research centers in Romania and internationally. My research agenda will continue to be focused on RGCs, neuronal cell type development, and comparative studies between mouse and human systems. We will use our animal models to investigate pathogenetic mechanisms in visual system disorders, and explore vision restoration strategies based on bio-electronic interfaces, gene therapy or cell replacement/reprograming. This rich program will hopefully joined by PhD candidates from all connected fields, and will be strongly interdisciplinary.

Rezumat

In aceasta teza de abilitare prezint un sumar al cercetarii mele si progresului meu academic si profesional dupa obtinerea titlului de Doctor (Ph.D.) la Johns Hopkins University School of Medicine. De asemenea, prezint cateva dintre proiectele mele de viitor, legate de infintarea unui program de cercetare in genetica moleculara si neurostiinte la Universitatea Transilvania.

Traiectoria mea academica (sectiunea B.III.1 din teza) este oarecum neobisnuita. Am obtinut licenta in medicina (M.D.) de la Universitatea de Medicina si Farmacie Iuliu Hatieganu din Cluj, dupa care am urmat un stagiu de cercetare in patologie (fellowship) la Universitatea Maryland. Apoi am schimbat orientarea catre stiinte fundamentale, urmand un program de masterat (Master of Arts) in stiinte biologice la Columbia University in New York, urmat de programul de doctorat (Ph.D.) in Biochimie si Biologie Celulara si Moleculara de la Johns Hopkins University School of Medicine.

Din toamna lui 2010 am devenit Cercetator Principal (Principal Investigator) la Institutul National de Sanatate al SUA (National Institutes of Health - NIH) in Bethesda, Maryland, si am condus un grup de cercetare al Institutului National al ochiului (NEI) constand din studenti de colegiu, doctoranzi si cercetatori postdoctorali timp de 11 ani. In toamna lui 2021 m-am alaturat centrului de Cercetare si Dezvoltare al Universitatii Transilvania din Brasov, in cadrul Facultatii de Medicina, unde am initiat un grup de cercetatori dedicat geneticii moleculare si neurostiintelor.

Cercetarile mele sunt publicate in 59 de lucrari evaluate "inter pares", toate in jurnale indexate WOS/ISI, citate de 3109 ori cu un indice Hirsch de 27 (WOS, Decembrie 28 2021). Din acestea, 48 de lucrari au fost publicate dupa obtinerea titlului de doctor, incluzand publicatii in Nature, Cell, Neuron, PNAS, si alte jurnale prestigioase de mare impact. Cateva articole sunt sub recenzie, acceptate si pe cale de a fi publicate sau depositate in BioRxiv. Sunt membru in comitetul editorial la PlosOne si Frontiers in Neuroscience, am participat ca recenzent de lucrari pentru peste 20 de reviste stiintifice indexate WOS/ISI, si am participat ca recenzent de propuneri de proiect pentru multe organizatii de finantare a cercetarii in SUA si Europa. Am fost mentorul a 23 de studenti postdoctorali, doctoranzi, masteranzi si studenti in programe post-baccalaureat, si am functionat ca membru in multe comitete de recrutare, promovare sau management la NIH si pentru alte institutii academice.

In teza voi discuta pe scurt stadiul de dezvoltare si cunoastere al studiului tipurilor celulare neuronale (sectiunea B.I), si voi rezuma criteriile anatomice, fiziologice, moleculare si functionale pentru definitia tipurilor celulare, scotand in evidenta lipsa studiilor cuprinzatoare care sa combine toate aceste criterii intr-un concept unitar. In ultimii 16 ani, studiile mele au fost concentrate in majoritate pe celulele retinale ganglionare (RGC), neuronii care transmit informatia vizuala de la ochi la creier. Folosind RGC ca si exemplu ilustrez cum pot fi definite tipurile neuronale si cum pot fi studiate functia lor in sistemul vizual si dezvoltarea lor embriologica. Apoi prezint mecanismele transcriptionale care regleaza formarea tipurilor de RGC prezentand detaliat familia de factori de transcriptie Brn3/Pou4f, subiectul principal al studiilor mele in ultimii 15 ani.

In sectiunea B.II prezint contributiile mele la aceste domenii. Capitolul B.II.1.1 descrie studiile mele doctorale asupra tehnicilor de recombinare aleatorie rara folosite in studiul tipurilor celulare neuronale. Aceasta revizie a muncii doctorale este necesara, pentru ca cercetarile mele din perioada de postdoctorat si ca leader de grup la NEI sunt bazate metodologic si conceptual pe instrumentele si temele pe care le-am initiat in timpul doctoratului. Sectiunile B.II.2 pana la B.II.8 descriu principalele mele contributii organizate dupa subiect si/sau metodologia utilizata.

O parte semnificativa din munca mea a constat din elaborarea de noi strategii pentru manipulari genetice si/sau celulare (B.II.1) prin generarea de alele knock-in conditionale in paralel cu alele sintetice care exprima recombinaza Cre. Prin intersectia acestor linii de animale modificate genetic, anumite tipuri celulare de interes pot fi marcate si/sau manipulate. In studii mai recente am inclus si o a doua recombinaza (Dre) in manipularile noastre genetice. Majoritatea acestor manipulari genetice mi-au folosit mie si colaboratorilor mei la intelegerea modului in care factorii de transcriptie controleaza specificarea tipurilor de RGC. Am descoperit mecanisme celulare autonome, coduri combinatorice transcriptionale si interactiuni cu factori neurotrofici (B.II.2). In consecinta, am studiat potentiale mecanisme moleculare pentru specificare tipurilor RGC prin analiza tintelor transcriptionale a factorilor Brn3 in procesul de specificare al RGC, atat in grupul nostru cat si prin colaborari externe (B.II.2 si B.II.3). O directie de studiu extrem de productiva a fost analiza ipRGC (B.II.4), o clasa speciala de RGC care au sensibilitate intrinseca la lumina prin expresia fotopigmentului Opn4/Melanopsina. Folosind manipulari genetice bazate pe linii de animale pe care le-am dezvoltat, am colaborat cu colegi la Hopkins, U. of Maryland, si NIH, participand la subclasificarea ipRGCs in doua populatii, raspunzatoare, respectiv, pentru ritmul circadian si constrictia pupilara.

Cascade transcriptionale si de signaling inrudite regleaza dezvoltarea a mai multe clase de neuroni senzoriali de proiectie (incluzand RGC, celulele somatosenzoriale din ganglionul spinal si trigeminal, si neuronii ganglionilor vestibulari si auditor). De aceea, folosind alele noastre cu knock-in conditional, am putut sa facem progrese semnificative in descrierea anatomica si clasificarea acestor clase de neuroni (B.II.5).

Pentru a analiza consecintele manipularilor noastre genetice asupra electrofiziologiei RGC si a functiei vizuale la soricei, am desfasurat un program viguros de dezvoltare de tehnici, construind aparatura si software pentru analize functionale ex vivo si in vivo (B.II.6). Acestea ne-au dat posibilitatea sa participam la caracterizarea unei intregi serii de defecte retinale congenitale, boli genetice si modele de boala.

Expertiza si educatia mea initiala a fost centrata pe mecanizmele imunopatologice ale bolilor autoimune sau degenerative. In mod mai specific, mi-am initiat cariera de cercetator in SUA ca cercetator postdoctoral in patologie, studiind efectele complementului asupra semnalizarii celulare si transcriptiei in celule somatice, la Universitatea Maryland, sub indrumarea lui Horea Rus si Florin Niculescu. Acese studii, desfasurate inaintea doctoratului (B.II.7.1) au culminat in clonarea si caracterizarea functionala a RGC-32 (redenumit mai nou Rgcc), o gena implicata in controlul ciclului celular si modificarilor fenotipice suferite de o varietate de celule somatice si imunitare. Ulterior, in timpul studiilor mele de PhD, Postdoctorale si de Investigator Principal, am continuat colaborarea cu laboratorul domnului profesor Rus, si,

impreuna, am generat soarecele Knock-out de RGC-32, pe care l-am folosit pentru a demonstra implicarea RGC-32 in cateva modele pe animal pentru boli autoimune si fibroza (Sectiunea B.II.7). Sunt interesat in mod special de rolul RGC-32 in patologie, deoarece date noi din grupul nostru cat si al altora a deomnstrat rolul RGC-32 in tranzitia epitelial-mezenchimala, si reprogramarea femotipica a oligodendrocitelor si astrocitelor in urma stimulilor inflamatorii. Aceste schimbari apar in cateva boli neurologice, incluzand unele cu determinare in sistemul vizual (Glaucom, scleroza multipla sau degenerenerescenta maculara legata de varsta).

Pentru viitor mi-am propus sa dezvolt un centru de cercetare in genetica moleculara si neurostinte la Universitatea Transilvania, sa progresez la pozitia de Cercetator Stiintific I sau Profesor, si sa creez un program de cercetare multidisciplinara implicand studenti la toate nivelurile. Sper sa pot realiza aceste scopuri interactionand cu registrul foarte larg de experti de Universitatea Transilvania, de la alte centre din tara, si internationale. Agenda stiintifica va fi in continuare concentrata pe RGC, dezvoltarea (pre - si postnatala) a tipurilor celulare neuronale, si studii comparative intre rozatoare si oameni. Vom folosi modelele noastre experimentale pentru a investiga mecanismele patogenetice in bolile sistemului vizual si vom explora strategiile de restabilirea vazului bazate pe interfete bio-electronice, terapii genetice, sau terapii bazate pe celule stem sau reprogramare celulara. Acest program foarte divers va fi sustinut de doctoranzi din toate domeniile conectate si va fi puternic interdisciplinar.

B Scientific, professional and academic achievements

B.I Introduction to Molecular Genetics in Visual Neuroscience

B.I.1 Neuronal cell type definition and development – the case of Retinal Ganglion Cells (RGCs)

Understanding the normal function of the nervous system and how it can be affected in various physiological or pathological conditions requires a thorough understanding of its building elements, the neuronal cell types. The definition of a cell type has been initially based on the anatomic descriptions of Santiago Ramon y Cajal, and typically consists of the description of dendrites, axonal trajectory and terminal arborizations (Amthor et al., 1983, 1983, 1989b, 1989a; T. C. Badea & Nathans, 2004, 2011; Bae et al., 2018, 2018; Boycott & Wassle, 1974; Coombs et al., 2006; Dacey et al., 2003; Helmstaedter et al., 2013; Martersteck et al., 2017; Masri et al., 2019; Ramón y Cajal, 1972; Rockhill et al., 2002; Rodieck & Watanabe, 1993; Sun et al., 2002a, 2002b). Anatomic classifications work well when the differences between neurons are large, e.g. axonal arbors projecting to different brain nuclei, distinctions in dendritic arbor area, complexity and lamination within various tissues etc. However, in many cases the morphological differences between different neurons are subtle or aligned along a continuum of features. Under such circumstances anatomic parameters that can efficiently separate neuronal cell types into reliably distinct clusters are harder to find, resulting in “lumping” of multiple cell types into the same cluster or incorrect assignment. Serial electron microscopy reconstructions of increasingly larger volumes of neuronal tissue hold the promise of complete and systematic neuroanatomical descriptions, however in most cases only small to medium size neurons can be captured in their entirety (Bae et al., 2018; Helmstaedter et al., 2013), except for small model organisms such as C. Elegans or D. Melanogaster.

Intracellular or extracellular recordings that allow the collection and classification of various physiological parameters of neurons have constituted a further step forward in neuronal cell type classification, and soon after it was realized that physiological properties are in many cases aligned with anatomical features, resulting in coherent anatomo-physiological neuronal cell type classifications (Goetz et al., 2021; Levick, 1975). However single cell physiology approaches are tedious and subject to a certain stochasticity of sampling resulting in skewed cell classifications or omission of rare cell types. Approaches to collect physiological information from larger defined populations of neurons are multielectrode array (MEA) recordings (Meister et al., 1991, 1994) and imaging of intracellular Ca increases secondary to action potential generation (Ca imaging)(Baden et al., 2016; Feller et al., 1996; Tank et al., 1988; Yuste & Katz, 1991). MEA recordings sample randomly neuronal populations, and generally have a bias towards action potentials derived from large neurons or neurons in close vicinity to the electrode. Ca imaging

has the advantage of collecting activity information from all cell bodies within a certain preparation (surface or relatively flat volumes), but is of course limited by the access of the probe to the tissue, and the slower temporal dynamic of Ca transients compared to neuronal action potentials (see for instance (T. Badea et al., 2001). Another aspect of the complete definition of a neuron are its neurotransmitter profile and synaptic connectivity, on the input and output side. Neurotransmitter profiles and synapses at many pairs of neurons have been described using pharmacological agents and electrophysiology. However we still have little information about exactly what the synaptic partners of each cell type are, how many synaptic inputs are received and how many synaptic outputs are generated with each other neuronal partner.

All aforementioned anatomic, electrophysiological and connectivity features of a given neuron depend on a multitude of molecular mechanisms for proper function and development. These in turn involve the expression of wide array of genes encoding the necessary molecular components, which are in turn regulated at transcriptional level by transcription factors(Hobert, 2011; Jessell, 2000; Komiyama & Luo, 2006; Sanes & Zipursky, 2020). These complex gene expression patterns can be captured by a variety of differential gene expression approaches(Kawai et al., 1993; *Molecular Cloning: A Laboratory Manual (Fourth Edition)*, n.d.; Taub et al., 1983; Velculescu et al., 1995). These approaches have been recently superseded by the deep sequencing of RNA, either at the single cell level or from isolated, purified cell populations(Macosko et al., 2015; Margulies et al., 2005; Schuster, 2008). Using these approaches we can relatively quickly enumerate large numbers of gene products expressed in specific neuronal cell populations. While in some rare instances unique markers for specific cell types have been identified, the overall result of these investigations is that individual cell types can be identified at molecular level only by combinations of molecular markers(Macosko et al., 2015; Peng et al., 2019; Rheaume et al., 2018; Tran et al., 2019; Yan et al., 2020). Although the analysis of functional consequences of gene expression in these cell populations will keep the neuroscience community busy for the foreseeable future, these studies will probably be conducted at cell group (multiple cell types) level. Given this complex combinatorial pattern of gene expression, genetic targeting of individual cell types – defined by unique dendritic and axonal arbor anatomies, synaptic partner sets, electrophysiological properties, and circuit functions – will require combinatorial genetic approaches, combining multiple genetic loci or combinations of genetic and other types of delivery (e.g. viral vectors, electroporation, membrane-crossing peptides, etc.)(Birling et al., 2009; Bischof & Basler, 2008; Grindley et al., 2006; Jefferis & Livet, 2012; Jensen & Dymecki, 2014; Sauer & Henderson, 1988; Turan et al., 2013).

The work I have carried out during my PhD, my postdoc and during my 11 years tenure as a Principal Investigator are focused on designing and applying novel molecular genetic approaches to facilitate the study of individual cell types. I have centered my work on the study of mouse Retinal Ganglion Cells (RGCs). RGCs are the retinal neurons that carry the visual signal to the brain, and are major targets of several prevalent blinding diseases, collectively known as Glaucomas. Additionally, I have participated in unravelling the mechanisms of tissue reaction to inflammation, based on the discovery of a cell cycle regulatory gene that is induced by

inflammatory signals, including Complement and TGFbetta, and may have regenerative properties.

I will first provide an overview of the understanding of RGC type definition and development. I will then break down my scientific and academic achievements by the main directions, and highlight main results, publications and other research output resulting from this work. Academic career path, mentoring and other activities will be described in separate chapters.

B.I.2 Retinal Ganglion Cells are conduits of visual information

In the mammalian retina, the visual information is captured by photoreceptors, which transmit their signals through bipolar neurons to Retinal Ganglion Cells (RGC) that then relay the stimulus to various centers in the brain(Dowling, 2012; Polyak, 1941; Ramón y Cajal, 1972; Rodieck, 1998). Visual information undergoes a first level of computation in the retina (https://webvision.med.utah.edu/), and the extracted features of the visual stimulus are conveyed to the brain through several parallel channels, sub served by 40-50 distinct RGC cell types (Field & Chichilnisky, 2007, 2007; Masland, 2001, 2012; Troy & Shou, 2002; Wassle, 2004). RGC types can be distinguished by their physiological properties, target nuclei in the brain, and distinct dendritic arbors within the retina, and, in a few instances, by molecular markers (Amthor et al., 1983; T. C. Badea & Nathans, 2011; Baden et al., 2016; Bae et al., 2018; Goetz et al., 2021; Martersteck et al., 2017; Rheaume et al., 2018). Visual information computations are helped by the presence of 10-12 bipolar cell types(T. C. Badea & Nathans, 2004; Boycott & Wassle, 1991; Ghosh et al., 2004; Grunert et al., 1994; Grünert & Martin, 2020; Helmstaedter et al., 2013), as well as horizontal(T. C. Badea & Nathans, 2004; Grunert et al., 1994; Grünert & Martin, 2020; Peichl & Gonzalez-Soriano, 1994; Thoreson & Dacey, 2019) cells and 25-35 amacrine cell types(T. C. Badea & Nathans, 2004; Boycott et al., 1969; MacNeil & Masland, 1998; Mariani, 1990), and the synaptic interactions between bipolar, amacrine and ganglion cells are performed at the level of a sharply stratified neurite layer, called the Inner Plexyform Layer (IPL). The resulting streams of information are then relayed to distinct retinorecipient areas in the brain by the axons of RGCs.

Much is known about the connectivity, functional properties and central projections of RGCs (Baden et al., 2016; Bae et al., 2018; Goetz et al., 2021; Martersteck et al., 2017), but how these features are mapped onto cell types and visual information channels, is understood only for a few RGC cell types (Chichilnisky & Baylor, 1999; Crook et al., 2011; Dacey & Lee, 1994; De Monasterio & Gouras, 1975; Dhande et al., 2013; D'Souza et al., 2021; Guler et al., 2008; Hattar et al., 2002, 2003; Huberman et al., 2009; Jacobs et al., 2007; Jacoby & Schwartz, 2017, 2018; Joesch & Meister, 2016; Johnson et al., 2018; Kay et al., 2011; Nath & Schwartz, 2016; Rivlin-Etzion et al., 2011; Smallwood et al., 2003; Tien et al., 2015; Watanabe & Rodieck, 1989; Yonehara et al., 2008, 2009; Zhang et al., 2012).

Each RGC type is tilling the retina in a relatively even fashion. RGCs with smaller dendritic arbors are more densely distributed, while large dendritic arbor RGCs occur in smaller numbers. In most species with fovea or central area, the size of dendritic arbors increases and density of

RGCs decreases with the distance from the central area, reflecting lower visual acuity at the edges of the visual field. Dendrites of distinct RGC cell types are laminated in a very precise and reproducible fashion in distinct layers of the IPL, and the lamination depth is a strong predictor of RGC functional class. In addition to the diversity of dendritic arbors, RGCs also display a wide range of central projections (Hong et al., 2011; Martersteck et al., 2017). Besides the axons projecting into the Lateral Geniculate Nucleus, a relay center that transmits visual information to the cortex, and hence to our conscious visual perception, RGCs also project to a wide variety of nuclei involved in other visual functions which are not necessarily conscious, but may cooperate with "cortical" vision. Thus, RGC projections to the Suprachiasmatic Nucleus (SCN) convey general light levels information and thereby help to entrain our circadian clock in synchrony with the light dark cycles of our environment (circadian photoentrainment)(Guler et al., 2008; Hattar et al., 2003). RGCs projecting to the Olivary Pretectal Nucleus (OPN), inform a much faster visual reflex, which controls the diameter of the pupil (Pupilary Light Reflex - PLR) in response to light levels in the environment; this results in pupil dilation in dim light, and pupil constriction in bright light(Guler et al., 2008; Hattar et al., 2003). Furthermore, RGCs that respond to motion in three preferential directions, separated from each other by 120 degrees, specifically project to the Lateral, Dorsal and Medial Terminal Nuclei (LTN, DTN and MTN), and constitute the visual arm of the Vestibulo-Ocular Reflex, which determines the motion status of our head and body within the surrounding world (Oculo - Vestibular Coordination)(Yonehara et al., 2008, 2009). Finally, most RGCs also innervate the Superior Colliculus (SC), the part of the dorsal mesencephalon, which represents a phylogenetically conserved visual nucleus, and is in lower vertebrates the equivalent of the visual brain. Retino-tectal projections are used to compute more sophisticated visual functions such as eye and head movement while fixating visual objects, tracking moving objects within the visual scene, etc… Collectively, these subconscious functions, executed in the background, can be brought into our awareness under pathological conditions, where their crucial roles in normal physiology become evident.

B.I.3 Cell type Development

Although some information on the development of RGCs as a cell class is available, the mechanisms by which the very distinct and complex dendrite arbors and the well-segregated central inputs of individual cell types are forming are largely unexplored (Cardozo et al., 2020; Graw, 1996; Herrera et al., 2019; Marquardt & Gruss, 2002; Mason & Slavi, 2020; Nguyen-Ba-Charvet & Rebsam, 2020; Sitko & Goodrich, 2021; Wilson & Houart, 2004). Thus, a large body of work has defined many of the transcriptional cascades involved in the development of the retina as an organ, and the specification of different retinal cell classes (Lyu & Mu, 2021). Specifically, transcription factors (TF) of the bHLH (Math5), lim homeobox domain (Isl1, Dlx1 and 2), and pou domain IV (Brn3b and Brn3c) families have all been shown to play a role in RGC cell formation, as deletions of these TFs result in more or less severe RGC deficits. However, the transcriptional mechanisms of individual RGC cell type formation are essentially unexplored. Also, though several classes of signaling, cell adhesion and guidance molecules have been

implicated in the development of RGCs, how these molecules participate in the definition of individual cell types, and how transcriptional codes regulate their cell type distribution are still open questions.

B.I.4 Brn3 transcription factors are determinants of RGC cell type definition and development

Brn3a, Brn3b and Brn3c, transcription factors of the POU IV class, are excellent candidates for participating in the diversification of RGC cell types(Erkman et al., 1996; Gan et al., 1996, 1999; Liu et al., 2000, 2001; McEvilly et al., 1996; Pan et al., 2005, 2008; Wang et al., 2002; Xiang et al., 1993, 1995, 1996; Xiang, Gan, Li, Chen, et al., 1997; Xiang, Gan, Li, Zhou, et al., 1997). All Brn3s are expressed early (embryonic day E11.5-E12.5) during RGC differentiation, essentially in newly formed, postmitotic RGCs. Whereas in the adult Brn3a and Brn3b appear to be expressed in about 75-80 % of RGCs, Brn3c is restricted to a minority of these neurons (20 %). Brn3b homozygous mutants are viable, and exhibit a large (80 %) depletion of RGCs, whereas Brn3c single mutants do not appear to exhibit any obvious retinal phenotype, though they suffer from balance and hearing disorders, as a result of loss of inner ear hair cells. Brn3a homozygote mutants die perinatally, and exhibit, amongst other defects, a reduction in dorsal root ganglia (DRGs), and trigeminal ganglion (TG) neurons. Thus, the consequence of deleting Brn3a from the retina was poorly understood previous to our work. Interestingly, double knock-outs of Brn3b and Brn3c exhibit a more severe loss of RGCs compared to Brn3b null animals, and replacing the endogenous Brn3b gene with a Brn3a open reading frame results in a partial rescue of RGC numbers. All these observations suggest the possibility that Brn3 transcription factors participate in a combinatorial code which orchestrates the specification of individual RGC cell types.

B.II Personal contributions to the field

Note: Sections **B.II.1.1** and **B.II.7.1** represent work done during and/or before my PhD, but I have included them in the narrative, as they represent the foundation of subsequent work, and are necessary for the understanding of the rationale of my postdoctoral and lab head work. Subsequent sections will present accomplishments and papers by subject matter in chronological order. Papers I have co-authored and that are relevant for each subchapter are on a separate "personal" bibliographic list and will be mentioned in the subchapter headings marked by #number according to the list.

B.II.1 Development of novel molecular genetic approaches for cell type analysis.

B.II.1.1 Sparse random recombination (PhD work, #51 Badea 2003; # 49 Badea 2004): Up to the late 1990's, the field of neuronal cell type specification was lacking reliable molecular markers for the identification of neuronal cell types. Thus cell type classifications had to rely on anatomic or physiologic classifications. Methodologically, this meant typically

electrophysiological recordings coupled with die filling and anatomic tracing, processes that are extremely laborious and require a high level of expertise. Thus, the goal of my doctoral studies in Johns Hopkins University School of Medicine was to design molecular genetic tools for the characterization of neuronal cell types.

The ability to genetically manipulate neuronal circuits depends on defined genetic loci with expression limited to a given neuronal cell type, or a tractably small number of cell types. Major efforts have been previously directed at expression analysis of neuronal cell populations, in the hope of defining useful markers and genetic drivers. In parallel, mouse and fly genetic loci are being tagged with reporter constructs, contained in Bacterial Artificial Chromosomes, or limited to short promoter and enhancer elements. These approaches should yield in time many useful markers and genetic elements, which will allow the marking and genetic manipulation of more and more restricted neuronal cell populations. However, visualization of complex neuronal arbors is best achieved when only a few individual neurons are labeled, allowing the tracing of individual fibers, as in the classic

Golgi staining used by Ramon y Cajal. Therefore, as a PhD student in the Nathans' lab, I developed a genetic strategy for visualizing neuronal morphology. In brief, we generated a knock-in mouse line ubiquitously expressing the drug inducible CreER recombinase. Crossing this line with Cre-loxP recombination reporters that express Alkaline Phosphatase (AP), and activating the CreER with 4-hydroxytamoxifen results in the sparse labeling of neurons, with complete visualization of both dendritic and axonal arbors. Moreover, by controlling the timing and dosage of 4-hydroxytamoxifen, this approach can be used to perform clonal analysis and lineage tracing. We published the description of mouse lines and methodology in J. of Neuroscience (Badea et al. 2003). Using this approach, I conducted the first comprehensive survey of all mouse retinal neurons, with a quantitative analysis of neuronal morphologies (Badea 2004).

B.II.1.2 Cre recombination with zero background (ROSA26rtTACreER; #43 Badea 2009)

During our experiments involving crossing many different CreER and loxP lines, we observed that the efficiency of CreER – loxP recombination depends not only on the level and timing of expression of the CreER driver, and the amount of delivered 4HT, but also, in quite a significant fashion, on an intrinsic "recombination availability" of the target locus (Vooijs et

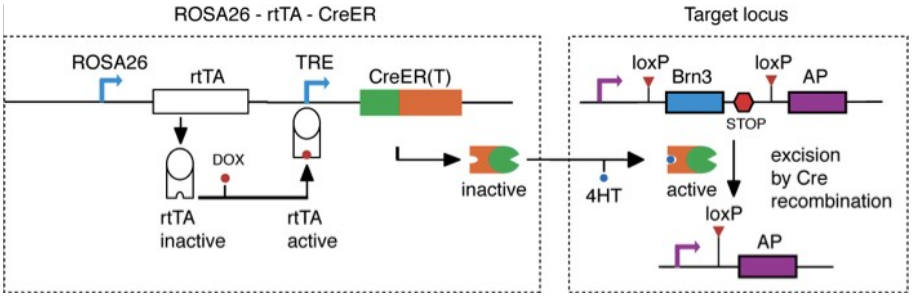


Figure 1. Dual pharmacological control of Cre-loxP recombination: In the ROSA26-rtTA-CreER knock-in line, rtTA is constitutively transcribed under the control of the ROSA26 promoter, activated by the addition of Doxycycline, binds the tetracycline response element (TRE) and induces transcription of CreER. CreER is then activated by 4HT, to induce recombination at target loci. Both Doxycycline and 4HT can therefore be used to titrate the recombination activity.

al., 2001). Using the Rosa26-CreER mouse line (T. C. Badea et al., 2003), in conjunction with relatively inefficient recombination targets, such as the Z/AP reporter, we observed no background recombination in the absence of 4HT, and around 1 – 10 % recombination with either the early delivery or high dosage of 4HT. However, crossing other target loci to the ROSA26CreER line, ~1 % recombination levels were noticed even in the absence of 4HT administration, and when moderate to small (100 – 200 microgram) 4HT doses were delivered, recombination of the targets was close to 100 %. Mosaic analysis of genetic manipulations requires both precise timing and zero background recombination. To achieve this goals we developed a new genetic element in which CreER transcription is under the control of a tetracycline response element, and therefore Cre-loxP recombination is under dual pharmacologic control of Doxycycline and 4HT (Figure 1).

B.II.1.3 Inverted exon Cre recombination (ROSA26; #43 Badea 2009)

Most conditional constructs contain two open reading frames (ORF) arranged in tandem fashion. The general strategy requires that the first ORF (e.g. Brn3 in Figure 1) but not the second ORF (e.g. AP in Figure 1) be transcribed in the absence of Cre activity. This is insured by a transcriptional STOP sequence, containing tandem repeats of terminators, inserted upstream of the second loxP site. Once the Cre recombinase is activated, the first ORF, together with the STOP sequence, is removed, and only the second ORF is transcribed, uniquely labeling the cells in which recombination has occurred. However, in this strategy, there is a certain amount of read-through, meaning that the transcripts generated at the promoter continue through the STOP cassette and proceed to generate copies of the second ORF, which can reach significant levels for extremely potent promoters, and/or very sensitive reporters. To address this issue, we generated a new type of Cre-loxP reporter, in which the open reading frame of the reporter cDNA is interrupted by an intron, carrying a loxP site, which can recombine with a second loxP site placed in reverse orientation after the STOP codon of the AP reporter. This configuration insures that no reporter message is transcribed in the absence of Cre activity, however the recombination is reversible in the presence of sustained Cre activity, with both active and inactive configurations being equally efficiently recombined. The construct was placed under the control of the ROSA26 locus, tested, and found to be a far more sensitive recombination target than Z/AP, by about 1000 fold.

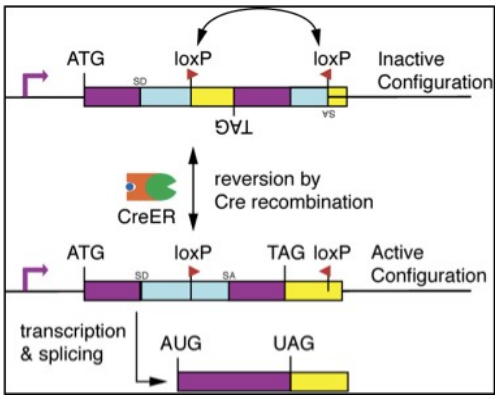


Figure 2. ROSA26-APi reporter. The full ORF of the AP gene is broken up in two exons (purple), interrupted by an intron (light blue) which carries a splice donor (SD) at the 5' end and a splice acceptor (SA) at the 3' end. The loxP sites (in reverse orientation) in the middle of the intron as well as in the 3'UTR of the gene (yellow), insure inversion of the fragment after Cre recombination. The construct in inactive configuration has been targeted to the ROSA26 locus. Cre activity induces reversion to the active configuration, which can be correctly transcribed, spliced and translated to yield the active reporter.

B.II.1.4 Conditional Knock-in Alleles for Genetic mosaic analysis in mice (Brn3CKOAP, #37 Badea 2012, #42 Badea 2011, #45 Badea 2009).

In order to more precisely correlate gene expression with RGC type, we generated targeting alleles for the three Brn3 TFs, following a novel conditional reporter strategy (Figure 3).

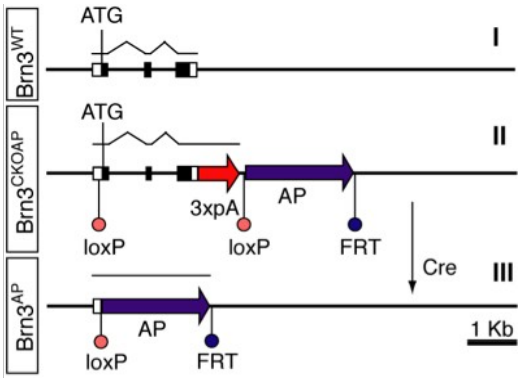


Figure 3. Conditional knock-in strategy: Upon Cre activity, the targeted gene is deleted and replaced with the reporter AP.

These conditional knock-in reporter alleles (Brn3aCKOAP,Brn3bCKOAP, and Brn3cCKOAP) contain loxP sites flanking the Brn3/Pou4f genes, inserted in the 5' and 3' UTRs. In the 3'UTR, a strong transcriptional STOP signal (3x SV40 polyA) is inserted before the 3' loxP site, and an AP cDNA is inserted after. Upon Cre mediated recombination, the sequence between the two loxP sites including the endogenous gene and the STOP signal are deleted and replaced by the AP reporter that is now expressed under the control of the endogenous locus, at the original transcription start site. Therefore the AP specifically labels the neurons that express the transcription factor, and allows for anatomic reconstruction of their dendritic morphologies and brain projections. If the allele is combined with a full KO allele on the sister chromosome ($Brn3^{CKOAP/KO}$), or if the mouse is homozygous for the conditional allele ($Brn3^{CKOAP/CKOAP}$), Cre mediated recombination results in full knockout cells, expressing the AP cDNA. Using this approach heterozygote $Brn3^{AP/WT}$ and homozygote null cells ($Brn3^{AP/KO}$ or $Brn3^{AP/AP}$) can be studied in parallel. Thus the anatomy, physiology and development of Brn3 expressing cells can be derived, either when they are phenotypically wild type, or mutant with respect to the Brn3 gene under study. This approach is basically a genetic mosaic analysis, as is currently used in Drosophila, and has been since used by us and several other groups in RGCs, as well as other Brn3 expressing neurons.

B.II.1.5 Combined use of two recombinases for intersectional genetics (roxP-STOP-roxP approach, Brn3aCKOCre andBrn3bCKOCre, #34 Sajgo 2014) Given the large overlap of gene expression pattern in neurons, combinatorial genetic approaches are powerful instruments in the analysis of visual circuits and their development. We have therefore decided to join the effort to develop genetic tools using a second recombinase, Dre, a close homologue of the Cre recombinase, but operating on distinct molecular target sites(Anastassiadis et al., 2009, 2010; Sauer & McDermott, 2004). We have generated two Dre dependent conditional knock-in Cre expressing mouse lines. In these lines, named $Brn3a^{CKOCre}$ and $Brn3b^{CKOCre}$, the Brn3a or Brn3b coding exons, are linked to a 3xSV40 polyA transcription STOP signal flanked by Dre recombinase target sites (roxP sites), and followed by the Cre recombinase (Figure 4). Upon Dre recombination, the endogenous Brn3 gene is removed, and replaced by the Cre recombinase, resulting in conditionally knocked-in Cre alleles. The Cre, now expressed specifically from either the Brn3a or Brn3b locus will activate any desired downstream target. The functionality of our

system was demonstrated by positive AP histochemistry in triple transgenic animals CAG:Dre; Brn3a^{CKOCre}; ROSA26^{AP} and CAG:Dre; Brn3b^{CKOCre}; ROSA26^{AP}. CAG:Dre is a transgenic line expressing Dre recombinase in a ubiquitous fashion, beginning with the germline(Anastassiadis et al., 2009)and ROSA26^{AP} is a ubiquitously expressed (embryonic day 5 and forward) knock-in homologous recombination Cre reporter line, previously generated by us (section B.II.1.3 and #43 Badea 2009).

To our surprise, both CAG:Dre; Brn3a^{CKOCre}; ROSA26^{AP} and CAG:Dre; Brn3b^{CKOCre}; ROSA26^{AP} lines showed ubiquitous, early expression of AP, indicating that the conditional Brn3 Cre knock-in lines were expressed early (at least E9.5) throughout the body. In contrast, CAG:Dre; ROSA26^{AP} mice did not show any staining enforcing previous reports that Dre does not recognize loxP sites, the targets of the Cre recombinase. Expression of Brn3a and Brn3b in the male and female germline was corroborated by literature and gene expression profiling data, as well as Brn3a^{AP} and Brn3b^{AP} expression in the germline, thus reinforcing the need for generating conditional alleles that can bypass germline effects.

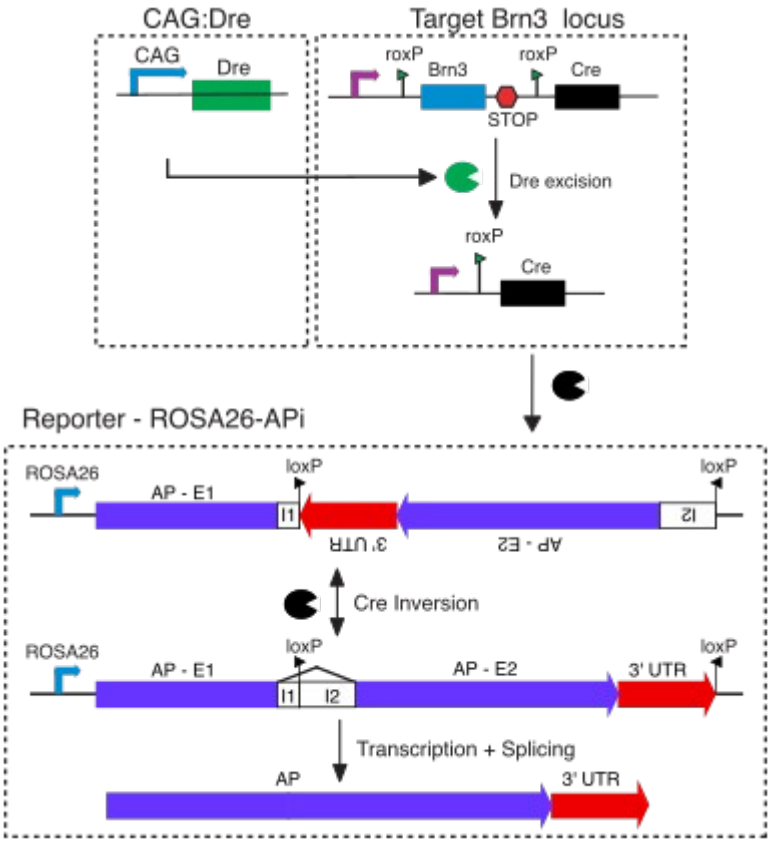


Figure 4. Dual recombinase Strategy. Dre recombinase (left-top) is generated from an ubiquitous promoter, and excises the DNA between the two roxP sites, thus removing the endogenous Brn3 gene and replacing it with the cDNA for the Cre recombinase (right – top). The Cre recombinase can now induce the inversion at the ROSA26-APi reporter (bottom).

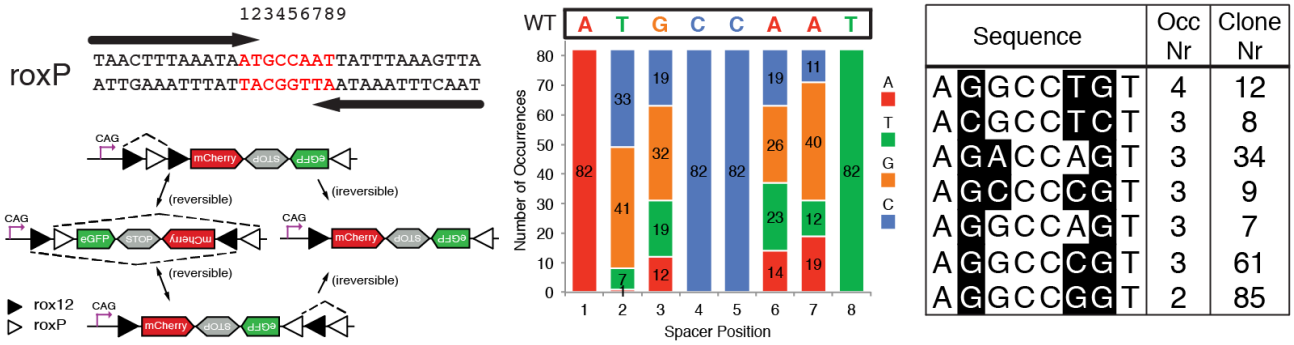
Unfortunately, Brn3a^{CKOCre}; ROSA26^{AP} and Brn3b^{CKOCre}; ROSA26^{AP} control retinas and brains showed a small but significant background recombination effect, most likely due to incomplete transcriptional termination at the STOP signal. Thus, although the proof of concept was successful, the conditional lines are not optimal for the desired application.

Germline recombination in our Brn3a^{CKOCre} and Brn3b^{CKOCre} alleles has resulted in generation of Brn3a^{Cre} and Brn3b^{Cre} knock-in alleles, which, despite the germline expression of both Brn3a and Brn3b, can be used as Cre drivers for viral injections or electroporations of Cre dependent constructs in the expression domain of the two genes, most relevant to us, in RGCs. We have used them to express a Cre-dependent AAV1-CAG-FLEX-tdTomato construct by intra-

ocular injections at postnatal day 0 (P0) and found tdTomato+ RGCs as early as P3, and fully filled dendritic arbors and axons as early as P7, all the way into the adult.

B.II.1.6 Identification and characterization of novel Dre recombinase target sites (rox12, FREX, #31 Chuang 2016).

As described in section B.II.1.5 we have demonstrated the feasibility of sequential Dre to Cre recombination. However, one of the technical limitations of our initial roxP - Brn3 - 3xSTOP - roxP design was that a significant amount of read-through is inducing background recombination in Brn3^{CKOCre}; ROSA26^{AP} crosses, even in the absence of Dre. We therefore designed an inversion-excision strategy, similar to the FLEX approach used for Cre recombinase target constructs. This approach crucially depends on the description of alternative lox sites (e.g. lox2272) that recombine with themselves, but do not recognize the wild type loxP site. To replicate this approach in the Dre- roxP system, we screened several random nucleotide substitution libraries based on the roxP site, using a drug selection approach in bacteria, and identified several novel Dre target sites that do not recombine with WT roxP. Amongst these, we identified optimal substitution sites in the rox sequence spacer that generate incompatibility with the original roxP site, and tested them for self-recombination specificity and efficiency in purified biochemical system, and by co-expression with Dre in bacterial and eukaryotic (HEK293) cultures.



Sequence	Occ Nr	Clone Nr
AGGCCTGT	4	12
ACGCCTCT	3	8
AGACCAGT	3	34
AGCCCCGT	3	9
AGGCCAGT	3	7
AGGCCCGT	3	61
AGGCCGGT	2	85

Figure 5. Selection and Identification of novel roxP sites. Top left, WT roxP sequence, with spacer region highlighted in red. Middle, outcome of screening for base pair substitutions at positions 2,3,6,7. The number of occurences are indicated for each base at each position. Right, the most frequently recovered specific sequences. Note that clone Nr. 12, henceforth rox12, occurred 4 times in 82 trials. Bottom left, inversion-excision strategy. In the unrecombined orientation, the CAG promoter drives eGFP expression. Dre can induce a first, reversible inversion step either at the roxP or rox12 sites, resulting in the CAG promoter driving mCherry expression. In the second, irreversible excision step, the triple-rox sequences are simplified to only one black or white rox site, by reactions between the serially arranged rox12 or roxP sites.

In addition, we tested these new sites for lack of cross-reactivity with the Cre system, by transforming plasmids carrying the new sites in Cre-Expressing E Coli strains and HEK293-Cre cells. The most effective novel rox site (termed rox12), that had minimal crossreactivity with the roxP sequence, highest self-recombination efficiency in the presence of Dre, and lacked recombination in the presence of Cre, was then combined with the roxP site to generate an inversion-excision (FREX) cassette. Efficient inversion-excision was then validated in HEK293-Dre cells, and lack of cross reactivity with the Cre system confirmed in HEK293-Cre cells (Figure 5).

B.II.1.7 Inversion – Excision strategy using Dre recombinase and rox12 target sites (Brn3cCKOCre #11 Parmhans 2021)

Based on the validated tools and approach described in B.II.1.7, we then applied the FREX inversion-excision strategy to the Brn3c locus and generated a Dre-dependent conditional knock-in Cre recombinase line (Brn3c^{CKOCre}, Figure 6). The sequential Dre-to-Cre recombination was validated using the same triple cross as described previously, CAG:Dre; Brn3c^{CKOCre}; ROSA26^{AP} and appropriate controls. Serial recombination was succesful, and CAG:Dre; ROSA26^{AP} mice had no background recombination. However, for an unknown reason, Brn3c^{CKOCre}; ROSA26^{AP} mice exhibited still a large amount of background recombination, despite the inversion of the Cre (confirmed by PCR, sequencing and southern blotting). Intriguingly, the background reporter expression is only present in neurons expected to express Brn3c, suggesting that, whatever mechanism is in use, it is under the control of the Brn3c regulatory elements. It is not clear whether a reverse transcription using either the genomic locus or the generated mRNA is occurring.

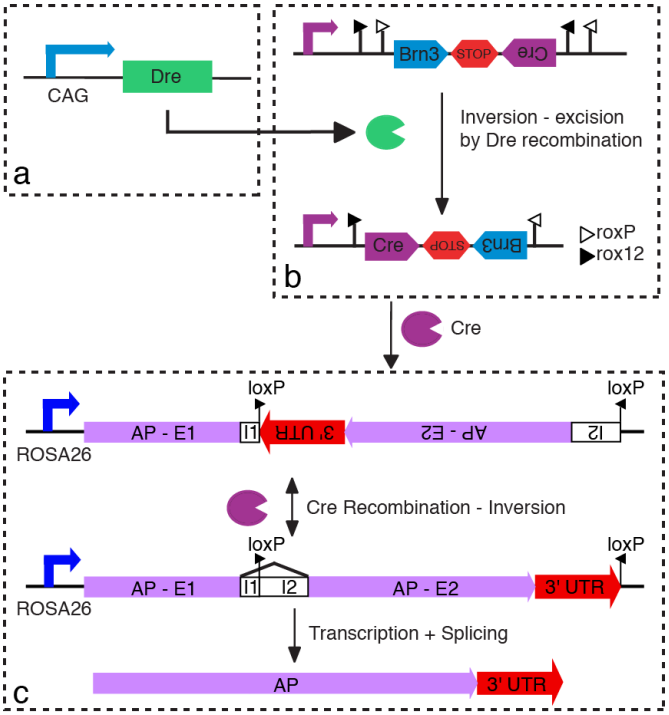


Figure 6. Serial recombination through Inversion-Excision Strategy at the Brn3c locus. The approach is identical to the one in Figure 4 with the exception of the Dre-dependent inversion-excision that uses tandem-inserted rox12-roxP sites at the Brn3c locus (panel b). After recombination, Cre is expressed under the control of the Brn3c promoter, and the Brn3c ORF is placed in reverse orientation.

B.II.2 Transcriptional Control of RGC type specification

The technical advances described in section B.II.1 were directed and tested in particular in one direction, namely the understanding of how transcription factors regulate Retinal Ganglion Cell development. I have described in the introduction what was known about transcriptional control of RGC development when I entered the field. Advances since have been quite substantial, and I will point out our own discoveries in the following sections.

B.II.2.1 The role of Pou4f/Brn3 transcription factors in the combinatorial code for RGC specification (Brn3CKOAP, #45 Badea 2009, #42 Badea 2011, #37 Badea 2012, #36 Shi 2013, #11 Parmhans 2021)

To genetically label RGC cell populations, we used the expression of three POU domain transcription factors Brn3a, Brn3b and Brn3c, expressed specifically in postmitotic RGCs

beginning in early differentiation (embryonic day E11.5-E12.5). Specifically, I developed Cre recombinase dependent conditional knock-in reporter mouse lines at the loci of Brn3a (Brn3a^{CKOAP}), Brn3b (Brn3b^{CKOAP}), and Brn3c (Brn3c^{CKOAP}) (see section B.II.1.4). The fact that the reporter (AP) replaced the endogenous gene in the locus facilitated the characterization of morphological consequences of gene ablation in individual neurons, thus revealing cell-autonomous effects. This represents a mosaic genetic analysis strategy, comparable to the one used with great success in flies (Lee & Luo, 1999). Using this approach, we determined that Brn3a, Brn3b and Brn3c are expressed in distinct but partially overlapping RGC populations and may participate in a combinatorial transcriptional code determining RGC types. Brn3a is expressed in a majority of RGCs in the adult retina, but is excluded from Opn4 expressing ipRGCs. Brn3b is also broadly expressed, however conspicuously absent from ON-OFF direction selective RGCs (ON-OFF DS RGCs), and a few other cell types. Brn3c is expressed at high levels in a far more restricted RGC population. Significantly, a majority of RGC types express overlapping combinations of Brn3a, b and c, suggesting that RGC specification does not happen through expression of unique transcription factors, but rather through combinatorial codes of expression. Subsequent work using the recently generated Brn3c^{Cre} allele shows that Brn3c is transiently expressed in several other RGC types, although at lower levels (see section B.II.1.7, #11 Parmhans 2021). This is definitely possible for Brn3a and Brn3b, although further studies are necessary.

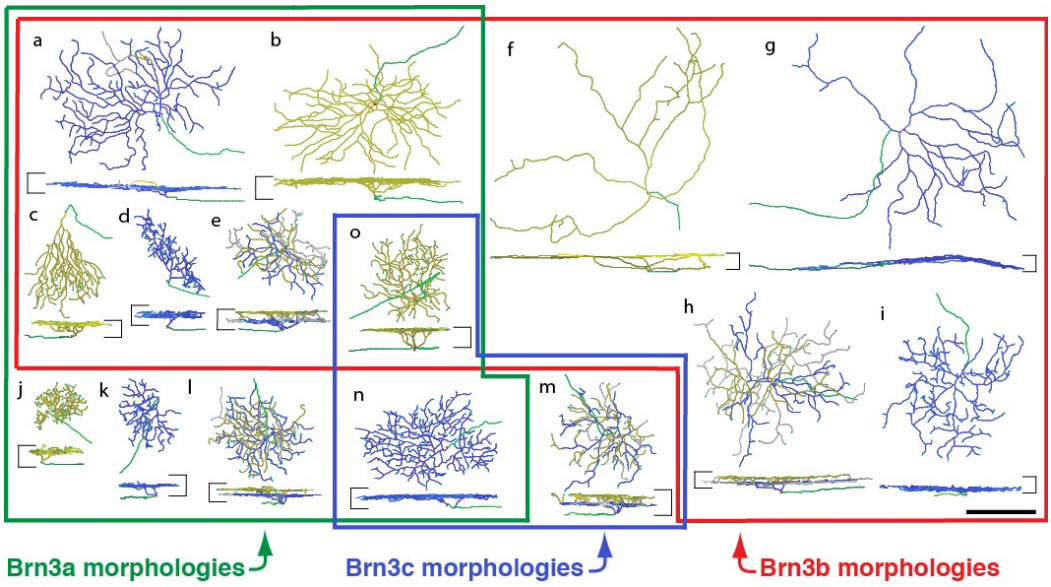


Figure 7. Combinatorial expression of Brn3 transcription factors in RGCs. Note the partial overlap of expression, with individual cell types expressing Brn3a, Brn3b or Brn3c either separately or in various combinations.

In addition, we have found that removing Brn3a or Brn3b from RGCs results in cell autonomous dendritic arbor defects. In Brn3a$^{AP/KO}$ RGCs bistratified dendritic arbor morphologies are heavily overrepresented, suggesting a role of this transcription factor in either the survival of monostratified neurons, or the choice between mono or bistratified arbor morphologies. In addition, RGCs with small-dense dendritic arbors (similar to midgets in human/primate or betta cells in the cat) were completely ablated. Brn3b$^{AP/KO}$ RGCs exhibit a variety of intraretinal axon defects. In addition, the dendritic arbor areas of Brn3b$^{AP/KO}$ RGCs are significantly enlarged, potentially as a result of a reduced coverage of the retina derived from the significant RGC cell

loss previously described for these mutants. Midget-like RGCs are also severely reduced in Brn3b$^{AP/KO}$ retinas, and bistratified RGCs co-stratifying with the ChAT bands are significantly enriched. However, removal of Brn3c does not seem to affect the number, dendritic arbor morphology, or central projections of these neurons.

How does this partial overlap of expression and function contribute to RGC diversity? Previous findings point at some degree of genetic interaction between the three Brn3 factors during RGC specification. For instance, substituting the coding exons of Brn3b with Brn3a results in partial rescue of RGC numbers, although the effect on RGC type distribution is unknown (Pan et al., 2005), while Brn3b$^{KO/KO}$; Brn3c$^{KO/KO}$ double knock-outs have more severe RGC loss than Brn3bKO/KO (Wang et al., 2002). To study the genetic interactions and proposed functional redundancy of Brn3 TFS in RGC development, we have generated Brn3b$^{KO/KO}$;Brn3a$^{KO/AP}$, Brn3c$^{KO/KO}$;Brn3a$^{KO/AP}$ and Brn3b$^{KO/KO}$;Brn3c$^{KO/AP}$ double knock-outs, in which RGCs were labeled respectively by Brn3a^{AP}, Brn3a^{AP} or Brn3c^{AP}, and studied the effects of deleting each transcription factor alone or in combination on the selected Brn3^{AP} expressing population (#36 Shi 2013). Although no genetic interactions were found in Brn3a – Brn3c double KOs, several interesting conclusions emerge from the analysis of Brn3a-Brn3b and Brn3b-Brn3c double KO retinas. About 25% of Brn3a^{AP} RGCs survive in the absence of Brn3b, however combined loss of Brn3a and Brn3b results in an almost complete depletion of Brn3a^{AP} RGCs, and RGCs in general, thus arguing that at least a fraction of Brn3a^{AP} RGCs can be generated in the absence of Brn3b, and that Brn3a can function independently of Brn3b in RGC development. Furthermore, amongst the Brn3a^{AP} RGCs lost in Brn3b KO retinas, several morphological types do not express Brn3b in the adult. Brn3b loss results in reduced numbers of Brn3c^{AP} RGCs, coupled with a distinct and very specific deletion of the OFF morphology of Brn3c^{AP} RGCs (which in adults are Brn3b^{+}), and an overall increase in dendritic arbor area increase in surviving ON Brn3c^{AP} RGCs (which in adults are Brn3b^{-}). Thus Brn3b loss of function exhibits apparent cell autonomous and non-cell autonomous effects on Brn3a and Brn3c expressing RGCs. It is possible that Brn3b is expressed earlier in the developmental history of these cells, or that signaling or lateral contact interactions could explain the observed results.

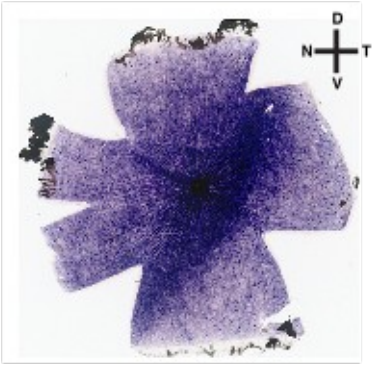


Fig 8 Alkaline Phosphatase (AP) staining reveals RGCs of ArCe in Brn3cCre; Brn3bCKOAP mice.

Closer investigation of the Brn3b$^{KO/KO}$; Brn3a$^{AP/KO}$ retinas showed a very small population of RGCs positive for the general RGC markers Neurofilament Light chain (NFL) and Islet 1 (Isl1) and the ipRGC marker Melanopsin. Since Isl1 is involved in transcriptional regulation of RGC development, in a pathway parallel to the one controlled by Brn3b, we stained Isl1 knock-out retinas for Melanopsin, and found that no staining remained. This finding, together with our previous observation that SCN projecting M1 ipRGCs are not expressing Brn3b (#45 Badea 2009, #39 Chen 2011), enables us to extend the transcriptional code of RGC cell type regulation to a partial overlap between Brn3s and Isl1. Thus, Brn3b has cell autonomous and non-cell autonomous effects on Brn3a^{AP} and Brn3c^{AP} RGCs, and has additive effects with Brn3a with

respect to RGC survival. Isl1, known to play a broad role in RGC development, seems to also specifically regulate (Brn3b^{+} and Brn3b^{-}) ipRGCs and/or Melanopsin.

Intersection of Brn3c and Brn3b expression patterns in RGCs, as labelled in Brn3c$^{Cre/WT}$; Brn3b$^{CKOAP/WT}$ mouse retinas (#11 Parmhans 2021), revealed another surprising fashion in which transcriptional codes can shape RGC development and function, namely by influencing topographic distribution of RGC types in the retina. Brn3c^{Cre}; Brn3b^{AP} RGCs exhibited an area of increased density, running from dorso-temporal to naso-ventral across the retina, in a crescent reminiscent of an Area Centralis in other species. This ArCe could constitute the equivalent of the human fovea, although at lower spatial resolution, and represent a good animal model for studying high visual acuity pathology in humans. This pattern is not visible when staining for either Brn3b or Brn3c (or indeed any other gene), and therefore the intersection of the expression of the two transcription factors defines this subdomain (Figure 8).

B.II.2.2 Transcriptomic program of RGC type specification regulated by Brn3 transcription factors (#21 Sajgo 2017)

What are the transcriptional programs regulated by Brn3 transcription factors? How do Brn3 target genes contribute to RGC type specification? What are the genetic programs ensuring developmental specificity at early stages of axon and dendrite formation?

To answer these questions, we have established an immunoaffinity purification strategy based on our Brn3^{CKOAP} alleles. After recombination, Brn3 expressing cells are marked on the surface with the genetically encoded reporter, AP, which is a GPI linked surface molecule. We used mouse monoclonal antibodies against AP and dynabead-coupled anti-mouse secondary antibodies to perform a immunomagnetic separation of Brn3^{AP} RGCs, followed by RNA extraction and Deep sequencing of the RNA from Brn3^{AP} RGCs and retinal supernatants. We identified: (i) RGC specific transcripts by comparing Brn3^{AP} RGCs to retinal supernatants, (ii) potential RGC type markers by comparing Brn3a^{AP} vs Brn3b^{AP} RGCs, (iii) genes up- or down regulated by loss of Brn3a or Brn3b by comparing Brn3$^{AP/WT}$ to Brn3b$^{AP/KO}$ RGCs, (iv) genes required during axon guidance vs. genes required during dendritic arbor formation, by comparing Brn3b^{AP} RGCs at embryonic day 15 (E15) and postnatal day 3 (P3).

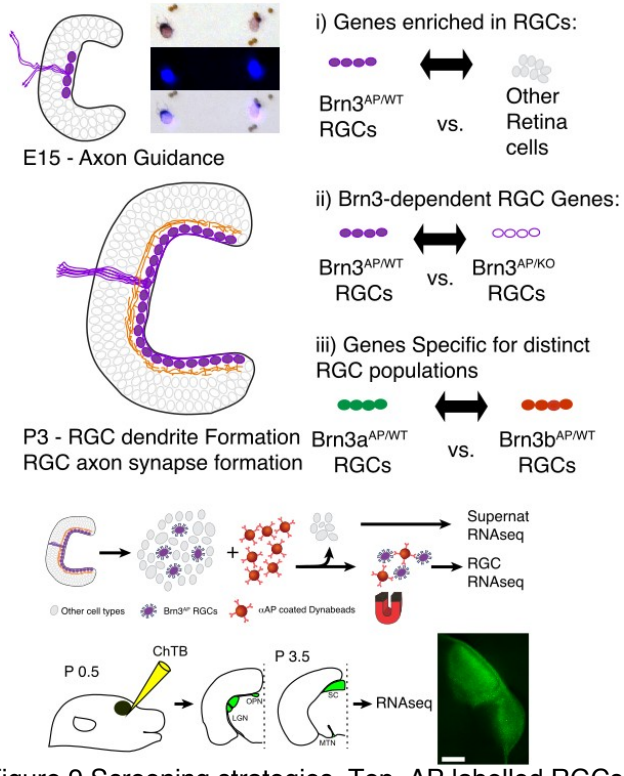


Figure 9 Screening strategies. Top, AP labelled RGCs (purple) were purified at E15 and P3 from Brn3a and Brn3b WT and KO retinas using magnetic isolation. Bottom, Retinorecipient layers (green) were identiifed by Alexa488-ChTB injections in the eyes at P3 and dissected. RNA was extracted from either RGCs, retina or brain nuclei and analyzed by RNAseq

About 3000 transcripts are enriched in RGCs when compared to the retina, and about 900 transcripts are regulated by Brn3b or Brn3a. The transcripts selectively expressed in RGCs, and/or affected by Brn3b loss differ significantly between E15 and P3, suggesting distinct expression profiles for the different stages of development. Deep Sequencing data is rendered extremely noisy because of the significant amplification that occurs between the RNA extraction and the sequencing of the individual fragments. We therefore followed up our Deep Sequencing screen with a validation screen, performed by in situ hybridization. For the P3 data, we selected 233 target genes and performed ISH on P3 retinal sections. For the E15 data, we intersected our candidate lists with the eye ISH data available through the Allen Brain Institute mouse developmental atlas, and identified 265 genes that had been analyzed by them. Of these 498 genes, about two thirds were indeed enriched in RGCs relative to the retina tissue. Gene Ontology analysis of these data sets revealed that a large part of the identified target genes belonged to pathways and molecular classes associated with development in general and neuronal development in particular. Amongst these we focused on two classes of molecules: (i) transcription factors, (ii) cell surface molecules/signaling receptors. Genome wide annotation work had identified some 2437 mouse genes that have gene regulatory function. Amongst these, 1647 are expressed in RGCs, but only 322 genes are enriched in RGCs compared to retinas, and 95 are under Brn3 control. Thus, transcriptional control of RGC development and function is accomplished through a large array of TFs, but only a small subset is specific to RGCs in the retinal context, and an even smaller number is regulated by Brn3b and/or Brn3a. However, based on the combinatorial usage of these factors, the number of possible combinations among these more restricted number of factors still generates a really large number of combination, allowing for the specification of significantly more than the 40-50 mouse RGC types believed to exist.

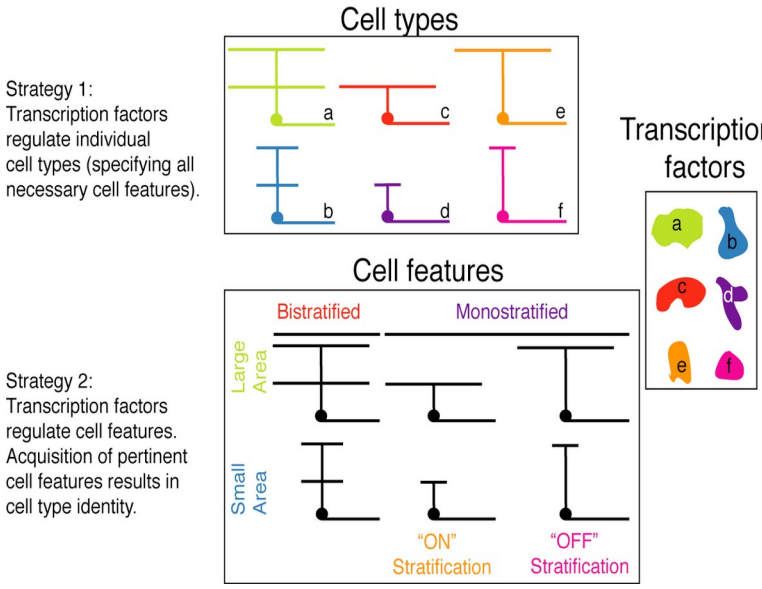


Figure 10 Proposed Mechanism for transcriptional control of cell type specification:
Strategy 1: TFs control individual cell types.
Strategy 2: TFs control features. Intersectional expression of TFs endows cell types with specific features resulting in cell type specification.

How do these transcriptional regulators coordinate the differentiation and specification of the diverse and characteristic neuronal arbor morphologies and partners of synaptic interaction characteristic of each RGC? In large part, surface adhesion molecules, guidance receptors and synaptic molecules guide the axonal and dendritic growth cones to the correct targets, ensure correct lamination and synaptic partners. We identified in the mouse genome a set of 822 genes belonging to these molecular classes and queried them with our RGC specific genes. About a

quarter (237 genes) were differentially expressed in various RGC subpopulations, in a variety of patterns, including some that were previously characterized by us or others. Thus, we have the molecular code that allows RGCs to correctly target their respective retinorecipient areas and laminae, and form their elaborate dendritic and axonal arbors, while choosing the correct synaptic partners.

We complemented these RGC profiles with RNAseq analysis of retinorecipient brain nuclei at P3, an age when RGC axons are actively engaged in synaptogenesis in the targets. The identified molecules, some of which form exquisite patterns of lamination in the optic tectum, or are specific for distinct retinorecipient areas, provide the potential interaction partners of RGC guidance cues.

To address the potential functions of molecules identified in our screen, we overexpressed a small subset in HEK293 cells, and find that several of them have the capacity to induce membrane processes reminiscent of neurites. We therefore suggest that cell-autonomous mechanisms may contribute to neuronal arbor formation, in addition to cell-cell and/or cell-matrix interactions mediated by transmembrane receptors/adhesion molecules. Using a Cre dependent, AAV-based overexpression approach to determine the subcellular localization of some of our targets in vivo in Brn3b^{Cre} RGCs, we find distributions consistent with roles in vesicle trafficking within neurites or at synapses. Finally, out of a set of 79 genes proposed to be associated with Glaucoma in human genetics studies, only 12 appeared to be enriched RGCs, potentially revealing molecular pathways associated with susceptibility to RGC damage.

Results in sections B.II.2.1 and B.II.2.2 converge onto the following model of transcriptional control of neuronal cell type specification. Transcription factors are not linked one-to-one with specific neuronal cell types. Rather, TFs, or their combinations, encode specific features of neurons (mono-stratified or bistratified arbors, large or narrow receptive fields, ON vs. OFF lamination). By intersecting the expression domains of these transcription factors, individual RGC types are endowed with the features they provide, resulting in the intersectional definition of types (e.g. small receptive field, dense dendritic arbors in the OFF sublamina of the IPL).

B.II.2.3 Brn3a involvement in midget-like RGC development (#21 Sajgo 2017, #18 Muzyka 2018).

In the previous sections I showed that Brn3a is expressed in at least ten distinct RGC cell types, and that retina- specific Brn3a ablation results in complete loss of specific RGC cell types, and bias towards bistratified RGCs. Amongst the RGC types completely ablated in Brn3a$^{KO/KO}$ retinas are cells with small, dense dendritic arbors that laminate in more than one sublamina. These morphologies are reminiscent of extrafoveal midgets in primates, betta cells in rabbit or X cells in cats, and their physiologies have only been recently recorded from in the mouse (Goetz et al., 2021; Jacoby & Schwartz, 2017; Zhang et al., 2012). Based on dendritic arbor morphologies, these cells might represent the mouse equivalents of the ON and OFF sustained, high spatial resolution detectors described in other species. This cell population overlaps at least

partially with recently characterized RGCs expressing FoxP2, Opn5 and Tusc5, and exhibiting small object motion sensitivity (D'Souza et al., 2021; Goetz et al., 2021, 2021; Rousso et al., 2016). The selective loss of these cells in Brn3a$^{KO/KO}$ retinas offers us the opportunity to study their molecular mechanisms of differentiation and the physiological and circuit functions of these cells.

Based on experiments described in section B.II.2.2, at P3 about 200 transcripts were differentially expressed between Brn3a$^{AP/WT}$ and Brn3a$^{AP/KO}$ RGCs. Given that midget-like cells are missing from Brn3a$^{AP/KO}$ RGCs, we wondered whether the differentially expressed molecules are markers and/or molecular determinants for their development. We therefore selected a list of 30 candidates amongst these molecules, based on high levels of expression in Brn3a$^{AP/WT}$ RGCs, at least two-fold downregulation in Brn3a$^{AP/KO}$ RGCs, and at least two-fold enrichment in Brn3a$^{AP/WT}$ RGCs versus the retina. In addition, we used protein domain structure and literature evidence to select candidates with molecular functions related to transcription and RNA processing, cell adhesion and neurite formation, and potential synaptic functions.

We performed in situ hybridization at P0, P3, P7, P12 and adult, on Rax:Cre; Brn3a$^{CKOAP/WT}$ and Rax:Cre; Brn3a$^{CKOAP/KO}$ mouse retinas, which have a complete recombination of the CKOAP allele, and found that nearly all selected candidates had GCL specific staining at P3. However for many of the candidates expression profiles changed over time, so a much more limited set preserved RGC specificity throughout all developmental stages, and an even narrower set were differentially expressed between Brn3a$^{AP/WT}$ and Brn3a$^{AP/KO}$ RGCs. Interesting patterns of expression where detected amongst molecules associated with transcription (FoxP2), intracellular signaling (Hpca, Mapk10), vesicle traficking (Snap91, Tusc5), synapse associated proteins (Gabra1, Grm4, Ntrk1, Pnkd and Rims1), adhesion molecules (Cdh4, Pcdh20) but also secreted proteins involved in axon guidance (Nptx1, Nptx2, Sez6l2). Some of these targets have been since confirmed by others, and we and other groups are trying to understand their involvement in RGC development and function. Many, such as Nptx1, Nptx2, Pnkd, Sez6l2 and Rims, have been involved in neurologic or psychiatric pathology. It remains to be established whether these Brn3a target genes are under direct transcriptional control by Brn3a or whether their reduced expression in Rax:Cre; Brn3a$^{CKOAP/KO}$ retinas is the result of ablation of midget-like RGCs.

B.II.2.4 Crosstalk between transcription and neurotrophic signaling in RGC type regulation (#20 Parmhans 2018, #5 Muzyka 2021).

RGC of each type are more or less evenly tiled across the retina. However, they are almost all derived from precursor neuroblasts that express Atoh7/Math5 (see section B.II.2.5). It is largely believed that cell type specification is a postmitotic event, particularly in the retina. Given that RGC type dendritic arbor areas and hence cell number densities vary widely across the retina, it is hard to design a model by which early neuroblasts that will generate retinal clones generate an exact number of downstream progeny of predetermined cell types in each category. How then do the various emerging postmitotic cells sense the local density of each cell type and commit to one RGC type fate or another?

Direct cell-cell contact decisions mediated by notch-delta interactions would liekly work only for closely apposed cells. One potential mechanism could be provided by the excess developmental production of RGCs, followed by the ablation of those who fail to establish functional interactions in the retinorecipient targets and hence obtain neurotrophic support. This mechanism requires that transcriptional mechanisms interact with neurotrophic signaling in establishing RGC type identity.

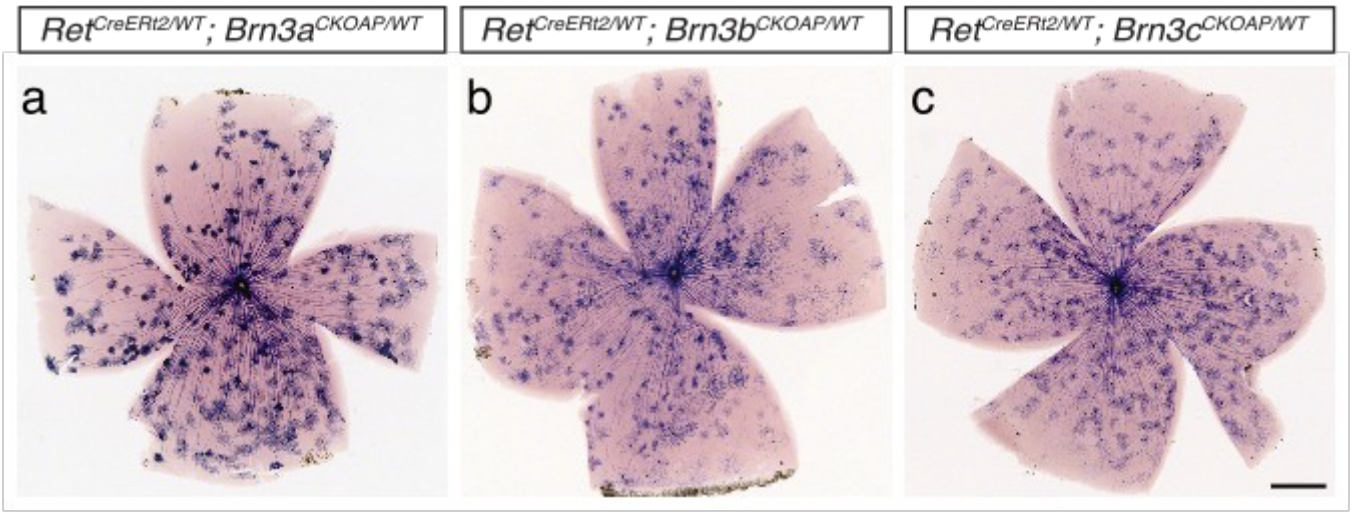


Figure 11 Examples of RGC populations labelled in retinas from Ret x Brn3 intersections. Induction of recombination for these examples was achieved by 4-HT injection at P14 or adult age. Genotype for each retina is indicated at the top.

As described, Brn3s are part of the combinatorial transcriptional code of RGCs and other sensory projection neurons. We took advantage of this knowledge to mine our RGC expression database for genes which had been studied in other systems, and for which genetic tools had been developed. One such opportunity emerged from the study of the Ret neurotrophin receptor which is expressed in DRGs, but also in the retina (Brantley et al., 2008; Luo et al., 2007, 2009). Using a Ret^{CreERt} allele crossed with a variety of generally expressed reporters, it was demonstrated that Ret has a dynamic expression pattern during DRG development, beginning with certain types ofmechanoreceptors, and then expanding to classes of mechano and nociceptors (see also section B.II.5.1). Since we found enriched Ret expression in RGCs at both E15 and P3, we analyzed retinas from Ret^{CreERt}; $ROSA26^{AP}$ mice. In collaboration with Drs. Niu and Luo at University of Pennsylvania, we defined the developmental profile of retinal cell types expressing the GDNF receptor Ret. It was previously known that Ret is expressed in adult horizontal as well as some amacrine and retinal ganglion cells, but the developmental profile and specific Ret^{+} cell populations were unknown. We found that Ret expression is largely RGC specific at E15, expands to horizontal cells by E17 and a mixed subpopulation of GABAergic and Glycinergic amacrines beginning with P1. These results suggested a partial overlap of expression between cRet and the Brn3 transcription factors. We therefore analyzed the RGC distribution in retinas of Ret^{CreERt}; $Brn3a^{CKOAP}$, Ret^{CreERt}; $Brn3b^{CKOAP}$ and Ret^{CreERt}; $Brn3c^{CKOAP}$ mice, and found that only subsets of RGC types were labelled in each cross. In particular, while the Ret^{CreERt}; $Brn3b^{CKOAP}$ cross labelled six distinct cell types, four major RGC types were labelled in

the Ret^{CreERt}; Brn3a^{CKOAP} cross, and only one cell population (ON-dense – similar to anatomies assigned to the local edge detector in other species) was labelled in the Ret^{CreERt}; Brn3c^{CKOAP} cross (Figure 11). These findings highlight the potential for combinatorial cross-talk between neurotrophic signaling and transcription factors in RGC specification.

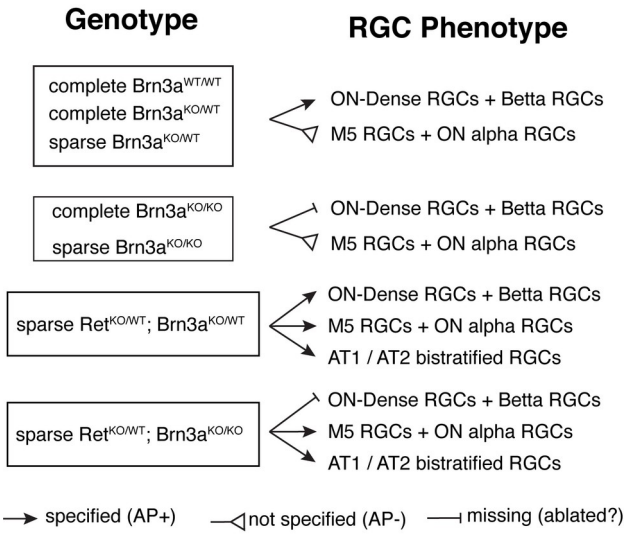


Fig 12 Summary of RGC phenotypes in retinas with different Ret and Brn3a gene dosages.

Since the Ret^{CreERt}; Brn3a^{CKOAP} labelled mostly bistratified and midget-like RGCs when recombination was induced late in postnatal development, we sought to take advantage of this cross in order to get a better understanding of the requirement for Brn3a in midget-like RGC development. We therefore induced sparse random recombination in Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/WT}$ and Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/KO}$ embryos (E15), pups (P0) or adults (P22), and quantified the resulting neuronal morphologies. We found, somewhat expected, that early (E15 and P0), but not late (P22) complete ablation of Brn3a resulted in loss of midget like RGCs.

However, more surprisingly, we found that embryonic (E15) random sparse Brn3a dosage reduction in both Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/WT}$ and Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/KO}$ embryos resulted in a dramatic alteration of RGC type distribution, with two ON laminating RGC types (M5 and OnalphaS) becoming Brn3a positive, and two novel abnormal bi- and tri-stratified RGCs being generated. In addition, small bistratified morphologies, present in retinas from both Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/WT}$ and Ret$^{CreERt2/WT}$; Brn3a$^{CKOAP/KO}$ mice, where missing from the E15 inductions. This effects are surprising, since they appear in both Brn3a$^{AP/KO}$ (homozygote null) and

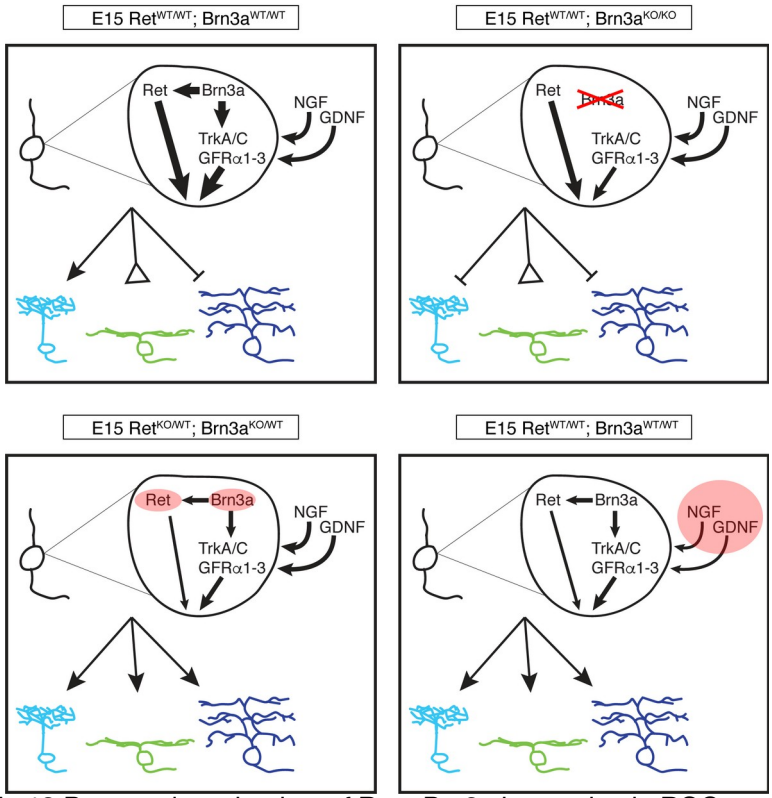


Fig 13 Proposed mechanism of Ret - Brn3a interaction in RGC type specification. **Top left:** proper balance between Brn3a + Ret + Trk dosage results in correct RGC specification. **Top right:** full Brn3a loss results in complete ablation of midget-like RGCs. **Bottom left:** dosage reductions in Ret and Brn3a result in wrong RGC type induction. **Bottom right:** in WT animals, normal dosages of Ret, Trk and Brn3a respond to variable dosages of NGF or GDNF family neurotrophins, resulting in context-dependent cell fate decisions.

Brn3a$^{AP/WT}$ (heterozygote) RGCs, only under circumstances where they lose one copy of the Brn3a gene embrionically, compared to the surrounding tissue (mosaic dosage desequilibrium). This phenomenon does not occur when Brn3a heterozygosity or homozygous null alleles are generated homogeneously across the entire retina, or when the sparse random Brn3a^{CKOAP} allele induction is recombined using other, biologically inert Cre drivers (e.g. the Paxα:Cre or Rosa^{26rtTACreErt}). Thus, only early, sparse double dosage reduction at the Ret and Brn3a loci results in dramatic shifts in RGC type specification and/or dendritic arbor changes (Figure 12). This genetic interaction suggests that the two genes are involved in a pathway that can drive RGC type specification and dendrite morphology, likely by a competitive mechanism with surrounding RGCs. What could be the molecular nature of this mechanism? By interrogating the gene expression profiling data described in section B.II.2.2, and extensive immunofluorescence experiments in Brn3a and Ret knockout retina preparations, we established that Brn3a only mildly modulates Ret transcription, while Ret knockouts exhibitvnormal Brn3a and Brn3b expression. We also found that Brn3a loss of function significantly affects distribution of Ret co-receptors GFRα1-3, and neurotrophin receptors TrkA and TrkC in RGCs. Brn3a loss of function also affects the expression of several intracellular components of the downstream Ret signaling cascade. Based on these observations, we propose that Brn3a and Ret converge onto developmental pathways that control RGC type specification, potentially through a competitive mechanism requiring signaling from the surrounding tissue (Figure 13).

B.II.2.5 Crosstalk between Brn3s and other transcription factors impacting RGC development (#36 Shi 2013, #8 Brodie-Kommit 2021, #7 Chen 2021, #16 Kiyama 2019)

As described in section B.II.2.2, some 1500 transcriptional regulators are expressed and more than 300 are enriched in RGCs, of which nearly 100 are regulated by Brn3 TFs. It is therefore obvious that Brn3s must interact with other TFs in order to generate cell type specific gene expression patterns, resulting in RGC type specification. There is an extensive literature on other TFs involved in RGC type specification, reviewed in #21 Sajgo and #18 Muzyka. Here I will highlight only our contributions and collaborations revealing Brn3 interactions with other transcriptional regulators.

In section B.II.2.1, we discussed the parallel pathway established by Isl1 in RGC type formation. Work from other labs demonstrated that Isl1 knock-outs have reduced numbers of RGCs, accompanied by some loss in Brn3a and Brn3b numbers (Elshatory et al., 2007; Elshatory & Gan, 2008; Mu et al., 2008; Pan et al., 2008; Wu et al., 2015). Conversely, Brn3b ablation results in loss of RGCs, reduction of Isl1 and Brn3a numbers. We found that combined Brn3a-Brn3b ablation removed all RGCs from the retina, with the exception of Isl1^{+},Opn4^{+} ipRGCs. Complementary, Opn4 and/or ipRGCs seem to be under Isl1 transcriptional control (#36 Shi 2013).

Upstream of both Isl1 and Brn3 TFs, Atoh7/Math5 is considered the master regulator of RGC type development (Brown et al., 1998, 2001; Wang et al., 2001). It was previoulsy reported that ipRGCs are not completely ablated in Atoh7/Math5 knockout mice (Lin et al., 2004). In a recent

study from the lab of my long term collaborator, Dr. Hattar, I helped characterize a puzzling phenomenon occurring in Atoh7$^{KO/KO}$ mice in which RGCs were prevented from dying using a genetic apoptosis blockade (Bax$^{KO/KO}$ mice). Our contribution was to provide unique antibodies and help guide the initial stages of RGC characterization in Atoh7$^{KO/KO}$; Bax$^{KO/KO}$ mice. The puzzling finding is that, in these mice, cells expressing markers for RGC fenotype (RBPMS, Isl1, Brn3b and Brn3a) and extending axons are surviving in nearly normal numbers. However these faulty axons do not exit the optic nerve (#8 Brodie-Kommit 2021). The one possible interpretation is that, in the absence of Atoh7, faulty "RGCs" are specified, and these are lacking correct cues for leaving the eye. Other studies have shown that retinas in which Isl1 and Brn3b expression were driven in an Atoh7$^{KO/KO}$ background make a good number of RGCs, further supporting the conclusion that Brn3b and Isl1 in conjunction can specify the RGC cell fate (Wu et al., 2015). None of these papers has looked in any detail at the cell type composition of these populations.

Several TFs are believed to be working downstream of Brn3b (and Isl1) in RGC type specification (Lyu & Mu, 2021). Using our conditional ROSA26^{iAP} and Brn3a^{CKOAP} alleles, in combination with Tbr1^{CreERT2} or Tbr2^{CreERT2} alleles, Dr. C-A Mao at University of Texas was able to isolate the RGCs expressing Tbr1 and Tbr2, two transcription factors regulated by Brn3b (#7 Chen 2021, #16 Kiyama 2019). Together, we used our AP histochemistry and clearing techniques in order to enumerate the RGC morphologies, and the effects of Tbr1 and Tbr2 ablation on RGC numbers and anatomies. Tbr1 is expressed in and required for the development of OFF-DS RGCs (J-RGCs, or JamB expressing RGCs), while Tbr2 is expressed in several RGC types including ipRGCs and Brn3a^{+} subtypes, and plays an essential role in ipRGC survival and Opn4 expression.

Taken together, these studies underline the complexity of the combinatorial transcriptional code of RGC specification, and hence the need for further developing novel intersectional genetics approaches for the labelling and manipulation of neuronal cell types.

B.II.3 Molecular Determinants of RGC type specification

As described in section B.II.2.2, Brn3 transcription factors control a large number of genes with potential functions in neuronal morphology, physiology and connectivity. These results pose the next challenge: how can the functions for each gene be tested within the specific RGC types they are expressed in? The challenge is not made easier by the fact that many of the identified molecules are part of large molecular families, thus raising the possibility of functional redundancy and subtle functional differences between individual family members. Therefore the strategies for loss and gain of function experiments have to be carefully designed. I will illustrate the successes and challenges of these approaches with our studies in two such molecular families: Semaphorins and Copines.

B.II.3.1 Involvement of Semaphorins and Plexins in IPL lamination (#38 Matsuoka 2011, #41 Matsuoka 2011b).

Dendritic arbors of RGCs, together with the axon arbors of bipolar cells and the neurites of amacrine neurons are sharply laminated in the IPL. The mechanisms by which this lamination is achieved are poorly understood, though several cell adhesion molecule families have been implicated in the process (Atkinson-Leadbeater & McFarlane, 2011; Baier, 2013; Garrett & Burgess, 2011; Missaire & Hindges, 2015; Prigge & Kay, 2018; Sanes & Zipursky, 2020).

As part of a collaborative project with the Kolodkin lab at Johns Hopkins, we identified extracellular/transmembrane Semaphorins and their Plexin ligands as potential regulators of retina IPL lamination. The results of this effort demonstrate very clearly that Semaphorins function as repulsive cues that help to delineate the very sharp lamination boundaries of the IPL (#38 Matsuoka 2011, #41 Matsuoka 2011b). They do so via signaling through their receptors, Plexins, expressed on the neurites of retinal neurons. Therefore, in mice lacking either Semaphorin ligands, or Plexin receptors, the stratification levels of different neuronal arbors are shifted, resulting in abnormal lamination of the IPL, and in some instances functional defects at circuit level.

Specifically, removing Sema 6A and its ligand, Plexin A4 from retinal neurons results in the partial displacement of axon arbors for Dopaminergic Amacrine cells as well as their synaptic partners, the dendrites of M1 melanopsin cells. In addition, in mice lacking Sema5A/5B or their receptors PlexinA1/A3, the OFF strata of the IPL are severely disrupted, with neurites of many amacrine, bipolar and retinal ganglion cells being shifted sclerad, and invading the Inner Nuclear Layer (INL), essentially creating a novel, abnormal plexyform layer in the middle of the INL. In contrast, the ON-OFF and ON strata of the IPL look relatively normal.

My involvement in these studies consisted in guiding Ryota Matsuoka, a graduate student in the Kolodkin lab through the phenotypic analysis of his Sema and Plexin mutants, assisting him with some of the RGC axon projection studies, and performing RGC physiology experiments on these mutants. S. Kumar, an IRTA postbac fellow in my group, has analyzed the multielectrode array recordings of RGCs from Sema5A/5B mutant retinas, generated by me, and determined that OFF, but not ON-OFF and ON RGC responses are dramatically reduced. Direction selective responses, mostly mediated by ON and ON-OFF RGCs were also preserved, and so was the ability of the mutant mice to track moving gratings stimuli (OKR responses).

Together, these studies strongly suggest that negative cues play a major role in setting up the correct lamination of the retina, and the correct co-stratification of the neuronal arbors that are synaptically connected.

B.II.3.2 Copines: Ca-dependent membrane adaptors with function in synaptic plasticity (#21 Sajgo 2017, #15 Goel 2019, #1 Goel 2021).

One molecular family identified in our screen for RGC Brn3 transcriptional targets are the Copines, comprised of nine members in the mammalian system, of which five appeared to be enriched or indeed specific to RGCs when compared to other retinal tissues. Copines are intracellular adaptor molecules characterized by the presence of two C2 domains (Ca^{2+}-dependent membrane binding, often found in presynaptic proteins) coupled to an intracellular

vonWillebrand A domain, thought to mediate protein-protein interactions. Previous work has shown that Copines can translocate to the plasma membrane in a Ca^{2+}-dependent fashion, and several members of the family have been identified in various screens for neuron specific molecules, axonal proteins, or synaptic vesicle fractions in the brain. Expression of several of the Copines identified in our screen (Cpne4, Cpne6 and Cpne9) is reduced in retinas of a genetic mouse model of Glaucoma (DBAj). Mutations in the Dictyostelium and Arabidopsis orthologues of Copines result in functional and morphological defects, apparently associated with incorrect intracellular vesicle trafficking. A recent study involved Cpne6 in hippocampal spine structural plasticity and $Cpne6^{KO/KO}$ mice exhibited deficits in learning and memory. These observations suggest that Copines may be regulating interactions of membrane compartments (potentially intracellular vesicles, synaptic vesicles or plasma membrane, neurite processes) with the cytoskeleton, resulting in morphological changes or vesicle trafficking/release, in a Ca^{2+} dependent manner. They could therefore play a role during early postnatal development, when RGCs exhibit waves of correlated spontaneous activity, resulting in massive Ca^{2+} influx, believed to be important for RGC dendritic arbor sculpting and axonal synapse refinement in retinorecipient targets (activity dependent pruning).

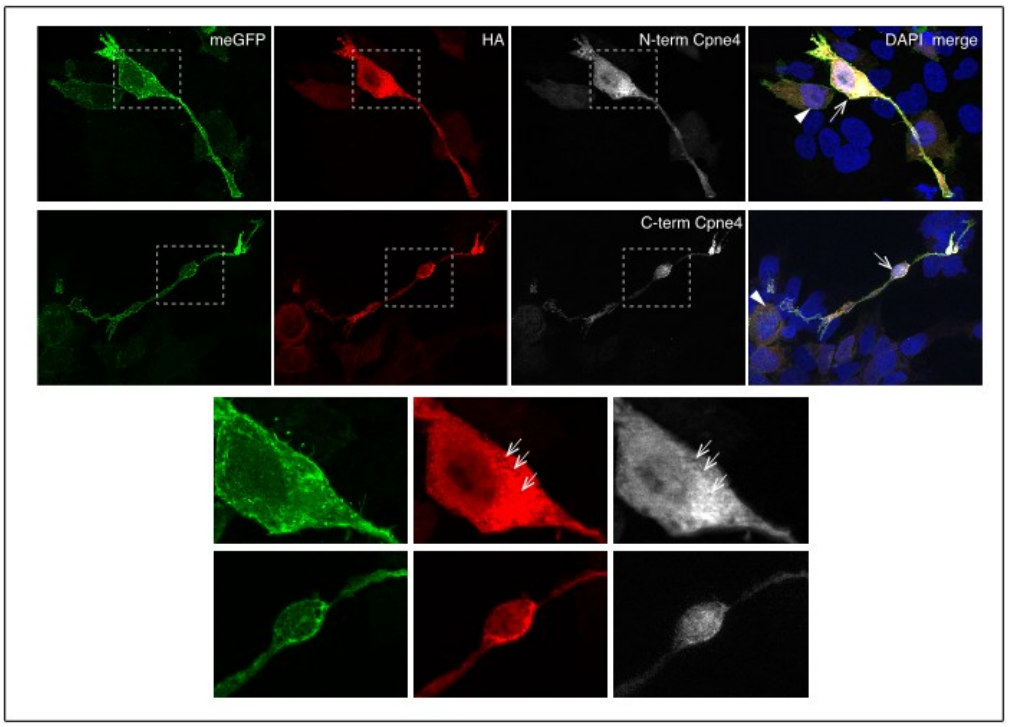


Figure 14 Cpne4 effects on HEK293 morphology. Top two rows, HEK293 cells overexpressing Cpne4 exhibit elongated processes reminiscent of neurites. Membrane attached eGFP (meGFP) labels membranes while Cpne4 (labelled here with either anti-Cpne4 antibodies or anti-HA antibodies) is present in these processes, on the plasma membrane or in fine intracellular vesicles (shown in insets in the bottom row.

Our RNAseq screen for RGC specific molecules revealed that Cpne4, Cpne5, Cpne6, Cpne8 and Cpne9 were enriched in RGCs when compared to the retina, at P3 but not E15. We have performed In situ hybridization on $Brn3b^{WT/WT}$ and $Brn3b^{KO/KO}$ mouse retinas at P0, P3, P7, P12 and adult using probes against the 3' UTRs of the five Copines, and find that Cpne4 is expressed specifically in a subset of RGCs, while the other four are expressed dynamically in many or most RGCs and subsets of inner nuclear layer cells, with Cpne9 showing the broadest and most intense pattern, essentially covering the whole Ganglion Cell Layer and Inner Nuclear Layer by the adult stage. As predicted by our RNASeq data, Copine expression begins largely at P3, coincident with RGC

dendrite formation and synaptogenesis. Several of our target Copines are Brn3b dependent, as their levels decrease drastically in Brn3b$^{KO/KO}$ retinas. We find that overexpression of Cpne4 in HEK293 cells results in elaboration of cellular processes reminiscent of neurites (Figure 14). In addition, we find that Cpne4-HA, overexpressed in Brn3b$^{Cre/WT}$ RGCs using AAV delivery, is localized to dendritic processes and the soma, and adopts a punctate pattern, reminiscent of synaptic puncta.

We then used commercially available and in house generated antibodies for Cpne4, Cpne5, Cpne6 and Cpne9 to characterize the cell type distribution and subcellular localization of these molecules. Cpne5, Cpne6 and Cpne9 are expressed in a majority of GCL and inner INL cells, suggesting a broad expression in RGCs and amacrine neurons. In addition, Cpne9 is also highly expressed in a population of bipolar cells (type 9 bipolars, as reported elsewhere).

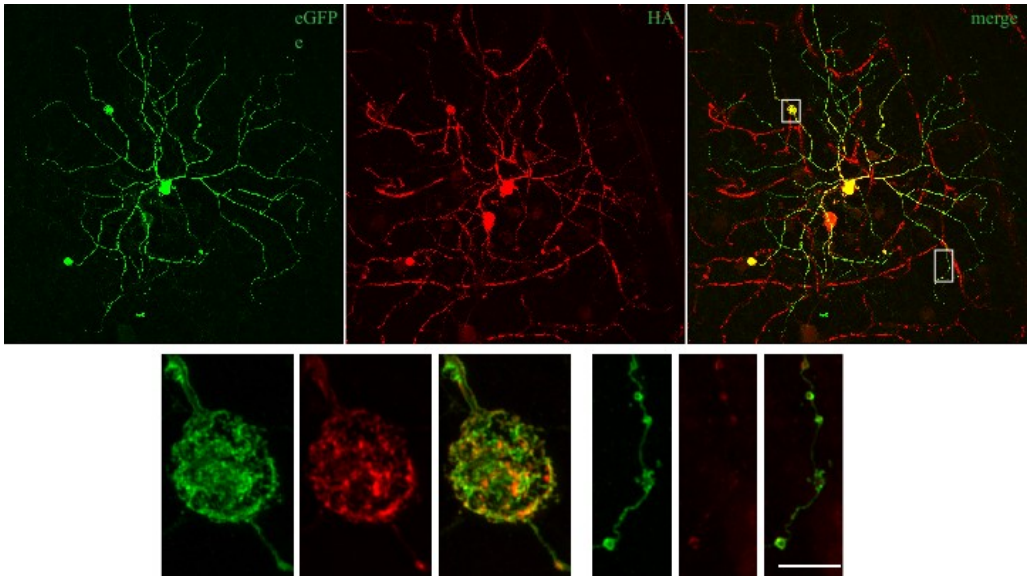


Figure 15 Cpne4 effects on RGC morphology. Top: example of RGC infected with a virus expressing HA-tagged Cpne4 in conjunction with membrane attached eGFP. Dilations of the dendritic arbor are visible along the dendrites (bottom row - left insets). These "blebs" contain plasma membrane (meGFP) and Cpne4 (HA staining), and are significantly larger than the small "beads" typically seen along dendritic arbors (bottom row - right insets).

Cpne4 expression appears to be restricted to RGCs, and one large field amacrine cell population. It's expression in the GCL is significantly reduced in Brn3b$^{KO/KO}$ retinas, and the remaining signal is evenly split between the surviving RGCs and amacrine cells. Since it is the most RGC-specific of Copines, we further investigated Cpne4 function and binding partners. Potential Cpne4 interactors were identified using yeast two-hybrid screens with Cpne4-vWA baits and mass spectrometry analysis on retina protein pull-downs with GST-Cpne4 and GST-vWA. Amongst the validated Y2H interactors, we fond several proteins with functions in neuronal arbor formation and endocytic/autophagy pathways. The mass spectrometry screen yielded a set of some 200 proteins, amongst which metabolic, signaling, intracellular vesicle traffick and neurite/process formation pathways were highly enriched. Consistent with a role in vesicle/membrane trafficking, overexpression of Cpne4 in RGCs resulted in swellings of the dendritic arbor, filled with Cpne4 attached to membranes/vesicles, suggestive of a disruption of the vesicle trafficking along the dendrite (Figure 15). However, no major defects were noticed in the overall morphologic arrangement of the dendritic arbor (area, lamination, etc.). Our next objective will be the understanding of organelle interactions for Cpne4, and the generation of loss of function alleles in mice.

B.II.4 Characterization, Development and Function of ipRGCs

While rods and cones are the main photoreceptors of the vertebrate retina, other cell types can also express photopigments and exhibit light sensitivity, in a species-dependent manner. Intrinsically photosensitive RGCs (ipRGCs) are unique in that they express the photopigment Opn4/Melanopsin, and can modulate their membrane voltage and alter their firing pattern even in the absence of synaptic input relaying light information from photoreceptors via bipolar cells. Melanopsin expression in ipRGCs and its involvement in circadian photoentrainment and pupillary light reflex were discovered in 2001, by Ignacio Provencio, Samer Hattar, King Y Yau, and David Berson (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2000). However, the roles of ipRGCs in visual function are still not fully explored. During my postdoctoral training at Johns Hopkins and as an Investigator at the NEI, I collaborated with Samer Hattar and Phyllis Robinson in several studies that elucidated some important questions of ipRGC and Melanopsin function and physiology.

B.II.4.1 Circadian Photoentrainment (CP) and Pupilary Light Reflex (PLR) are controled by two distinct types of ipRGCs (#46 Güller 2008, #45 Badea 2009, #39 Chen 2011)

While both CP and PLR can be elicited in mice lacking conventional photoreceptors (rods and cones), the two reflexes are ablated when Opn4/Melanopsin is additionally knocked out, thus demonstrating that ipRGCs can directly receive light input (Hattar et al., 2003). In order to demonstrate that ipRGCs themselves are required for CP and PLR, I assisted Samer Hattar and KY Yau to insert a diptheria toxin A subunit (DTA) cDNA in the Opn4 locus, and ablate all Opn4-expressing RGCs, resulting in complete loss of CP and PLR. In addition to suggesting the experiment and providing critical reagents, I also assisted with performing the tracking of RGC axons into retinorecipient areas, and demonstrating that, indeed,

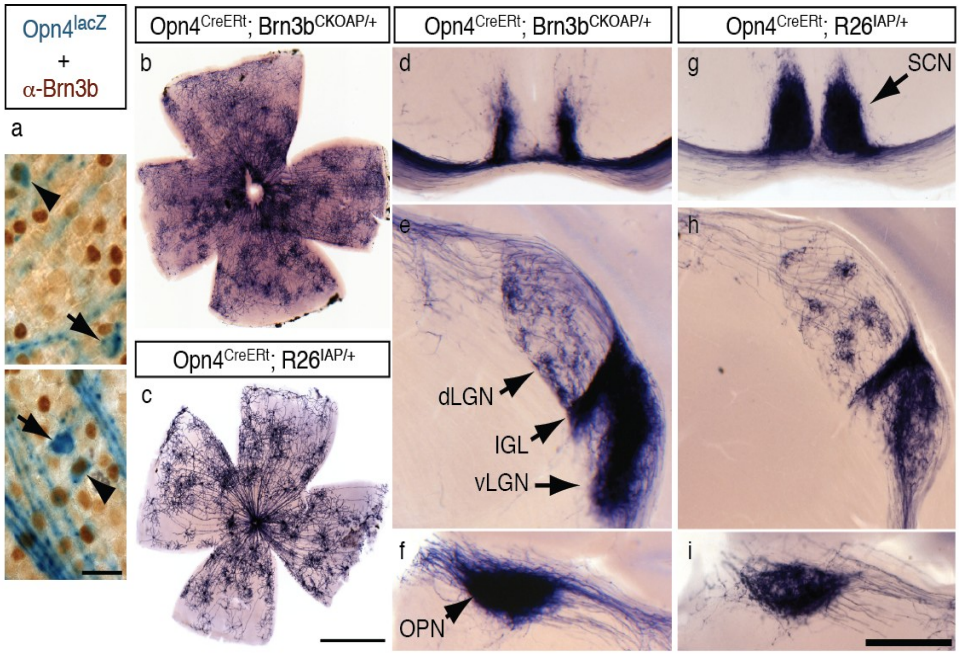


Figure 16 Opn4 and Brn3b expression overlap. a, Brn3b protein is coesxpress with some but not all Opn4^{lacZ} RGCs (a). Opn4^{CreERt} induction of the general AP reporter (Rosa26^{iAP}) labels RGCs that project to the SCN, IGL, vLGN and OPN (c, g-i), while Opn4^{CreERt} ; Brn3b^{CKOAP} induction labels RGCs that project to OPN, IGL, vLGN, but only label the lateral aspects of the SCN (b, d-f).

the Suprachiasmatic Nucleus (SCN) and Olivary Pretectal Nucleus (OPN) had lost their inputs. This then confirmed that ipRGCs control CP and PLR by providing visual input to the SCN and OPN (#46 Guller 2008). In parallel, my work with the Brn3a^{CKOAP} and Brn3b^{CKOAP} alleles was showing that, while both Brn3a^{+} and Brn3b^{+} RGCs projected to the OPN, neither had projections to the SCN . Moreover, CP is affected with partial penetrance in Brn3$^{KO/KO}$ mice, while RGC projections to the OPN are greatly diminshed and the PLR nearly absent (#45 Badea 2009). Overall, our genetic conditional reporter analysis strongly suggested that some Brn3b, but not Brn3a or Brn3c RGC dendritic arbor morphologies and central projections were overlapping with those of Melanopsin (Opn4) positive ipRGCs. Taking advantage of this partial overlap, in collaboration with the Hattar group in Hopkins, we defined two distinct ipRGC cell populations: (I) Opn4^{+}Brn3b^{-} and (II) Opn4^{+}Brn3b^{+}. ipRGCs expressing Melanopsin but not Brn3b belong exclusively to the M1 morphological cell type and project to the suprachiasmatic nucleus (SCN), responsible for circadian photoentrainment. M1 and non-M1 ipRGCs that express both Brn3b and Melanopsin send very few projections to the laterocaudal aspect of the SCN, however innervate the Olivary Pretectal Nucleus (OPN, which relays information necessary for pupil constriction reflex – PLR), and parts of the lateral geniculate nucleus and superior colliculus. Taking advantage of this knowledge, together with S-K. Chen and S. Hattar we genetically ablated specifically the Brn3b positive, Melanopsin positive neurons in mice, and demonstrated that in these animals PLR is almost completely removed, whereas circadian photoentrainment is unaffected. These results demonstrate that one neuronal cell population, having the same dendritic arbor morphology and molecular marker (melanopsin), can be separated into two cell types, based on the distinction of axonal targets in the brain (SCN vs. OPN), and their roles in two distinctive circuits (Circadian Photoentrainment vs. PLR). This raises new complexities for anatomists and physiologists involved in classifying neuronal populations, as it shows that the definition of a cell type may require physiological as well as several layers of molecular and anatomical evidence.

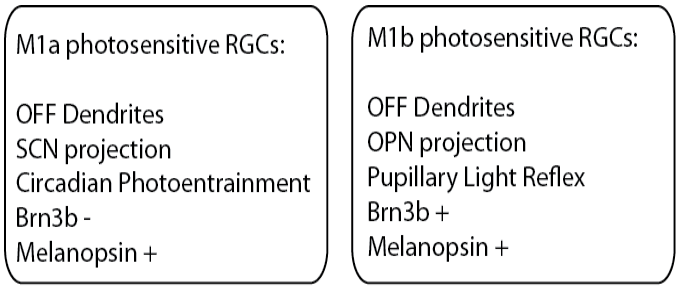


Fig 17 Properties of Brn3b- (left) and Brn3b+ (right) M1 ipRGCs

This study further supports our previously stated hypothesis that Brn3b might be involved in the development of axonal processes in RGCs. In this particular instance, the distinction in axonal targeting of RGCs to separate nuclei is brought about by a distinction in Brn3b expression.

It is quite interesting to note that the light information for circadian phototentrainment, a visual behavior integrating light information over extremely long time periods is transmitted to the brain by a different channel than pupil constriction, a reflex which can adapt pupil diameter to light conditions within seconds.

B.II.4.2 ipRGCs Development (#40 McNeill 2011, #36 Shi 2013, #7 Chen 2021)

Given the partial overlap between Brn3b and Opn4 in RGCs (Section B.II.4.1), and our conclusion that Brn3b is required for the development of most but not all RGC types, it is interesting to understand how Opn4$^+$ ipRGCs develop. Opn4$^+$ ipRGCs survive combined ablation of Brn3a and Brn3b but are completely missing from retinal Isl1 knockouts (Section B.II.2.1, #Shi 2013). In addition, Opn4 expression and maintenance and ipRGC specification require Tbr2 (Section B.II.2.5, #7 Chen 2021). Intriguingly, some Opn4$^+$ cells escape even Atoh7 ablation (Lin et al., 2004) and (#8 Brodie-Kommit 2021). Thus, ipRGCs, and in particular the M1 population, that projects to the SCN, are a very distinct RGC subpopulation, that is controlled in a very distinct fashion from other, “conventional” RGCs. ipRGCs are born around the same time as other RGCs (E11-E15), and innervation of their main target nuclei, SCN and OPN is largely postnatal, similar to the timing of “conventional” RGC axon arrival in the LGN and SC, as we have shown in collaboration with Dr. Hattar and Guido (#40 McNeill 2011). For this particular study, my contribution consisted in contributing experiments and reagents to contrast ipRGC development with "conventional" RGCs. Given that the SCN is the first retinorecipient station in the path of RGCs form the eye to the brain, located right above the optic chiasm, in the hypothalamus, an evolutionary ancient vertebrate brain nucleus, it is tempting to speculate that ipRGCs are the primordial light sensing neurons of primitive vertebrates providing basic light sensing capacity, upon which further developmental programs have elaborated, to innervate more sofisticated brain regions that allow for more complex visual computations. In support of this, Opn4, and its downstream signaling cascades are phillogenetically related to rabdomeric photopigments, like the ones used by Drosophila photoreceptors, neurons, which, like ipRGCs extend axons from the fly eye to the brain (Contreras et al., 2021).

B.II.4.3 Physiology of Melanopsin and ipRGCs (#27 Keenan 2016, #25 Somasundaram 2017)

Given the large number of candidate RGC specific genes we discovered as Brn3 targets we needed to develop a relatively fast approach for interogating their function in RGC development and physiology. We therefore designed a set of Cre dependent AAV constructs that couple the expression of a tagged version of the gene of interest with the expression of a membrane attached eGFP, in order to visualize the infected cells and easily asses their anatomy and physiology (section B.II.2.2). We have applied this approach to the analysis of response properties of intrinsically photosensitive RGCs that carried mutant versions of the photopigment Melanopsin in a collaborative project lead by Drs. P. Robinson of University of Maryland Baltimore County and S. Hattar. This research, for which we contributed Cre-dependent AAV targeting vectors, multielectrode array recordings of normal and mutant retinas (recordings and analysis done by P. Somasundaram A. Ghahari in my lab, see section B.II.6.2 below) and behavior apparatus and expertise (section B.II.6.5 below), demonstrated that phosphorylation of the c terminal tail of Melanopsin plays a key role in the shut-off of the photopigment, and

regulates the length of both ipRGC light responses and Pupillary Light Reflex recovery times. In the absence of c-terminal phosphorylation, ipRGC light responses cannot be turned OFF, and persist in the absence of light stimulation, resulting in abnormal, prolonged pupil constriction, long after the light stimulus has been turned off (#25 Somasundaram 2017). We also used our viral vectors to dissect the contributions of rods, cones and ipRGCs in the transient and sustained phases of the PLR (#27 Keenan 2016).

B.II.5 Transcriptional control of projection somatosensory neurons

Transcriptional control of neuronal cell type specification is conserved across evolution, all the way to Sea Urchin, Nematodes (C. Elegans) and Fruit Flies (D. Melanogaster) (Hobert, 2011; Jessell, 2000; Komiyama & Luo, 2006). The specification of various sensory neurons in these species relies on homologues or orthologues of the three mamalian Brn3/POU4f genes. Vertebrates use several major classes of projection sensory neurons: proprioceptors (muscle and tendon tension), mechanoreceptors (various form of touch and vibration), nociceptors (pain), thermoreceptors (temperature) and itch receptors, have cell bodies located either in the Dorsal Root Ganglia (DRG - for body somatosensation) or Trigeminal Ganglion (TGG - for head somatosensation), send their sensory endings in the skin, muscles and bones throughout the body), and their axonal projections to various stations in the spinal chord and brainstem, in order to convey the information to more central nuclei, and ultimately to the concious perceptual centers in the cortex. In a similar fashion, auditory (Spiral Ganglion - SG) and vestibular (Vestibular Ganglia - VG) projection sensory neurons receive inputs from hair cells in the cochlea, semicircular canals, sacula and utricula and send it via their axonal projections to the central nuclei in the brainstem. These trajectories resemble to some extent those of RGCs, and neurons of the DRG, TGG, SG and VG express Brn3 transcription factors in a variety of combinations. Using our combinatorial genetic strategies, we characterized the distribution of Brn3 transcription factors in these classes of projection sensory neurons. As a corollary to this findings, molecular pathways that are employed across these neuronal cell classes could be re-used in the visual system, as was the case for the cooperation of the Ret neurotrophic factor and Brn3s (see section B.II.2.4 and section B.II.5.1).

B.II.5.1 Brn3 combinatorial code in DRGs (#37 Badea 2012, #35 Niu 2013, #9 Oliver 2021)

Understanding the anatomy of projection sensory neurons is particularly difficult, since both the target territory and the central projections can be at really long distance from the cell bodies, placed in the DRG or TGG. We have used sparse random recombination and immunostaining in conjunction with a variety of cell type markers to describe the anatomies of DRG neurons expressing Brn3a, Brn3b and Brn3c. Specifically, Brn3c^{AP} neurons were peptidergic nociceptors, Brn3b^{AP} DRG neurons were mechanoreceptors, while Brn3a^{AP} is expressed in most described DRG cell types, including mechano, proprio and nociceptors. Using AP histochemistry in sparsely recombined Brn3^{CKOAP} animals, we were able to image and

reconstruct individual arbors of sensory endings derived from individual neurons for the first time in the mouse. In addition, we demonstrated that Brn3a defective Brn3a$^{AP/KO}$ DRGs exhibited a specific loss of hair follicle associated sensory fibers, coupled with a depletion of projections to the dorsal horn of the spinal chord, most likely signifying a dramatic loss of mechanoreceptor DRGs (#37 Badea 2012). This approach was further developed and applied in follow-up experiments by many labs, resulting in refined characterization of projection sensory neurons.

As an example of such cross-talk, in our collaboration with Drs. Niu and Luo at U.Penn, we used our genetic approaches to define the distribution of Ret^{+} mechano - and Parvalbumin^{+} proprioceptive projection sensory neurons in the dorsal columns of the spinal chord (#35 Niu 2013). The sensitivity of our AP reporters allowed our collaborators to follow the centrally projecting axons of these somatosensory neurons throughout the entire spinal chord to their targets in the brain stem.

In addition, we collaborated with Dr. de Nooij at Columbia University, (providing mouse lines and antibodies), to characterize a subpopulation of proprioceptive DRG neurons that also express Brn3c, namely group Ib Golgi Tendon Organ afferents, thus extending our previous observation (#9 Oliver 2021).

B.II.5.2 Brn3b and Brn3c distribution in cranial nerves and the brainstem (#30 Sajgo 2016, #11 Parmhans 2021)

Pou4f expression in projection sensory neurons of the two chemical senses (taste and

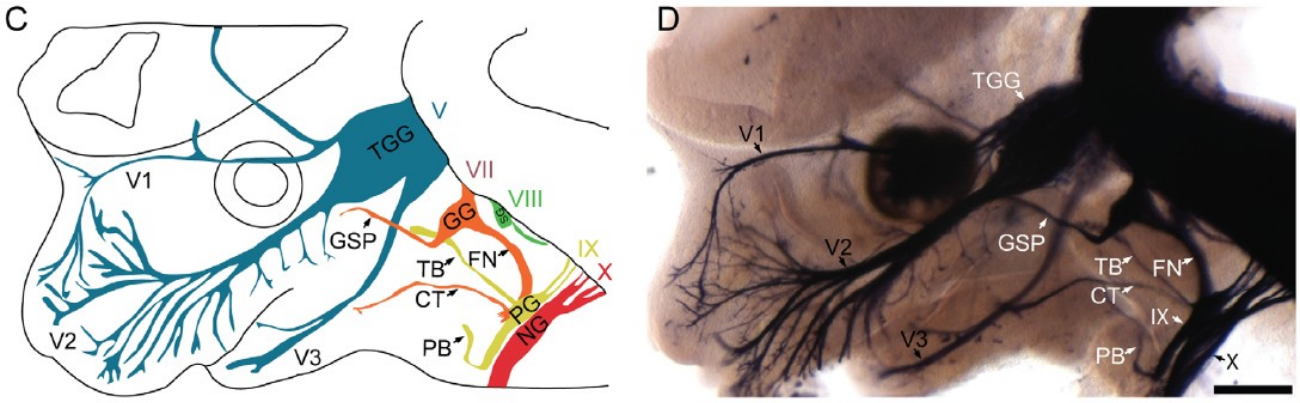


Figure 18 Brn3b^{+} cranial nerves together with their ganglia, labelled in a Rosa26^{CreER}; Brn3b^{CKOAP} E13 embryo.

smell) of vertebrates have not been reported, although the fruit fly (D. Melanogaster) homologue gene, Acj6, is a major regulator of olfactory neurons. We therefore conducted a developmental profile of Brn3b expression in cranial nerves and nuclei of the brainstem. We found that Brn3b is dynamically expressed in the somatosensory component of cranial nerves II, V, VII, and VIII and visceromotor nuclei of nerves VII, IX, and X and other brainstem nuclei, but that no olfactory or taste pathways are positive for Brn3s (e.g. Figure 18). Interestingly, all Brn3s also label bulb pontine and mesencephalic nuclei of the sensory pathways (#37 Badea 2012, #30 Sajgo 2016, #11 Parmhans 2021). Thus, in the mouse, Brn3 combinatorial codes are utilized by all major sensory pathways with the exception of taste and smell. These findings suggest a “marking” of

the ascending sensory pathway by Brn3s. Together with previous work, it appears that at the level of cranial nerves, Brn3s are participating in sensory and visceral pathways, Phox2 transcription factors mark mostly visceral pathways, while Islet transcription factors define motor neurons and sensory projection neurons. These findings are interesting from an evolutionary perspective, but have also practical implications, as molecular mechanisms, genetic tools and strategies can be shared between the various systems to refine our circuit analysis in the visual system.

B.II.6 Visual functional evaluation in genetically modified mice.

Understanding of the circuitry, functions and pathological mechanisms of the visual system in humans is highly dependent on animal models. Mammals with visual systems more similar to those of humans (primates, monkeys, pigs, dogs) are necessary at certain levels of the analysis (e.g. mechanisms of cortical function, final tests of therapeutic approaches), and embryonic stem cell-derived retina organoids derived from healthy donors or human patients help unravelling molecular mechanisms of disease. However, the basic understanding of molecular neuroscience and circuit layout, as well as the heavy duty pharmacologic and toxicity studies need mice as a small, more ethically acceptable and genetically tractable disease model. With regard to the analysis of the function, development, treatment and repair of the visual system, understanding not just the anatomy and molecular layout, but also the visual function in mice is paramount. While mice rely far more on their other senses compared to vision for navigating the world and making decisions, their visual system is quite developed. Training mice to visual cues has made huge progress, and is complemented by a large array of visual tasks that rely on more simple reflexes that allow quick and reproducible testing of visual function within the retina and at higher stations of processing. Over the years, we applied and/or helped develop several of these methodologies, based on the needs for the analysis of our genetically modified animals. In trying to understand RGC types, we also thrive to understand their functions within the visual circuit at whole animal level, by challenging our genetically modified animals with a variety of visual tasks.

Our genetic labeling approaches in mice mutant for the Brn3 transcription factors revealed that Brn3b$^+$ RGC populations target distinct retinorecipient areas of the brain and that loss of function in the Brn3 factors result in discrete defects in specific RGC subpopulations. For instance, loss of Brn3b resulted in a) complete ablation of ON-DS RGC projections to the Medial Terminal Nucleus and the vertical optokinetic reflex; b) significant reduction in horizontal optokinetic reflex and loss of ON-DS RGC inervation to the Lateral and Dorsal Terminal Nuclei and the Nucleus of the Optic Tract; c) severe impairment of ipRGCs projecting to the Olivary Pretectal Nucleus accompanied by almost complete loss of the pupillary light reflex d) mistargeting of some Brn3b$^+$ RGCs to the Suprachiasmatic Nucleus and partially penetrant defects in circadian photoentrainment. (see section B.II.2.1). These findings convinced us of the necessity of further exploring visual function assays in order to document the functional consequences of our genetic manipulations.

In recent years, several subpopulations of RGCs were targeted for electrophysiological recordings using patch clamp physiology, based on the availability of mouse lines genetically modified to selectively express fluorescent reporters in them. Nevertheless, in many cases, it is desirable to have broad access to the entire RGC population, in order to assess functional changes induced by manipulations that could impact the function of the whole retina, or many RGC types simultaneously. We therefore applied and/or developed in vitro analyses of whole retinal function, using multi-electrode array recordings of retinal preparations subjected to visual stimuli of a varied nature.

B.II.6.1 Multielectrode array (MEA) recordings of RGCs in mice with mutations in the Frizzled or Semaphorin pathways (#44 Ye 2009, #41 Matsuoka 2011)

The simultaneous recording of activity patterns in large populations of neurons is one of the major goals of modern neuroscience. In the context of Retinal Ganglion Cells, this has been achieved by two approaches, MEA recordings or Ca imaging. Ca imaging allows the unambiguous identification of every detectable RGC cell body in a preparation, but the connection between fluorescent signals resulting from Ca influx into the cell and the bona fide Action Potential discharges is somewhat ambiguous, given the much slower dynamics of intracellular Ca concentration changes produced by AP firing (T. Badea et al., 2001; Rupprecht et al., 2021; Tank et al., 1988; Yuste & Katz, 1991). MEAs allow the identification of AP spike trains from individual RGCs, but the number of cells detected is highly dependent on the preparation, and only very rarely can all RGCs from a preparation be isolated (Meister et al., 1991, 1994). In order to have relatively rapid access to a large population of RGCs in normal and pathological conditions, I implemented a MEA pipeline to study RGC responses to a battery of visual stimuli. One of the difficulties of this approach is that a large fraction of RGCs exhibit center-surround type receptive fields, and therefore the optimal response features of each cell can only be studied with stimuli centered on the “center of mass” of the dendritic arbor. To circumvent this issue, we combined stimuli that sweep broadly across the retinal preparation with checkerboard stimuli that permit the mapping of the receptive field with high resolution, and subsequently determined the responses of each cell when the sweeping stimuli overlapped optimally with the RGC receptive field. Our set of stimuli, created with the Psychophysics Toolbox, consisted of a) full field (2 sec ON + 2 sec OFF) black-white alternations, b) 25 tiles (200 µm diameter in a 5 x 5 tiled square) each 2 sec ON + 2 sec OFF, c) Checkerboard stimulus movie (24,000 frames): Each movie frame consisted of a 25 x 25 grid of black or white squares presented for 53.6 msec. Each square was 40 x 40 µm in the optical plane of the retina, and arrangement of squares were pulled at random from the computer’s random number generator, d) Direction selectivity stimuli (DSbars): bars sweeping in each of 8 directions at intervals of 45 degree were presented (10 sweeps/direction) at a speed of 300 µm/second (at the retina). Four bar shapes/colors were used: narrow (100 µm) or wide (240 µm), black or white. For all stimuli, white refers to screen Lookup table (LUT) values [255 255 255] and black to Lookup table values [0 0 0], and the screen background was set to LUT [126 126 126]. Given screen brightness and

light path conditions, these stimuli are expected to stimulate the retina under photopic conditions. These stimuli were presented to ex vivo retinal preps from wild type or genetically engineered mice, and spike (Action potentials) trains were collected from the 60 electrodes of a multielectrode array system built by Multichannel Systems. Spike trains for individual RGCs were diagnosed for each electrode using a clustering algorithm based on iterative K-means approach. Responses to each stimulus were determined for all individual RGCs, and visual response parameters were derived, including latency, ON vs. OFF and direction selectivity indices and receptive field sizes.

We applied this methodology in several contexts in which genetic manipulations induce global changes to retinal anatomy and function. In mice mutant for Frizzled 4, a Wnt receptor that also signals in response to norrin (the gene affected in Norrie disease), the retinal vasculature is severely underdeveloped, leading to lack of function in retinal neurons that otherwise develop normally. However, we demonstrated that RGCs from retinas with impaired vascularization, as seen in Frizzled 4 mutants, are able to respond to visual stimuli when isolated from the animal and supplied with oxygen and metabolites via a perfusion system, but are unable to do so when presented with similar stimuli in the live behaving animal. In addition, we applied our MEA pipeline to the analysis of retinas lacking Semaphorins 5a/5b, and demonstrated that OFF-type responses were severely impaired, consistent with the anatomic defects of the OFF layers of the IPL in these mutants (see section B.II.3.1).

B.II.6.2 Exploring and applying novel MEA spike sorting approaches based on feature extraction (#26 Ghahari 2016, #25 Somasundaram 2017, #19 Ghahari 2018).

The separation of individual neuronal responses recorded simultaneously on extracellular electrodes is based on the ability to describe, sort and classify action potential (spike) shapes characteristic of each neuron. This is a computationally intensive task, and especially difficult if real-time spike sorting is to be achieved. Historically, in order to save computer power and/or storage space, spikes were detected through hardware circuits, cut out of the continuous recording waveform digitized and stored as Spike-cutouts (e.g. 76 observations for one spike recording of 3 milliseconds recorded at 25000 Hertz) for further analysis. From here on, two general approaches were possible. One consists of extracting principal components from the 76 timepoints of the waveform, and applying a variety of clustering algorithms in order to separate the waveforms of each neuron. This, understandably is a very computationally costly alternative, and is hard to apply in real time. A second general approach is to extract basic features from the spike waveform (e.g. time – to peak, maximal voltage amplitude, minimal voltage amplitude, duration, etc.), and then perform clustering on this dataset that has benefited from the dimensionality reduction. For the experiments described in section B.II.6.1, spike sorting was done using the full Waveform – PCA approach, resulting in long spike-sorting processes, often lasting for several days and multiple iterations.

Therefore, Alireza Ghahari, a postdoctoral fellow in my lab, designed a novel spike sorting pipeline, that applied advanced data filtering (based on eigenvector decomposition), spike detection (using an adaptive threshold) and feature extraction methodologies in order to generate a fast and robust spike sorting protocol. He used i) negative maximum deflection ii) positive maximum deflection, iii) time delay between negative and positive deflections, and iv) spike width at threshold as the four defining features of the spike form (Figure 19). Cluster numbers and initial centroid assignment is determined using a leader-follower clustering algorithm, based on the 4 features, followed by an error minimization protocol, applied to refine clustering. A quality control procedure, involving inter-spike interval assessment, amplitude scale factor analysis and intra-electrode correlation is applied, and initial clustering parameters are adjusted for clusters failing quality control. Finally, cells detected on multiple electrodes are identified by comparing templates of clusters detected in these electrodes.

In order to increase the robustness of the algorithm, a series of diagnostic tests trigger iterations of the filtering, detection and sorting steps based on improved parameters derived from the analysis of the resulting clusters. This approach could be implemented in conditions of low computational power coupled with the need of realtime spike detection, as is the case for human-machine interfaces requiring neuronal control of prosthetic limbs. Alireza applied his approach to the identification of ipRGCs in retinal preparations from mice carrying either wild type or mutant forms of Melanopsin, and was able to show that ipRGCs missing phosphorylation sites on their c-terminal tail were exhibiting prolonged light responses, many hundreds of seconds after turning off the stimulus (see section II.4.3, #25 Somasundaram 2017).

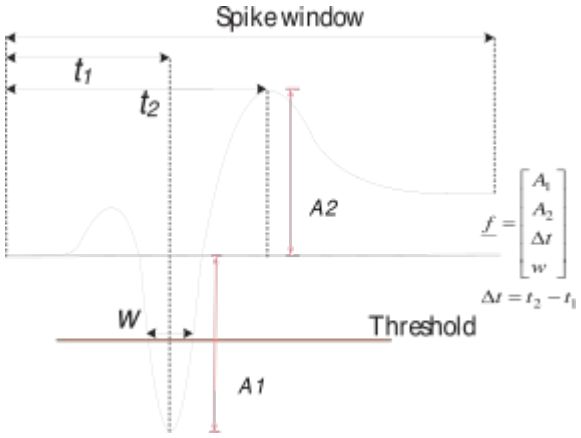


Fig 19 Spike Features for analysis and clustering of individual units. t0 marks -0.5 msec from waveform negative deflection passing the threshold. t1, A1 are timepoint and amplitude for maximal negative deflection. t2, A2 are timepoint and amplitude for maximal positive deflection.
w = width of negative deflection at threshold.

B.II.6.3 Identifying stimulus response properties in Brn3b$^{KO/KO}$ RGCs using GPU-based clustering methods (Kumar, Lin & Badea, ICON4N conference, Romanian Academy Bucharest 2021)

More recently, we implemented an approach employing the entire spike waveform, Kilosort, designed by M. Pachitariu, and which exploits GPU-based programming to perform cluster analysis. This approach allowed us to analyse RGC responses in retinas from Brn3b$^{KO/KO}$ mice, and compare them to wild type cells. We found that Brn3b$^{KO/KO}$ retinal preparations have less active RGCs, that respond disproportionately to “ON” stimuli, and have fewer OFF RGCs, that are larger in size compared to controls. As a population, Brn3b$^{KO/KO}$ RGC responses are delayed (longer latency relative to stimulus onset), and less sustained (shorter decay times) than the controls. ON-OFF RGCs are significantly enriched in Brn3b$^{KO/KO}$ retinas, with a modest, but significant enrichment in direction selective cells. These findings are consistent with the

enrichment in bistratified RGCs seen in Brn3b$^{KO/KO}$ retinas (section B.II.2.1), and offer a physiological counterpoint to our anatomical analyses.

B.II.6.4 Contributions to the characterization of novel methodologies for measuring cell monolayer impedance (#13 Gheorghiu 2020a, #14 Gheorghiu 2020b)

We have also assisted for many years Eugen and Mihaela Gheorghiu at IBD Bucharest in their efforts to establish a novel methodology based on whole tissue/cell electrical resistance for assessing cell health and metabolic status. These noninvasive impedance sensing methodologies could be employed to study the effect of various noxious elements in vitro. Our (Miruna Ghinia in my lab) specific contribution consisted in generating HEK293 cell lines genetically engineered to stably express Channel-Rhodopsin. These cells were instrumental in calibrating the impedance measurements in cell monolayers, by using light-induced HEK293-ChRh cell membrane conductance changes.

B.II.6.5 Characterization and contrast of Optomotor and Optokinetic reflexes in wild type and mutant mice (#32 Kretschmer 2015, #29 Wang 2016, #24 Wang 2017, #22 Kretschmer 2017, #2 Chuang-in press).

The retinal image of the surrounding world is continuously updated, as a result of eye, head, and body movements we are engaged in. However the visual perception is stable, and we correctly infer that our surroundings are at rest while we are moving in them. This seemingly trivial insight is the result of complex neuronal mechanisms involving visual, vestibular and proprioceptive signals, that report the status of body and head self motion as well as muscle contraction throughout the body and especially in the muscles that control eye motion within the socket. When a disconnect between the information provided by the three sensory systems occurs, for instance in the damage of the inner ear vestibular organ, our visual system attempts to compensate the error by rapid eye and/or head movements that take the form of the optokinetic nystagmus (Masseck & Hoffmann, 2009; Rucci & Victor, 2015; Simpson, 1984; Spering & Carrasco, 2015; Stahl, 2004). The relative contributions of head and eye movements to image stabilization in mice are poorly understood.

We have developed a novel dual functionality setup that can be used to automatically evoke compensatory eye movements (optokinetic reflex - OKR) and head movements (optomotor reflex - OMR) responses in mice (#32 Kretschmer 2015, Figure 20). OMR responses, typically reported by a human observer, are relatively easy to collect and find broad use in the evaluation of visual function in mouse models of genetic defects, disease or therapeutic intervention. OKR determinations are more challenging but yield more stereotypical, easily quantifiable results. Our apparatus records head and eye-movements automatically and determines various properties of the visual system in a robust, fast, and objective way, without the necessity for human intervention. Stimuli are presented on four computer screens surrounding the animal covering the whole field of view in form of a texture on the surface of a virtual sphere. Head and body movements of unrestrained mice sitting on a platform are

recorded by video tracking the snout and body axis of the animal in real time. Our self-developed video tracking algorithm continuously determines the location of the animal head that is used to readjust the position of the virtual sphere.

This is an important requirement for measuring parameters like spatial acuity that are dependent on the perceived size and therefore on the distance between the head and the presented stimulus. The measurement of eye movements is performed on mice with implanted head mounts, placed in a holder in the center of the virtual arena. When recording the eye movements, the system is calibrated in a semi-automated manner, allowing us to precisely quantify angular eye-velocities. Presentation of visual stimuli and detection of head or eye movements are all performed using an integrated software suite including a graphical user interface, developed in its entirety by us, and the apparatus is assembled from readily available parts, with the exception of the eye tracker that is a commercial EyeLink setup.

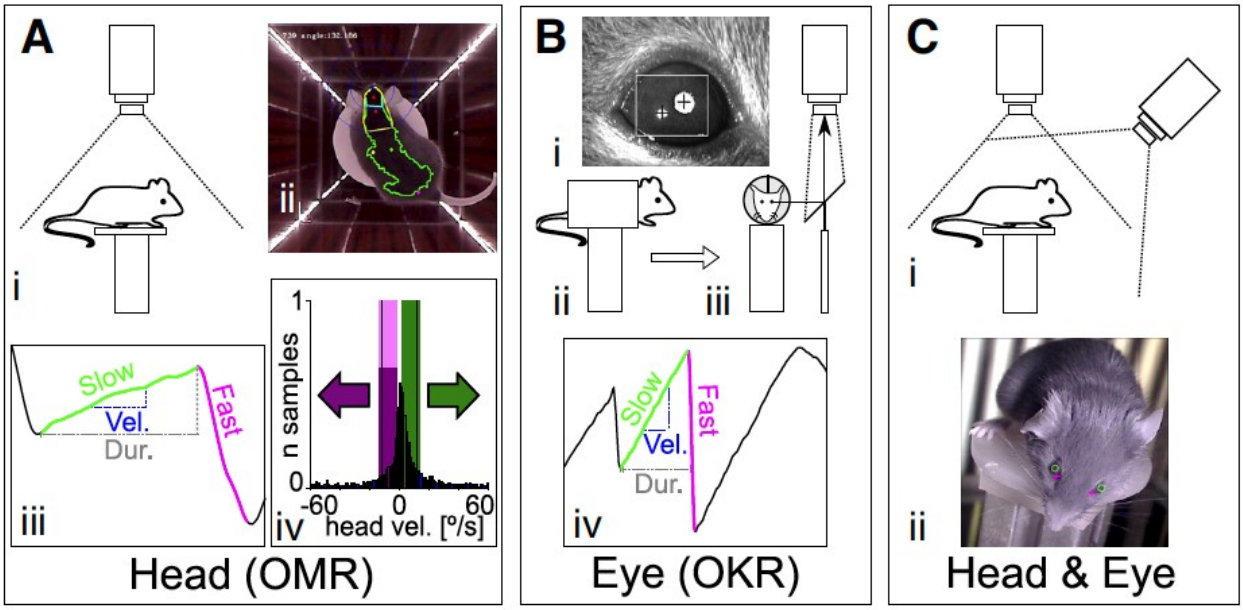


Figure 20 Imaging head (A), eye (B) or combined head and eye movements (A+B) in mice. Ai, camera is imaging a mouse from the top. Aii example image, with contour and markers outlining salient landmarks. Aiii example trace of head movement, with slow component in stimulus direction and fast component opposed to stimulus direction. AiV histogram of head velocities in stimulus direction (green) or opposed to it (magenta).

We have used the setup to determine the spatial frequency, contrast, and the velocity tuning of OKR and OMR in wild type (C57Bl6) and Brn3b$^{KO/KO}$ mice. We therefore are in position to provide the first direct comparison of OMR and OKR in mice under identical stimulation conditions. We found that the two reflexes have comparable tuning curves and are similarly affected in genetically modified mice with defects in RGCs (Brn3b$^{KO/KO}$), suggesting they use common circuitry conveying visual information from the eye to the brain. OKR eye movements have significantly higher gains than the OMR head movements, but neither can fully compensate global visual shifts. However conjugated eye and head movements can be detected in unrestrained mice performing OMR, suggesting they can be combined to achieve image stabilization.

A second important observation is that both head and eye can respond with qualitatively distinct types of movements to the stimulus. The first type has a slow component of relatively high gain and short duration and is generally followed by a resetting fast component (saccade). These responses are the characteristic responses typically reported in literature. The second category has a slow component of variable length and low gain and is not followed by a resetting fast (saccadic) movement. This type of response is more reminiscent of pursuit movements, and had not been previously reported in this context. We find that, surprisingly, in Brn3b$^{KO/KO}$ mice, which have a total loss of Accessory optic system, but preserve some LGN and SC innervation, there is a residual horizontal OMR and OKR response which mostly consists of the long, slow, sacade-less type of movements. These movements are suggestive of a smooth pursuit of the target, as used by predators when pursuing prey. Thus, our methodology and findings open the door to defining new visual stimulus response properties in mice, and hence to the genetic analysis of visual circuit development.

Given the ease with which the assay can be performed, and the robust, hard-wired nature of the reflex, OMR determinations are the most popular test for visual function in mice, in a variety of disease models and drug screening assays. We have used our apparatus to help characterize visual deficits in a variety of mouse models of retinal disease and therapeutics, beyond our Brn3 mutants. For instance, we were able to document OMR visual function deficits in mice lacking microglia, and improvements in visual function in a mouse model of retinitis pigmentosa in which microglia function was modulated by treatment with tamoxifen. More recently, we have colaborated with the Sung lab in Cornell to characterize visual function in a mouse model of Age-related Macular Degeneration (AMD), in RPE-specific Clic4 mutants. Given its popularity, the software/hardware solutions developed by Dr. Kretschmer were incorporated in a commercial device that is now sold under the name qOMR by Phenosys Inc. from Germany (https://www.phenosys.com/collaboration/visual-acuity-mice/).

B.II.6.6 Visually Evoked Defensive Behaviors in mice with mutations in Brn3 transcription factors (#12 Lees 2020).

Study of more complex visual functions is facilitated in humans and primates by the participation of the subject in the experiment, either by reporting perceptions (humans) or by performing a variety of learned tasks (primates). In mice, such experimental feedback is much harder to achieve, because of the limited number of salient motivations that can be induced in order to elicit specific responses or associate learned behaviors. Therefore, collectively the field is exploring the visual stimulus space that can elicit robust responses in mice. Visual reflexes such as the PLR and OMR/OKR and the activity dependency on light conditions (circadian photoentrainment) are well established and yield pretty robust responses, however the visual information driving them is conveyed to subcortical retinorecipient areas that participate in subcortical circuits and do not necessarily engage conscious vision. Visually evoked defensive behaviors (VEDBs), elicited by presentation of stimuli that can elicit a perception of threat (e.g. an aerial predator hovering overhead or quickly approaching) were shown in the 1980's to elicit strong responses in rats/mice (Blanchard et al., 1981; Blanchard & Blanchard, 1988). Lesions to

the Superior Colliculus (SC, the largest retinorecipient area in the mouse) and the dorsal peryaquaeductal gray (dPAG) of rodents were soon associated with defects in VEDBs. Recently, several labs engaged in a more precise characterization of VEDBs in mice (De Franceschi et al., 2016; Evans et al., 2018, 2019; Salay et al., 2018; Salay & Huberman, 2021; Yilmaz & Meister, 2013). To summarize their work, wild type mice tend to react by fleeing from overhead visual stimuli simulating an approaching predator, while stimuli mimicking a bird of pray cruising overhead (sweeping stimuli) tend to induce a complete arrest of movement, described as "freezing".

We studied VEDBs in our genetically modified animals, and found that in mice with RGC loss due to Brn3b ablation, the fleeing response to looming stimuli while leaving the minor freeze response intact, resulting in a net flight-to-freeze switch. Surprisingly, freezing responses to sweeping stimuli are significantly affected. The results are somewhat counterintuitive since Brn3b controls a vast majority of RGCs, and its ablation results in nearly 75 % of RGCs missing. Thus the expectation would have been that responses are lost, not switched. Our results pointed instead to specific RGC populations being involved in flight vs. freezing response and loom vs. sweep detection. We therefore presented looming and sweeping stimuli to mice missing three distinct RGC subgroups, namely 1) Brn3a^{+} bettaRGCs (missing from Rax:Cre; Brn3a$^{CKOAP/KO}$ mice – section B.II.2.3), 2) Opn4^{+}Brn3b^{+}RGCs (missing from Opn4^{Cre}Brn3b^{cDTA} mice, section B.II.4.1) and 3) Brn3c^{+}Brn3b^{+} RGCs (missing from Opn4^{Cre}Brn3b^{cDTA} mice, section B.II.2.1). To our surprise, all three genetic manipulations resulted in milder phenocopies of Brn3b knockout response deficits. These three genetic manipulations selectively ablate three RGC subpopulations with very little overlap, thus suggesting that the computation of looming and sweeping stimulus detection begins at the level of the retina, and multiple complementary RGC populations are involved in each.

B.II.7 Discovery and function of Response Gene to Complement 32 (RGC-32/Rgcc)

B.II.7.1 Discovery of RGC-32 and its role in complement response (work done before/during my PhD #58 Lang 1997, #50 Badea 2003, #56 Badea 1998, #52 Badea 1992)

The established role of natural immunity is to provide first response to damaged tissue and/or invading pathogens, using secreted factors, such as the blood clotting factors, complement cascade, interferon, interleukins, cytokines, etc. and cellular components such as circulating and resident macrophages, professional phagocytes (neutrophyls, eosinophils, mastocytes and basophils). But a distinct role, foreshadowed by early studies in the 80s, is that of clearance of cellular or tissue residue and priming tissue for "restitutio ad integrum", i.e. repair and regeneration. Injured tissues react to inflammatory responses that attempt to clear the noxious influence, but at the same time repair the damage. Somatic cells can be affected in a variety of ways by these signals. One type of adaptive response is the induction of stress response genes, assumption of less mature tissue phenotypes, or even reactivation of cell cycle. Recently, this line of research is being intensely pursued, with the aim of manipulating somatic cells into re-entering cell cycle, proliferating and then re-differentiating to produce novel mature

cells. This process, known as regeneration, is readily accessible in early vertebrates, such as fish and amphibians, and many groups are focused on unravelling the distinct molecular pathways that allow these fila to regenerate, while mammals can not. As a pathology fellow in the lab of Moon Shin, Horea Rus and Florin Niculescu, I was exposed to these ideas and participated in a pair of studies exploring dedifferentiation of cultured myotubes that were treated with sublytic complement membrane attack complexes (MACs, #58 Lang 1997, #50 Badea 2003). Similar studies were done on Olygodendrocytes by H. Rus and F. Niculescu, showing a loss of differentiation markers and reversion to younger phenotypes. During my three years as a pathology fellow, I undertook together with H. Rus and F. Niculescu a screening for genes induced in vitro in oligodendrocytes by challenge with terminal complement complexes (also known as MACs). The approach consisted of a differential display (essentially comparing randomly primed RNA from controls. Amongst the identified targets was a cDNA belonging to RGC32 (response gene to complement – 32 , literally the 32nd band on the gel). I went on to clone the mouse, rat and human genes, describe the expression in different tissue types, identify the localization of the human gene to chromosome 13 and raised an antibody against rat RGC-32. Based on this work, colleagues in the Rus lab went on to demonstrate that RGC32 overexpression can induce S-phase entry, DNA synthesis, and increase p34/cdc2 activity in aortic smooth muscle cells. Together, we showed that RGC32 can interact with the cell cycle activator cdc2, enhance its kinase activity, and that RGC32 is itself a substrate for the kinase. These studies coincided with my Master and PhD studies in Columbia and Hopkins, and were finally published in 2002.

B.II.7.2 RGC-32 as a potential cancer marker (#48 Fosbrink 2005)

Using the reagents we had generated, together with Matthew Fosbrink and Dr. Rus we were able to show that RGC-32 is a potential colon carcinoma marker. This work resulted in a patent for RGC-32 as a diagnostic tool in cancer.

B.II.7.3 Generation of RGC-32 knockout mice and RGC-32 involvement in inflammation and fibrosis (#33 Tegla 2015, #23 Rus 2017, #17 Tatomir 2018, #10 Tatomir 2021, #6 Tatomir 2021b, #4 Luzina 2021)

During my postdoctoral fellowship in Johns Hopkins, I continued my collaboration with the Rus lab, and, together with C. Cudrici, we isolated genomic clones for the mouse RGC-32 locus, and generated knock-out and conditional knock-out targeting constructs. The knock-out mice were long in the making, but finally in 2015, we reported the generation of the RGC32KO allele. RGC32KO mice are viable and fertile. However, RGC-32–deficient CD4+ T cells exhibited enhanced proliferation, IL-2 production, and Akt phosphorylation as compared with wild type CD4+ T cells, suggesting a inhibitory role of RGC-32 under Th0 conditions. More specifically, RGC-32 is preferentially induced in Th17 cells, a subset of CD4+ Th cells expressing IL-17, IL-21, IL-22 and IL-23R. In RGC32KO mice, the percentage of IL17+ Th cells is reduced relative to

the whole CD4+ population, and so are their signature set of interleukins. However other CD4+ T cell subpopulations (Th1, Th2 and Treg lineages) do not seem to be affected by the absence of RGC-32. The reason Th17 is impaired in RGC32KO seems to be related to defective TGFbetta signaling, and the induction cannot be rescued by providing a cocktail of TGFbetta, IL6 and IL21 or IL23. The impairment of Th17 cells in RGC32KO mice seems to be responsible for a milder progression of experimental autoimmune encefalomyelitis (EAE), a disease model for multiple sclerosis (MS). Indeed EAE can be induced in RGC32KO mice by transfer of wild type CD4+ cells, and RAG1-/- mice exhibit a more severe form of EAE if transplanted with wild type CD4+ cells than if they receive RGC32KO CD4+ T cells.

Another immune response associated cell type expressing RGC-32 is the astrocyte. Astrocytes are important for EAE, and their activation and phenotypic changes are also under TGFbetta control. In a series of papers using the RGC32-KO mice, the Rus lab explored the role played by RGC-32 in TGFbetta activation of astrocytes, and the implications of these interactions for EAE. Overall, RGC-32 appears to function downstream of TGFbetta in astrocyte activation, as RGC-32KO astrocytes had reduced TGFbetta responses in vitro. Specifically they reduced the synthesis of Extracellular matrix proteins, such as collagens I, IV and V and fibronectin, adopted more immature phenotypes (radial glia-like), and had distinct expression of cell-surface molecules that modulate cell morphology. Thus, it is possible that RGC-32 is a key signaling molecule involved in mediating inflammatory signals in autoimmune syndromes, by modulating TGF-betta dependent responses in both T cells and astrocytes. However, RGC-32 seems to work in the exact opposite fashion in a chronic model of pulmonary fibrosis, as RGC-32KO mice had more accentuated fibrosis in bleomycin-induced pulmonary fibrosis, and RGC-32 antagonized TGFbetta effects in lung fibroblasts. Thus, the modulatory effects of RGC-32 could be very diverse, depending on the cell type and experimental model employed.

B.II.8 Mathematical modeling of mosaic or homogeneous Frizzled 6 mutations in skin-hair patterning (#47 Wang 2006).

A somewhat isolated project that is nevertheless worth mentioning involved my mathematical modeling of hair follicle orientation patterning on the skin of Frizzled6 mice. Under wild type conditions, hair follicles exhibit a relatively orderly pattern on the hairy skin of P0 pups. This pattern becomes even more tightly aligned, with most hair follicles exhibiting nearly perfect alignment. However, Fzd6KO/KO pups exhibit fairly disordered hari follicle orientation at birth, that becomes even more disordered as the animal grows, and eventually arranges itself in whorls. This phenotype was discovered in the Nathans lab by one of my colleagues. I quantitated the hair follicle orientation defects and general progression of the phenotype, and derived a very simple mathematical rule for the self-organizing features of the hair follicles on themselves. Briefly, I found that an iterative rule in which the new orientation of the hair follicle was derived by vector addition of the average orientation of its 18 immediate neighbors in the previous cycle, scaled by a constant. This rule mimics the one developed for orientation of electron spins in a ferromagnet (Ising model).

B.III Academic Career Progression, Productivity and Recognition

B.III.1 Education and career progression:

I will briefly describe the education and professional stages of my career, beginning with my medical studies to the present.

1) September 1988 - September 1994 - **Medical Doctor (MD)**, University "Iuliu Hatieganu", School of Medicine Cluj-Napoca, Romania – Overall grade 9.90/10. License dissertation subject was the characterization of Anti-Neutrophil Cytoplasmic Antibodies (ANCA) in vasculitic syndromes – resulting in two publications (#59 Cristea 1995, #60 Cristea 1995b).

2) January 1995 - January 2000 - **Instructor**, Department of Immunopathology, University "Iuliu Hatieganu", School of Medicine, Cluj-Napoca, Romania. I only acted in this capacity for a brief period of time, and my position was put on hold and eventually cancelled, while I was pursuing my research career in the USA.

3) March 1995 - January 1998 **Research Fellow**, Department of Pathology, Medical School, University of Maryland at Baltimore. I worked under the guidance of Horea Rus and Florin Niculescu, on the tissue reaction to inflammation, in particular complement membrane attack complex (MAC). 3 papers were published (# 56 Badea 1998, #57 Rus 1997, #58 Lang 1997), and we performed a differential display that resulted in the discovery of RGC-32/Rgcc, as a gene upregulated in vitro in olygodendrocytes challenged with MACs.

4) January 1998 – June 1999 **Master of Arts (MA)**, Biological Sciences, Columbia University, New York. I worked in the laboratory of Rafael Yuste, on imaging of epileptiform events in brain slices, publishing one paper (#53 Badea 2001).

5) June 1999 – May 2004 **Doctor of Philosophy (PhD)**, Biochemistry, Cell and Molecular Biology, School of Medicine, Johns Hopkins University, Baltimore. I worked in the laboratory of Jeremy Nathans, on strategies for genetic random sparse recombination, and published 2 papers on this topic, in parallel with finishing some RGC-32-related papers (#55 Niculescu 1999, # 54 Niculescu 1999, #52 Badea 2002, #50 Badea 2003, #51 Badea 2003, #49 Badea 2004).

6) May 2004 – September 2010 **Postdoctoral Fellow**, Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, School of Medicine, Johns Hopkins University, Baltimore. I continued my work in the laboratory of Jeremy Nathans, designing novel conditional

knock-in strategies for gene manipulation and neuronal labelling. I co-authored 6 papers (#48 Fosbrink 2005, #47 Wang 2006, #46 Guler 2008, #45 Badea 2009, #44 Ye 2009, #43 Badea 2009b).

7) September 2010 – August 2021, **Investigator**, **Head of Retinal Circuits Development and Genetics Unit,** N-NRL, National Eye Institute, NIH, Bethesda Maryland. I managed a group of about five scientists, working on the development, function and pathology of RGCs. We published 41 papers, 21 from our group and 20 as collaborations (#1 to #41 on the personal paper list). As a group leader I mentored 18 undergraduate, postbac, master, PhD and postdoctoral students (description under section ldkjnvljdnv, mentoring and teaching activities).

8) Since September 2021 - **Senior Researcher (CS II)**, Research and Development Institute, Faculty of Medicine, Transilvania University of Brasov, Romania. I am in the process of establishing a molecular genetics and neuroscience lab, and have initiated a Neurogenetics research student club. Several manuscripts were completed while at UnitBv or are either submitted or accepted/in press.

B.III.2 Productivity:

At the time of writing, I have co-authored 115 publications indexed in Web of Science, of which 60 are peer reviewed original communications. I have totaled 3102 citations, with a Hirsch Index of 27.

Of the 60 peer-reviewed papers, 48 were published after 2004, the year of my PhD defense and graduation, of which 22 are as principal author (first, last, and/or corresponding). My work was published in top ranking journals, such as Nature (3), Cell (1), Neuron (2), Proceedings of the National Academy of Sciences (USA) (3), Cell reports (1), Molecular Cell (1), Nature Communications (1 published 1 in press), Science Advances (1), Journal of Neuroscience (4), Journal of Comparative Neurology, Journal of Neurophysiology, etc. Please see attached personal publication list.

I have co-authored one book chapter in a volume published at MIT press.

In the last 10 years, I have presented 13 invited lectures in the USA, France, Belgium, China and Japan, and I have been presenting our results at international meetings, at a rhythm of 1.5 meetings / year.

Our work on RGC-32 in colon carcinoma resulted in a patent for RGC-32 as a potential biomarker for cancer (WO/2006/110748) RESPONSE GENE TO COMPLEMENT 32 (RGC-32) IN DISEASE THE UNIVERSITY OF MARYLAND, BALTIMORE, Inventors: Rus, Horea, Badea, Tudor, Fosbrink, Matthew).

My work during postdoctoral studies was funded by the Howard Hughes Medical Institute.

My research group at the National Eye Institute was supported through intramural research awards, project (numbers 1ZIAEY000504 01 to 10, ranging from 900000 – 1800000 US$/year).

The Optomotor/Optokinetic Response testing apparatus designed by Dr. Friedrich Kretschmer and other members of my group was converted into a commercial device, available under the name qOMR from Phenosys, Gmbh, Germany.

Over the years we generated 10 genetically modified mouse lines, that are in broad use throughout the world, distributed through the Jax mice repository (more than 350 labs in 15 countries as of 2014).

B.III.3 Recognition:

B.III.3.1 Participation in professional associations, societies, and service on committees

Howard Hughes Medical Institute – Research Associate 2005 - 2010.
American Asssociation of the Advancement of Science – Since 1996.
Society for Neuroscience – Since 2001.
American Physiological Society – Since 2017
Association for Research in Vision and Ophthalmology – Since 2011
Federation of European Neuroscience Societies (FENS) – Since 2019
Stadtmann Tenure Track Investigator Recruitment Committee – 2012 – 2013
Animal Care and Use Committee - National Eye Institute / NIH 2012 – 2021

B.III.3.2 Reviewer and Editorial activity:

Reviewer for: Acta Histochemica; Biochimica et Biophysica Acta; BMC Biology; BMC Molecular Brain; BMC Molecular Medicine; Cell Death and Disease; Cellular and Molecular Life Sciences; Developmental Dynamics; Developmental Biology; eNeuro; FEBS Letters; Frontiers In Neuroscience; Genesis; Graefe's Archiv; Journal of Comparative Neurology; Journal of Neuroscience; Journal of Neurophysiology; Molecular Vision; Molecular Cellular Neuroscience; Molecular Medicine; Nature Communications; Neuroscience; Pigment Cell and Melanoma Research; PLOS One; Proceedings of the National Academy of Sciences (USA); Proceedings of the Royal Society (B)
Editorial Board: PLOSOne, Frontiers in Neuroscience (Neurodevelopment section)

B.III.3.3 Grant review and Academic Evaluation activity:

Evaluated grant proposals for: Agencie Nationale de Recherche (France); Association Retina France; Wellcome Funds (UK); Royal Netherlands Academy of Arts and Sciences; Israel Science Foundation.
Referent for tenure/promotion committees at: Baylor College of Medicine, University of Texas – McGovern Medical School and University of Virginia.

B.III.3.4 Awards

1. Young Investigator Award at the XVII International Complement Workshop October 11-16, 1998

Rhodes, Greece
2. First prize - poster presentation at the Gordon Research Conference on Visual Development, Salve Regina, Rhode Island, US, August 10-15, 2008 – related paper was published in Neuron (I.F. = 15 at the time of publication).
3. Ten Years Service Award – United States of America Government - 2020

B.III.4 Mentoring/Teaching/Supervisor activity

B.III.4.1 Teaching activity

Given my dual degree (M.D., Ph.D.), I already functioned as a faculty member and teaching assistant before obtaining my Phd:
1) Instructor, School of Medicine, at University "Iuliu Hatieganu," Cluj-Napoca, Romania, Spring Semester 1995 I Delivered lectures and directed laboratory sections for the Immunology course.
2) Teaching assistant, Neurobiology Course (Darcy Kelley and Stuart Firestein), Columbia University, New York, USA, 1998-1999 academic year. Lead discussion sections and graded exams.
3) Teaching assistant, Genetics course (Jeremy Nathans and Roger Reeves), Medical School at Johns Hopkins University, Baltimore, USA, 2002-2004 academic years. Graded exams and lead student discussions.

B.III.4.2 Mentoring activity

Lead a group of biomedical researchers for 11 years. Participated in recruitment and management committees at NEI and NIH levels for several years. Trained PhD, Postdoctoral, Master and Bachelor students. All trainees have secured jobs in Academia, Biotech companies or Medical School/Residency/Fellowships. They all have published scientific papers as first or co-authors.

During this time, I mentored:

Five Postdoctoral Fellows: Friedrich Kretschmer, Alireza Ghahari, Vladimir Muzyka, Manvi Goel, Raluca Pascalau.
Four PhD students: Graduate Partnership Program with Octavian Popescu at UBB Cluj, Romania: Miruna Ghinia, Szilard Sajgo. On advisory committee (co-mentorship with Phyllis Robinson, UMBC, Maryland): Preethi Somasundaram, Alexis Rubin.
Two M.S. Students: Walid Chatila (Georgetown) and Momina Tariq (George Mason)
Eight Postbac Fellows: Melody Shi, Sumit Kumar, Oluwaseyi Motajo, Katherine Chuang, Eileen Nguyen, Rebecca Lees, Annie Fuller, Armaan Akbar.
Four Undergraduates: Nadia Parmhans, Beverly Wu, Esika Savsani, Tyger Lin

C. Proposed Research Program and Career Development

My future research agenda is a direct continuation of the work I have done until now, taking into account the current status of the field and achievable goals in the near and long range future. The general aim is the study of the visual system, from anatomy, to function, development, and opportunities for therapeutic intervention and repair. For this purpose, we will draw parallels between the mouse and human visual circuit, understand the layout of cell types, reconstruct the conectomes, study the human and mouse visual transcriptomes, investigate the functional analogies, and exploit the molecular genetic capabilities in the mouse to derive information about the equivalent human circuits. With this knowledge, we will be in position to explore pathogenetic mechanisms in humans and generate and analyze disease models in mice. Our molecular insights will be instrumental for advancing our abilities to repair and regenerate retinal neurons, using the available bioelectronic interfaces, gene therapy and stem cell tools recently developed. This research program can be divided into several main directions, by methodology and scope.

We will deploy the genetic mouse lines we have generated in the past, and develop new ones in order to uniquely label RGC cell types and study their properties. We will conduct functional, electrophysiological, anatomical and molecular experiments in order understand RGC type function and development in the mouse. We will purify the genetically labeled cell populations, and perform deep sequencing analysis on them in order to characterize their transcriptomes. Using this information, we will then dissect the developmental pathways leading to the formation of these neurons.

To illustrate some specific opportunities available immediately to us, I present a few of the projects we are actively pursuing:

1) Early steps in RGC axon guidance The first mouse RGCs are defined at around Embryonic day 11 (E11), and soon thereafter extend their axons out of the forming eye cup and into the emerging optic stalk (E11 – E15). Early specification of RGCs and axon emergence from the eye cup are a critical time point from developmental, therapeutic (retinal organoids) and disease pathology (Glaucoma) perspectives and Pou4f/Brn3 transcription factors are the earliest markers/determinants of RGC specification, providing uniquely suited access to unravel these processes. We have so far derived, validated (and published) transcriptomic data for purified E15 $Brn3b^{WT/WT}$ and $Brn3b^{KO/KO}$ RGCs, and have characterized by immunohistochemical stains the very first RGCs being born and projecting axons out of the retina at E11. Future projects will involve the isolation and sequencing of $Brn3b^{AP}$ RGCs at E11. We plan to use these data to identify the molecules allowing RGC survival and axon projections out of the retina and into the optic stalk.

2) Activity-dependent molecular mechanisms in RGC development. RGCs develop their dendrite and terminal axonal arbors and establish synaptic connections in the first two weeks of postnatal development. This is a period of accelerated development of neurites for RGCs,

enlisting both molecular and activity-dependent mechanisms. Massive activity (Ca^{2+} waves) sweep through the retina, and are believed to help sculpt neuronal arbors and topography. Our transcriptomic analysis of Brn3 - dependent genes at postnatal day 3 (P3) followed by extensive validations using alternative strategies yielded, amongst other candidates, several members of the Copine molecular family. As detailed in section B.II.3.2, we will pursue mechanistic studies on the function of Copines in the retina, and their potential role in activity-dependent morphological changes in RGCs.

3) Mouse area centralis in $Brn3c^{Cre}$; $Brn3b^{CKOAP}$ mice. We discovered an area of densely packed $Brn3b^{+}Brn3c^{+}$ RGCs in $Brn3c^{Cre}$; $Brn3b^{CKOAP}$ mice, arranged in a dorso-temporal to ventro-nasal crescent, placed centrally, close to the optic nerve (Figure 8). These RGCs project to the Lateral Geniculate Nucleus (LGN) and Superior Colliculus (SC). Based on retinotopic to visuotopic transformations, this area is predicted to contribute to the contralateral information for the binocular visual field of the mouse. By specifically ablating this cell population, we have discovered that these RGCs are necessary for visually evoked evasive behaviors (flight), elicited by stimuli mimicking approaching predators. We plan to investigate the RGC type distribution of this area and study its involvement in high acuity vision and binocular vision in mice. This animal model is particularly important since it offers the opportunity to study high acuity, central vision in mice, the most advanced mammalian genetic animal model. This was not attempted until now.

4) Genetic Mosaics in Ret^{CreERt}; $Brn3a^{CKOAP}$ mice. As an example of early development switches, we have uncovered a unique genetic synergy between the neurotrophin receptor Ret and the transcription factor Brn3a. RGCs manipulated to have sparse (mosaic) double heterozygosity for Ret and Brn3a (*$Ret^{KO/WT}$; $Brn3a^{KO/WT}$*) at early (E15) but not late (P0) stages of development exhibit cell type specificity switches and/or dramatic dendritic arbor defects (see section B.II.2.4). This phenomenon is not observed when the entire RGC population is rendered *$Ret^{KO/WT}$; $Brn3a^{KO/WT}$*, pointing to a neurotrophic competition between RGCs, in which sparse double heterozygote cells are at a disadvantage. We intend to pursue the mechanisms by which Ret and Brn3a interact in order to isolate the interaction between transcription and neurotrophic support. This will be useful from both the basic science perspective (developmental biology, circuit assembly) as well as the therapeutic perspective (rational design of neuroprotective and regeneration strategies).

5) ON-dense Spiny neurons isolated in Ret^{CreERt}; $Brn3c^{CKOAP}$ mice. Sparse random recombination in adult Ret^{CreERt}; $Brn3c^{CKOAP}$ mice labeled one unique cell type, that according to previous electrophysiology surveys is involved in high resolution object motion detection. However, this is the only genetic approach that uniquely labels this line, revealing its projections to the superficial, motion processing layers of the LGN, and specific sublaminae of the superficial SC.

Repair and Regeneration Currently, the scientific community is in the process of generating atlases of human cell types, using single cell deep sequencing methodologies. This information, stored in databases and publicly available, will speed our capability of drawing parallels between information gained from the mouse and the human. This in turn will speed up the discovery of equivalent cell types, physiological processes and developmental pathways. We plan to exploit this information by comparative transcriptomic studies, and uncover, by analogy cell type specification in mouse and human RGCs. We will then use this information to study the developmental pathways for both systems.

Another recent methodological development is the discovery of retina organoids derived from Embryonic Stem Cells/ induced Pluripotent Stem Cells. These are nearly complete retinas, with stratified cell types comprising photoreceptors, horizontal cells, bipolar and amacrine cells, and finally RGCs. Retina organoids are grown in vitro, from human or mouse ES/iPS – derived neurospheres. Human retina organoids take nearly 6 months to fully develop, while mouse organoids cand be derived in about 2-3 weeks. The neurons within organoids are functional, and can be used to model various diseases. Ongoing efforts include attempts at surgically transplanting these in vitro retinas to animal models of blindness. However, retina oganoids in both humans and mice have two shortcomings: Photoreceptors do not develop outer segments and RGCs are born at some point, but eventually die off. Thus the current challenge consists in developing methodologies to overcome these limitations, and design meaningful strategies of reconnecting the retina organoids into the visual circuit.

We will use the insights we gained from our gene expression studies to identify the missing molecular signals that could ensure RGC survival and proper axon guidance, and test them in our mouse models and retina organoids. One such opportunity is the recently discovered link between neurotrophic signals and transcriptional regulation (see section B.II.2.4). It is widely believed that neuronal survival is assisted by neurotrophic support from innervated targets. However, in our hands, neurotrophic signaling seems to be more important for RGC type specification, compared to RGC survival per se.

Pathogenetic Studies. We plan to use our molecular insights in exploring pathogenetic mechanisms in diseases affecting RGCs (Glaucoma, ONH, Foveal Hypoplasia, Optic Neuritis in the context of multiple sclerosis).

Glaucoma, a leading cause of blindness worldwide, is believed to result from an interplay between high intra-ocular pressure and increased sensitivity of RGCs to mechanical stress. At one end of the spectrum, high IOP damages otherwise normal neurons, at the other normal IOP is poorly tolerated by sensitized RGCs. Management is surgical or pharmacological reduction of IOP, but typically, only delays of the eventual RGC death and vision loss can be achieved. Thus, in Glaucoma, the leading edge of research involves identifying the (genetic?) risk factors that render RGCs vulnerable to IOP, and discovering neuroprotective signals that can improve their survival.

Optic nerve hypoplasia and/or foveal hypoplasia are mostly congenital disorders, caused by albinism, retinopathy of prematurity or various genetic defects. Besides genetic counseling

and preventing premature births, the therapeutic goals once the ONH/FH are detected refer mostly to repair and regeneration approaches. Under these circumstances our animal models, combined with insights from patients will come in very handy.

Multiple Sclerosis is a neurodegenerative disease characterized by demyelination and inflammation of neuronal tissue, believed to have an autoimune mechanism. Involvement of the optic nerve (neuromyelitis optica) is often seen the context of MS, and in its animal model, Experimental Autoimmune Encephalomyelitis (EAE). Thus MS/EAE management will be one directed at understanding inflammation and immunity.

Although very distinct in etiology, these disorders share the destruction of RGCs, either early or late in life. In many cases, this destruction is modulated by inflammation. The major goals set by the field for these disorders are neuroprotection (using a variety of neurotrophic signals and/or mechanisms) or repair/regeneration from existing endogenous cells (e.g Muller Glia) or ES cells. In addition, control of the inflammatory process is essential in MS.

We intend to mine our transcriptomic data and use our insights to find potential targets for therapeutic intervention in these contexts. Of particular interests are transcription factors or signaling molecules that intervene in RGC survival or development, as these are likely candidates for helping with protection or rederivation of these cells, either from ES/iPSCs, or from other cell types of the retina.

In addition, we will investigate the mechanisms by which neuroinflammation affects the optic nerve and RGC axons. My interest in this particular direction is further peaked by the more recent realization that RGC-32/Rgcc, the gene I have cloned in the late 1990s, together with H. Rus, is a potential pathogenetic factor in EAE (see section B.II.7.3), and other models of faulty immune reactions resulting in fibrosis. My previous involvement in the understanding of RGC-32 function was mostly centered on the molecular biology: cloning the gene in mice, humans and rats, establishing its expression pattern in various tissues, raising the first antibody, making the knock-out and conditional knockout animal, assisting with gene expression profiling, etc. I now intend to extend our collaboration with the Rus lab and other scientists interested in inflammation, autoimmunity and tissue repair, and explore the involvement of RGC-32 in these processes, with a particular focus on the visual system.

From an Academic perspective, my goal is to advance to CSI (Scientific Researcher I) or Professor, and establish a strong neuroscience and molecular genetics program at Transilvania University. Towards this goal, I plan to establish strong collaborations with colleagues within the Faculty of Medicine and the Research and Development Institute (ICDT), and take advantage of the broad expertise in technical fields (material science, engineering, electronics, computer science and mathematics) at UnitBv, to tackle questions related to biology – electronic interfaces, mechanisms of function of the visual system, and therapeutic opportunities for the visual system. Scientific rogress for the foreseeable future will be made by technological advances, in genomics, stem cell and regeneration, bionic interfaces and human - machine crosstalk. Transilvania University is particularly suitable for this type of interactions, as all technical fields are well represented.

I intend to educate a new generation of scientists in Romania, and increase retention in the country by creating a vibrant and consistent research environment. I hope to establish good working relationships with various research institutes and universities in Romania, and have already started this process.

I also will strive to expand the visibility of Romanian research by collaboration and reach out to the international research community, using my broad network of collaborators throughout the world.

Some goals include establishing partnerships with well-known visual research centers in Europe, organize international conferences that can bring together researchers from all over the world to our center, and continue to publish and present our work in top journals and professional meetings.

Personal Publication List:

Book Chapters:

1. Niculescu, F., Badea, T., and Rus, H., (1998) Sublytic C5b-9 complexes induce proliferation of human aortic smooth muscle cells. Role of mitogen activated protein kinase and phosphatidylinositol 3-kinase. Proceedings of XIII World Congress of Cardiology. Monduzzi Editore, Bologna, pp1185-1190.

2. Badea T.C. and Nathans J. (2008) New genetic technologies for studying the morphology, physiology, and development of mouse retinal neurons. Eye, Retina, and Visual System of the Mouse. Chalupa, L.M. and Williams, R.W., Editors. MIT Press, Cambridge, Masssachussets.

Preprints (or under review/revision/accepted in other journals)

1: Layer-specific developmentally precise axon targeting of transient suppressed-by-contrast retinal ganglion cells (tSbC RGCs) Tien NW, Badea TC, Kerschensteiner D, bioRxiv 2021.11.26.470118; doi: https://doi.org/10.1101/2021.11.26.470118

2: Zfp503/Nlz2 is Required for RPE Differentiation and Optic Fissure Closure (2021) Boobalan E, Thompson AH, Alur RP, Dong L, Shih G, Vieta-Ferrer ER, Onojafe IF, Arno G, Lotery AJ, Guan B, Bender C, Memon O, Brinster L, Soleilhavoup C, Panman L, Badea TC , Minella A, Lopez AJ, Thomasy S, Moshiri A, Genomics England Research Consortium, Blain D, Hufnagel RB, Cogliati T, Bharti K, Brooks BP (In review).

3: Physiological Changes in Retinal Ganglion Cells Lacking the Transcription Factor Brn3b/Pou4f2 (2021) International Conference on Neuroscience, Neuroinformatics, Neurotechnology and Neuro- Psycho- Pharmacology Kumar SR, Lin T Badea TC. http://www.cpru.upb.ro/neuro2/index.html

Peer Reviewed Research Articles:

Since PhD completion.

1. Goel M, Aponte AM, Wistow G, **Badea TC**. (2021) Molecular studies into Copine-4 function in Retinal Ganglion Cells, PLOSOne ,16(11): e0255860, doi: 10.1371/journal.pone.0255860 & bioRxiv(2021), doi: 10.1101/2021.08.09.455730.

https://doi.org/10.1371/journal.pone.0255860

2. Chuang JZ, Yang N, Otsu W, Fu C, Nakajima N, Yang HH, Lee MP, Akbar AF, **Badea TC**, Guo Z, Nuruzzaman A, Hsu KS, Dunaief JL, Sung CH (2021). Modeling and mechanistic investigation of a novel dry AMD 1 mouse model with CLIC4 deleted in RPE, Nature Communications (accepted)

3. Liu S, Aldinger KA, Cheng CV, Kiyama T, Dave M, McNamara HK, Caraffi SG, Ivanovski I, Errichiello E, Zweier C, Zuffardi O, Schneider M, Papavasiliou AS, Perry MS, Humberson J, Cho MT, Weber A, Swale A, **Badea TC**, Mao C-A, Garavelli L, Dobyns WB, and Reinberg D. (2021) NRF1 Association with AUTS2-Polycomb Mediates Specific

Gene Activation in the Brain. Mol Cell. Vol 81 (22): 4663-4676.e8 Nov 18. doi: 10.1016/j.molcel.2021.09.020. & bioRxiv , (2021): doi: 10.1101/2021.03.30.437620

https://doi.org/10.1016/j.molcel.2021.09.020

4. Luzina IG, Rus V, Lockatell V, Courneya JP, Hampton BS, Fishelevich R, Misharin AV, Todd NW, **Badea TC**, Rus, H, Atamas SP (2021) Regulator of Cell Cycle protein (RGCC/RGC-32) protects against pulmonary fibrosis. American Journal of respiratory Cell and Molecular Biology (AJRCMB) 2021 Oct 20. doi: 10.1165/rcmb.2021-0022OC (online ahead of print).

https://doi.org/10.1165/rcmb.2021-0022oc

5. Muzyka, VV, **Badea TC**. (2021) Genetic Interplay Between Transcription Factor Pou4f1/Brn3a and Neurotrophin Receptor Ret In Retinal Ganglion Cell Type Specification. Neural Development vol 16: 5 September 21, & bioRxiv , no. (2020): 2020.03.23.004242. doi: 10.1101/2020.03.23.004242.

https://doi.org/10.1186/s13064-021-00155-z

6. Tatomir A, Beltrand A, Nguyen V, Courneya, JP, Boodhoo D, Cudrici C, Muresanu DF, Rus V, **Badea TC**, Rus H (2021) RGC-32 acts as a hub to regulate the transcriptomic changes associated with astrocyte development and reactive astrocytosis. Frontiers in Immunology Jul 29;12:705308. doi: 10.3389/fimmu.2021.705308. eCollection 2021.

https://doi.org/10.3389/fimmu.2021.705308

7. Chen CK, Kiyama T,Weber N,Whitaker CM,Pan P, **Badea TC**, Massey SC, Mao CA (2021). Characterization of Tbr2-expressing retinal ganglion cells, J Comp Neurol 2021 doi: https://doi.org/10.1002/cne.25208 & bioRxiv: 2020.2006.2017.153551. https://doi.org/10.1101/2020.06.17.153551

https://doi.org/10.1002/cne.25208

8. Brodie-Kommit J, Clark BS, Shi Q, Shiau F, Kim DW, Langel J, Sheely C, Ruzycki PA, Fries M, Javed A, Cayouette M, Schmidt T, **Badea T**, Glaser T, Zhao H, Singer J, Blackshaw S, Hattar S. Atoh7-independent specification of retinal ganglion cell identity. Sci Adv. 2021 Mar 12;7(11):eabe4983. doi: 10.1126/sciadv.abe4983.& bioRxiv , no. (2020): 2020.05.27.116954. doi: 10.1101/2020.05.27.116954

https://doi.org/10.1126/sciadv.abe4983

9. Oliver KM, Florez-Paz DM, **Badea TC**, Mentis GZ, Menon V, de Nooij JC. (2021) Molecular development of muscle spindle and Golgi tendon organ sensory afferents revealed by single proprioceptor transcriptome analysis. Nature Communications Mar 1; 12(1):1451 doi: 10.1038/s41467-021-21880-3 & bioRxiv , no. (2020): 2020.04.03.023986. doi: 10.1101/2020.04.03.023986.

https://doi.org/10.1038/s41467-021-21880-3

10. Tatomir A, Beltrand A, Nguyen V, Boodhoo D, Mekala A, Cudrici C, **Badea TC**, Muresanu DF, Rus V, Rus H (2021) RGC-32 regulates generation of reactive astrocytes in experimental autoimmune Encephalomyelitis. Frontiers in Immunology – Jan 25, doi: 10.3389/fimmu.2020.608294

https://doi.org/10.3389/fimmu.2020.608294

11. Parmhans N,Fuller AD, Nguyen E, Chuang K, Swygart DI, Wienbar SR, Lin T, Kozmik Z, Dong L, Schwartz GW, **Badea TC** (2021). Identification of Retinal Ganglion Cell Types and Brain Nuclei expressing the transcription factor Brn3c/Pou4f3 using a Cre recombinase knock-in allele. J Comp Neurol. 2021 Jun;529(8):1926-1953. doi: 10.1002/cne.25065. Epub 2020 Nov 10.

https://doi.org/10.1002/cne.25065

12. Lees RN, Akbar AF, **Badea TC** (2020). Retinal Ganglion Cell defects cause decision shifts in visually evoked defense responses. J. Neurophysiology, 2020 Nov 1;124(5):1530-1549. doi: 10.1152/jn.00474.2019. Epub 2020 Sep 30.

https://doi.org/10.1152/jn.00474.2019

13. Gheorghiu M, Stănică L, Ghinia Tegla MG, Polonschii C, Bratu D, Popescu O, **Badea T**, (co-corresponding author) Gheorghiu E. Cellular sensing platform with enhanced sensitivity based on optogenetic modulation of cell homeostasis. Biosens Bioelectron. 2020 Apr 15;154:112003. doi: 10.1016/j.bios.2019.112003. Epub 2019 Dec 31.

https://doi.org/10.1016/j.bios.2019.112003

14. Gheorghiu M, Stanica L, Polonschii C, David S, Ruckenstein A, Popescu O, **Badea T**, Gheorghiu E. Modulation of Cellular Reactivity for Enhanced Cell-Based Biosensing. Anal Chem. 2020 Jan 7;92(1):806-814. doi: 10.1021/acs.analchem.9b03217. Epub 2019 Dec 9. PubMed PMID: 31751507.

https://doi.org/10.1021/acs.analchem.9b03217

15. Goel M, Li T, **Badea TC**. Differential expression and subcellular localization of Copines in mouse retina. J Comp Neurol. 2019 Oct 1;527(14):2245-2262. Epub 2019 Mar 28.

https://doi.org/10.1002/cne.24684

16. Kiyama T, Long Y, Chen CK, Whitaker CM, Shay A, Wu H, **Badea T.C.**, Mohsenin A, Parker-Thornburg J, Klein WH, Mills SL, Massey SC, Mao CA. Essential Roles of Tbr1 in the Formation and Maintenance of the Orientation-Selective J-RGCs and a Group of OFF-Sustained RGCs in Mouse. Cell Rep. 2019 Apr 16;27(3):900-915.e5.

https://doi.org/10.1016/j.celrep.2019.03.077

17. Tatomir A, Tegla CA, Martin A, Boodhoo D, Nguyen V, Sugarman AJ, Mekala A, Anselmo F, Talpos-Caia A, Cudrici C, **Badea T.C.**, Rus V, Rus H. RGC-32 regulates reactive astrocytosis and extracellular matrix deposition in experimental autoimmune encephalomyelitis. Immunol Res. 2018 Aug;66(4):445-461.doi: 10.1007/s12026-018-9011-x.

https://doi.org/10.1007/s12026-018-9011-x

18. Muzyka VV, Brooks M, **Badea T.C.** Postnatal developmental dynamics of cell type specification genes in Brn3a/Pou4f1 Retinal Ganglion Cells. Neural Dev. 2018 Jun 29;13(1):15. doi: 10.1186/s13064-018-0110-0.

https://doi.org/10.1186/s13064-018-0110-0

19. Ghahari A, Kumar SR, **Badea T.C.** Identification of Retinal Ganglion Cell Firing Patterns Using Clustering Analysis Supplied with Failure Diagnosis. Int J Neural Syst. 2018 Oct;28(8):1850008. doi: 10.1142/S0129065718500089. Epub 2018 Feb 22

https://doi.org/10.1142/s0129065718500089

20. Parmhans N, Sajgo S, Niu J, Luo W, **Badea T.C.** Characterization of retinal ganglion cell, horizontal cell, and amacrine cell types expressing the neurotrophic receptor tyrosine kinase Ret. J Comp Neurol. 2018 Mar 1;526(4):742-766. doi: 10.1002/cne.24367. Epub 2017 Dec 19

https://doi.org/10.1002/cne.24367

21. Sajgo S, Ghinia MG, Brooks M, Kretschmer F, Chuang K, Hiriyanna S, Wu Z, Popescu O, **Badea T.C.** Molecular codes for cell type specification in Brn3 retinal ganglion cells. Proc Natl Acad Sci U S A. 2017 May 16;114(20):E3974-E3983. doi: 10.1073/pnas.1618551114. Epub 2017 May 2

https://doi.org/10.1073/pnas.1618551114

22. Kretschmer F, Tariq M, Chatila W, Wu B, **Badea T.C.** Comparison of optomotor and optokinetic reflexes in mice. J Neurophysiol. 2017 Jul 1;118(1):300-316. doi: 10.1152/jn.00055.2017. Epub 2017 Apr 19.

https://doi.org/10.1152/jn.00055.2017

23. Rus V, Nguyen V, Tatomir A, Lees JR, Mekala AP, Boodhoo D, Tegla CA, Luzina IG, Antony PA, Cudrici CD, **Badea T.C.**, Rus HG. RGC-32 Promotes Th17 Cell Differentiation and Enhances Experimental Autoimmune Encephalomyelitis. J Immunol. 2017 May 15;198(10):3869-3877, doi: 10.4049/jimmunol.1602158. Epub 2017 Mar 29

https://doi.org/10.4049/jimmunol.1602158

24. Wang X, Zhao L, Zhang Y, Ma W, Gonzalez SR, Fan J, Kretschmer F, **Badea T.C.**, Qian HH, Wong WT. Tamoxifen Provides Structural and Functional Rescue in Murine Models of Photoreceptor Degeneration. J Neurosci. Mar 22;37(12):3294-3310. doi: 10.1523/JNEUROSCI.2717-16.2017. Epub 2017 Feb 24

https://doi.org/10.1523/jneurosci.2717-16.2017

25. Somasundaram P, Wyrick GR, Fernandez DC, Ghahari A, Pinhal CM, Simmonds Richardson M, Rupp AC, Cui L, Wu Z, Brown RL, **Badea T.C.**, Hattar S, Robinson PR. C-terminal phosphorylation regulates the kinetics of a subset of melanopsin-mediated behaviors in mice. Proc Natl Acad Sci U S A. 2017 Mar 7;114(10):2741-2746. doi: 10.1073/pnas.1611893114. Epub 2017 Feb 21.

https://doi.org/10.1073/pnas.1611893114

26. Ghahari A, **Badea T.C.** Robust spike sorting of retinal ganglion cells tuned to spot stimuli. Annu Int Conf IEEE Eng Med Biol Soc. 2016 Aug;2016:1745-1749. doi: 10.1109/EMBC.2016.7591054

https://doi.org/10.1109/embc.2016.7591054

27. Keenan WT, Rupp AC, Ross RA, Somasundaram P, Hiriyanna S, Wu Z, **Badea T.C.**, Robinson PR, Lowell BB, Hattar SS. A visual circuit uses complementary mechanisms to support transient and sustained pupil constriction. Elife. Sep 26;5:e15392. doi: 10.7554/eLife.15392.

https://doi.org/10.7554/elife.15392

28. Ghinia MG, Novelli E, Sajgo S, **Badea TC**, Strettoi E. Brn3a and Brn3b knockout mice display unvaried retinal fine structure despite major morphological and numerical alterations of ganglion cells. J Comp Neurol. 2019 Jan 1;527(1):187-211. doi: 10.1002/cne.24072. Epub 2016 Jul 29

https://doi.org/10.1002/cne.24072

29. Wang X, Zhao L, Zhang J, Fariss RN, Ma W, Kretschmer F, Wang M, Qian HH, **Badea T.C.**, Diamond JS, Gan WB, Roger JE, Wong WT. Requirement for Microglia for the Maintenance of Synaptic Function and Integrity in the Mature Retina. J Neurosci. 2016 Mar 2;36(9):2827-42. doi: 10.1523/JNEUROSCI.3575-15.2016

https://doi.org/10.1523/jneurosci.3575-15.2016

30. Sajgo S, Ali S, Popescu O, **Badea T.C.** Dynamic expression of transcription factor Brn3b during mouse cranial nerve development. J Comp Neurol. 2016 Apr 1;524(5):1033-61. doi: 10.1002/cne.23890. Epub 2015 Sep 29.

https://doi.org/10.1002/cne.23890

31. Chuang K, Nguyen E, Sergeev Y, **Badea T.C.** Novel Heterotypic Rox Sites for Combinatorial Dre Recombination Strategies. G3 (Bethesda). 2015 Dec 29;6(3):559-71. doi: 10.1534/g3.115.025841.

https://doi.org/10.1534/g3.115.025841

32. Kretschmer F, Sajgo S, Kretschmer V, **Badea T.C.** A system to measure the Optokinetic and Optomotor response in mice. J Neurosci Methods. 2015 Dec 30;256:91-105. doi: 10.1016/j.jneumeth.2015.08.007. Epub 2015 Aug 14.

https://doi.org/10.1016/j.jneumeth.2015.08.007

33. Tegla CA, Cudrici CD, Nguyen V, Danoff J, Kruszewski AM, Boodhoo D, Mekala AP, Vlaicu SI, Chen C, Rus V, **Badea T.C.**, Rus H. RGC-32 is a novel regulator of the T-lymphocyte cell cycle. Exp Mol Pathol. 2015 Jun;98(3):328-37.
doi: 10.1016/j.yexmp.2015.03.011. Epub 2015 Mar 11.

https://doi.org/10.1016/j.yexmp.2015.03.011

34. Sajgo S, Ghinia MG, Shi M, Liu P, Dong L, Parmhans N, Popescu O, **Badea T.C.** Dre - Cre sequential recombination provides new tools for retinal ganglion cell labeling and manipulation in mice. PLoS One. 2014 Mar 7;9(3):e91435. doi: 10.1371/journal.pone.0091435. eCollection 2014

https://doi.org/10.1371/journal.pone.0091435

35. Niu J, Ding L, Li JJ, Kim H, Liu J, Li H, Moberly A, **Badea T.C.**, Duncan ID, Son YJ, Scherer SS, Luo W. Modality-based organization of ascending somatosensory axons in the direct dorsal column pathway. J Neurosci. 2013 Nov 6;33(45):17691-709. doi: 10.1523/JNEUROSCI.3429-13.2013

https://doi.org/10.1523/jneurosci.3429-13.2013

36. Shi M, Kumar SR, Motajo O, Kretschmer F, Mu X, **Badea T.C.** Genetic Interactions between Brn3 Transcription Factors in Retinal Ganglion Cell Type Specification. PLoS One. 2013 Oct 8;8(10):e76347. doi: 10.1371/journal.pone.0076347. eCollection 2013

https://doi.org/10.1371/journal.pone.0076347

37. **Badea T.C.**, (co-corresponding author) Williams J, Smallwood P, Shi M, Motajo O, Nathans J. Combinatorial expression of Brn3 transcription factors in somatosensory neurons: genetic and morphologic analysis. J Neurosci. 2012 Jan 18;32(3):995-1007. doi: 10.1523/JNEUROSCI.4755-11.2012

https://doi.org/10.1523/jneurosci.4755-11.2012

38. Matsuoka, R.L., Chivatakarn, O, **Badea, T.C.**, Samuels, I.S., Cahill, H., Katayama K, Suto F, Chédotal, A., Peachey, N.S., Nathans, J, Yoshida, Y., Giger, R.J.,, and Kolodkin, A.L. (2011). Class 5 transmembrane semaphorins control mammalian inner retinal lamination, neurite arborization, and function. Neuron 2011 Aug 11;71(3):460-73. doi: 10.1016/j.neuron.2011.06.009.

https://doi.org/10.1016/j.neuron.2011.06.009

39. Chen S.K., **Badea T. C.** (co-corresponding author), Hattar S. (2011) Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. Nature 2011 Jul 17;476(7358):92-5. doi: 10.1038/nature10206.

https://doi.org/10.1038/nature10206

40. McNeill D.S., Sheely C.J., Ecker J.L., **Badea T.C.**, Morhardt D., Guido W., Hattar S. (2011) Development of melanopsin-based irradiance detecting circuitry. Neural Dev. 2011 Mar 18;6:8. doi: 10.1186/1749-8104-6-8.

https://doi.org/10.1186/1749-8104-6-8

41. Matsuoka R.L., Nguyen-Ba-Charvet K.T., Parray A., **Badea T.C.**, Chedotal A., Kolodkin A.L. (2011) Transmembrane semaphorin signalling controls laminar stratification in the mammalian retina. Nature. 2011 Feb 10;470(7333):259-63. doi: 10.1038/nature09675

https://doi.org/10.1038/nature09675

42. **Badea T.C.**, (corresponding author) and Nathans J., (2011) Morphologies of mouse retinal ganglion cells expressing transcription factors Brn3a, Brn3b, and Brn3c: analysis of wild type and mutant cells using genetically-directed sparse labeling. Vision Res. 2011 Jan 28;51(2):269-79. doi: 10.1016/j.visres.2010.08.039. Epub 2010 Sep 6.

https://doi.org/10.1016/j.visres.2010.08.039

43. **Badea T.C.**, (co-corresponding author) Hua L.Z., Smallwood P.M., Williams J., Rotolo T., Nathans J., (2009) New mouse lines for the analysis of neuronal morphology using CreER(T)/loxP-directed sparse labeling. PLoS ONE 2009 Nov 16;4(11):e7859. doi: 10.1371/journal.pone.0007859.

https://doi.org/10.1371/journal.pone.0007859

44. Ye X., Wang Y., Cahill H., Yu M., **Badea T.C.**, Smallwood P., Peachey N.S., Nathans J. (2009) Norrin, Frizzled4, and Lrp5 signaling in endothelial cells control a genetic program for retinal vascularization. Cell 2009 Oct 16;139(2):285-98. doi: 10.1016/j.cell.2009.07.047.

https://doi.org/10.1016/j.cell.2009.07.047

45. **Badea T.C.**, Cahill H.C., Ecker J.L., Hattar S. and Nathans J. (2009) Distinct roles of transcription factors Brn3a and Brn3b in controlling the development, morphology, and function of retinal ganglion cells. Neuron 2009 Mar 26;61(6):852-64. doi: 10.1016/j.neuron.2009.01.020.

https://doi.org/10.1016/j.neuron.2009.01.020

46. Guler, A.D., Ecker, J.L., Lall, G.S., Haq, S., Altimus, C.M., Liao, H.W., Barnard, A.R., Cahill, H., **Badea, T.C.**, Zhao, H., Hankins, M.W., Berson, D.M., Lucas, R.J., Yau, K.W., and Hattar, S. (2008) Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature 2008 May 1;453(7191):102-5. doi: 10.1038/nature06829. Epub 2008 Apr 23

https://doi.org/10.1038/nature06829

47. Wang, Y., **Badea, T.** (co-first author), and Nathans, J. (2006) Order from disorder: Self-organization in mammalian hair patterning. Proceedings of the National Academy of Sciences USA 2006 Dec 26;103(52):19800-5. doi: 10.1073/pnas.0609712104. Epub 2006 Dec 15

https://doi.org/10.1073/pnas.0609712104

48. Fosbrink, M., Cudrici, C., Niculescu, F., **Badea, T.C.**, David, S., Shamsuddin, A., Shin, M.L., and Rus, H. (2005) Overexpression of RGC-32 in colon cancer and other tumors. Experimental Molecular Pathology 2005 Apr;78(2):116-22.
doi: 10.1016/j.yexmp.2004.11.001. Epub 2005 Jan 5

https://doi.org/10.1016/j.yexmp.2004.11.001

Papers Published before PhD completion

49. **Badea, T.C.**, and Nathans, J. (2004) Quantitative analysis of neuronal morphologies in the mouse retina visualized by using a genetically directed reporter. Journal of Comparative Neurology 2004 Dec 20;480(4):331-51. doi: 10.1002/cne.20304.

https://doi.org/10.1002/cne.20304

50. **Badea, T.C.**, Park, J.H., Soane, L., Niculescu, T., Niculescu, F., Rus, H., and Shin, M.L. (2003) Sublytic terminal complement attack induces c-fos transcriptional activation in myotubes. Journal of Neuroimmunology 2003 Sep;142(1-2):58-66. doi: 10.1016/s0165-5728(03)00261-3

https://doi.org/10.1016/s0165-5728(03)00261-3

51. **Badea, T.C.**, Wang, Y., and Nathans, J. (2003) A noninvasive genetic/pharmacologic strategy for visualizing cell morphology and clonal relationships in the mouse. Journal of Neuroscience 2003 Mar 15;23(6):2314-22. doi: 10.1523/JNEUROSCI.23-06-02314.2003.

https://doi.org/10.1523/jneurosci.23-06-02314.2003

52. **Badea, T.**, Niculescu, F., Soane, L., Fosbrink, M., Sorana, H., Rus, V., Shin, M.L., and Rus, H. (2002) RGC-32 increases p34CDC2 kinase activity and entry of aortic smooth muscle cells into S-phase. Journal of Biology Chemistry 2002 Jan 4;277(1):502-8. doi: 10.1074/jbc.M109354200. Epub 2001 Oct 30.

https://doi.org/10.1074/jbc.m109354200

53. **Badea, T.**, Goldberg, J., Mao, B., and Yuste, R. (2001) Calcium imaging of epileptiform events with single-cell resolution. Journal of Neurobiology 2001 Sep 5;48(3):215-27. doi: 10.1002/neu.1052.

https://doi.org/10.1002/neu.1052

54. Niculescu, F., Soane, L., **Badea, T.**, Shin, M., and Rus, H. (1999) Tyrosine phosphorylation and activation of Janus kinase 1 and STAT3 by sublytic C5b-9 complement complex in aortic endothelial cells. Immunopharmacology 1999 May;42(1-3):187-93. doi: 10.1016/s0162-3109(99)00014-4

https://doi.org/10.1016/s0162-3109(99)00014-4

55. Niculescu, F., **Badea, T.**, and Rus, H. (1999) Sublytic C5b-9 induces proliferation of human aortic smooth muscle cells: role of mitogen activated protein kinase and phosphatidylinositol 3-kinase. Atherosclerosis 1999 Jan;142(1):47-56.
doi: 10.1016/s0021-9150(98)00185-3.

https://doi.org/10.1016/s0021-9150(98)00185-3

56. **Badea, T.C.**, Niculescu, F.I., Soane, L., Shin, M.L., and Rus, H. (1998) Molecular cloning and characterization of RGC-32, a novel gene induced by complement activation in oligodendrocytes. Journal of Biological Chemistry 1998 Oct 9;273(41):26977-81. doi: 10.1074/jbc.273.41.26977

https://doi.org/10.1074/jbc.273.41.26977

57. Rus, H., Niculescu, F., **Badea, T.**, and Shin, M.L. (1997) Terminal complement complexes induce cell cycle entry in oligodendrocytes through mitogen activated protein kinase pathway. Immunopharmacology 1997 Dec;38(1-2):177-87.
doi: 10.1016/s0162-3109(97)00063-5

https://doi.org/10.1016/s0162-3109(97)00063-5

58. Lang, T.J., **Badea, T.C.**, Wade, R., and Shin, M.L. (1997) Sublytic terminal complement attack on myotubes decreases the expression of mRNAs encoding muscle-specific proteins. Journal of Neurochemistry 1997 Apr;68(4):1581-9. doi: 10.1046/j.1471-4159.1997.68041581.x

https://doi.org/10.1046/j.1471-4159.1997.68041581.x

59. Cristea, A., **Badea, T.**, Bodizs, G., and Olinic, N. (1995) Clinical evaluation of antineutrophil cytoplasmic autoantibodies in ANCA-associated diseases. Journal of Clinical Laboratory Immunology 46 (2): 85-94.

60. Cristea, A., **Badea, T.**, Bodizs, G., and Olinic, N. (1995) Antineutrophil cytoplasmic autoantibodies (ANCA) markers in diagnosis and monitoring systemic vasculitides. Romanian Journal of Internal Medicine 33 (1-2): 37-46.

General Bibliography

Amthor, F. R., Oyster, C. W., & Takahashi, E. S. (1983). Quantitative morphology of rabbit retinal ganglion cells. *Proc R Soc Lond B Biol Sci*, *217*(1208), 341--355.

Amthor, F. R., Oyster, C. W., & Takahashi, E. S. (1983). Quantitative morphology of rabbit retinal ganglion cells. *Proc R Soc Lond B Biol Sci*, *217*(1208), 341--355.

Amthor, F. R., Takahashi, E. S., & Oyster, C. W. (1989a). Morphologies of rabbit retinal ganglion cells with complex receptive fields. *J Comp Neurol*, *280*(1), 97--121. https://doi.org/10.1002/cne.902800108

Amthor, F. R., Takahashi, E. S., & Oyster, C. W. (1989b). Morphologies of rabbit retinal ganglion cells with concentric receptive fields. *J Comp Neurol*, *280*(1), 72--96. https://doi.org/10.1002/cne.902800107

Anastassiadis, K., Fu, J., Patsch, C., Hu, S., Weidlich, S., Duerschke, K., Buchholz, F., Edenhofer, F., & Stewart, A. F. (2009). Dre recombinase, like Cre, is a highly efficient site-specific recombinase in E. coli, mammalian cells and mice. *Dis Model Mech*, *2*(9–10), 508–515. https://doi.org/10.1242/dmm.003087

Anastassiadis, K., Glaser, S., Kranz, A., Berhardt, K., & Stewart, A. F. (2010). A practical summary of site-specific recombination, conditional mutagenesis, and tamoxifen induction of CreERT2. *Methods Enzymol*, *477*, 109–123. https://doi.org/10.1016/S0076-6879(10)77007-5

Atkinson-Leadbeater, K., & McFarlane, S. (2011). Extrinsic factors as multifunctional regulators of retinal ganglion cell morphogenesis. *Developmental Neurobiology*, *71*(12), 1170–1185. https://doi.org/10.1002/dneu.20924

Badea, T. C., & Nathans, J. (2004). Quantitative analysis of neuronal morphologies in the mouse retina visualized by using a genetically directed reporter. *Journal of Comparative Neurology*, *480*(4), 331–351. https://doi.org/10.1002/cne.20304

Badea, T. C., & Nathans, J. (2011). Morphologies of mouse retinal ganglion cells expressing transcription factors Brn3a, Brn3b, and Brn3c: Analysis of wild type and mutant cells using genetically-directed sparse labeling. *Vision Res*, *51*(2), 269–279. https://doi.org/S0042-6989(10)00434-7 [pii] 10.1016/j.visres.2010.08.039

Badea, T. C., Wang, Y., & Nathans, J. (2003). A noninvasive genetic/pharmacologic strategy for visualizing cell morphology and clonal relationships in the mouse. *J Neurosci*, *23*(6), 2314–2322. https://doi.org/23/6/2314 [pii]

Badea, T., Goldberg, J., Mao, B., & Yuste, R. (2001). Calcium imaging of epileptiform events with single-cell resolution. *Journal of Neurobiology*, *48*(3), 215–227. https://doi.org/10.1002/neu.1052

Baden, T., Berens, P., Franke, K., Roman Roson, M., Bethge, M., & Euler, T. (2016). The functional diversity of retinal ganglion cells in the mouse. *Nature*, *529*(7586), 345–350. https://doi.org/10.1038/nature16468

Bae, J. A., Mu, S., Kim, J. S., Turner, N. L., Tartavull, I., Kemnitz, N., Jordan, C. S., Norton, A. D., Silversmith, W. M., Prentki, R., Sorek, M., David, C., Jones, D. L., Bland, D., Sterling, A. L. R., Park, J., Briggman, K. L., Seung, H. S., & Eyewirers. (2018). Digital Museum of Retinal Ganglion Cells with Dense Anatomy and Physiology. *Cell*, *173*(5), 1293-1306 e19. https://doi.org/10.1016/j.cell.2018.04.040

Baier, H. (2013). Synaptic Laminae in the Visual System: Molecular Mechanisms Forming Layers of Perception. *Annual Review of Cell and Developmental Biology*, *29*(1), 385–416. https://doi.org/10.1146/annurev-cellbio-101011-155748

Berson, D. M., Dunn, F. A., & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science*, *295*(5557), 1070–1073. https://doi.org/10.1126/science.1067262

Birling, M.-C., Gofflot, F., & Warot, X. (2009). Site-specific recombinases for manipulation of the mouse genome. *Methods in Molecular Biology (Clifton, N.J.)*, *561*, 245–263. https://doi.org/10.1007/978-1-60327-019-9_16

Bischof, J., & Basler, K. (2008). Recombinases and their use in gene activation, gene inactivation, and transgenesis. *Methods in Molecular Biology (Clifton, N.J.)*, *420*, 175–195. https://doi.org/10.1007/978-1-59745-583-1_10

Blanchard, D. C., & Blanchard, R. J. (1988). Ethoexperimental approaches to the biology of emotion. *Annual Review of Psychology*, *39*, 43–68. https://doi.org/10.1146/annurev.ps.39.020188.000355

Blanchard, D. C., Williams, G., Lee, E. M. C., & Blanchard, R. J. (1981). Taming of wild Rattus norvegicus by lesions of the mesencephalic central gray. *Physiological Psychology*, *9*(2), 157–163. https://doi.org/10.3758/BF03332917

Boycott, B. B., Dowling, J. E., Kolb, H., Boycott, B. B., Dowling, J. E., & Wells, G. P. (1969). Organization of the primate retina: Light microscopy, with an appendix: A second type of midget bipolar cell in the primate retina. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *255*(799), 109–184. https://doi.org/10.1098/rstb.1969.0004
Boycott, B. B., & Wassle, H. (1974). The morphological types of ganglion cells of the domestic cat's retina. *J Physiol*, *240*(2), 397--419.
Boycott, B. B., & Wassle, H. (1991). Morphological Classification of Bipolar Cells of the Primate Retina. *Eur J Neurosci*, *3*(11), 1069--1088.
Brantley, M. A., Jain, S., Barr, E. E., Johnson, E. M., & Milbrandt, J. (2008). Neurturin-mediated ret activation is required for retinal function. *J Neurosci*, *28*(16), 4123–4135. https://doi.org/10.1523/JNEUROSCI.0249-08.2008
Brown, N. L., Kanekar, S., Vetter, M. L., Tucker, P. K., Gemza, D. L., & Glaser, T. (1998). Math5 encodes a murine basic helix-loop-helix transcription factor expressed during early stages of retinal neurogenesis. *Development*, *125*(23), 4821–4833.
Brown, N. L., Patel, S., Brzezinski, J., & Glaser, T. (2001). Math5 is required for retinal ganglion cell and optic nerve formation. *Development*, *128*(13), 2497–2508.
Cardozo, M. J., Almuedo-Castillo, M., & Bovolenta, P. (2020). Patterning the Vertebrate Retina with Morphogenetic Signaling Pathways. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *26*(2), 185–196. https://doi.org/10.1177/1073858419874016
Chichilnisky, E. J., & Baylor, D. A. (1999). Receptive-field microstructure of blue-yellow ganglion cells in primate retina. *Nat Neurosci*, *2*(10), 889–893. https://doi.org/10.1038/13189
Contreras, E., Nobleman, A. P., Robinson, P. R., & Schmidt, T. M. (2021). Melanopsin phototransduction: Beyond canonical cascades. *The Journal of Experimental Biology*, *224*(23), jeb226522. https://doi.org/10.1242/jeb.226522
Coombs, J., van der List, D., Wang, G.-Y., & Chalupa, L. M. (2006). Morphological properties of mouse retinal ganglion cells. *Neuroscience*, *140*(1), 123–136. https://doi.org/10.1016/j.neuroscience.2006.02.079
Crook, J. D., Manookin, M. B., Packer, O. S., & Dacey, D. M. (2011). Horizontal cell feedback without cone type-selective inhibition mediates "red-green" color opponency in midget ganglion cells of the primate retina. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(5), 1762–1772. https://doi.org/10.1523/JNEUROSCI.4385-10.2011
Dacey, D. M., & Lee, B. B. (1994). The "blue-on" opponent pathway in primate retina originates from a distinct bistratified ganglion cell type. *Nature*, *367*(6465), 731--735. https://doi.org/10.1038/367731a0
Dacey, D. M., Peterson, B. B., Robinson, F. R., & Gamlin, P. D. (2003). Fireworks in the primate retina: In vitro photodynamics reveals diverse LGN-projecting ganglion cell types. *Neuron*, *37*(1), 15--27.
De Franceschi, G., Vivattanasarn, T., Saleem, A. B., & Solomon, S. G. (2016). Vision Guides Selection of Freeze or Flight Defense Strategies in Mice. *Current Biology: CB*, *26*(16), 2150–2154. https://doi.org/10.1016/j.cub.2016.06.006
De Monasterio, F. M., & Gouras, P. (1975). Functional properties of ganglion cells of the rhesus monkey retina. *The Journal of Physiology*, *251*(1), 167–195. https://doi.org/10.1113/jphysiol.1975.sp011086
Dhande, O. S., Estevez, M. E., Quattrochi, L. E., El-Danaf, R. N., Nguyen, P. L., Berson, D. M., & Huberman, A. D. (2013). Genetic dissection of retinal inputs to brainstem nuclei controlling image stabilization. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(45), 17797–17813. https://doi.org/10.1523/JNEUROSCI.2778-13.2013
Dowling, J. E. (2012). *The retina: An approachable part of the brain* (Rev. ed). Belknap Press of Harvard University Press.
D'Souza, S. P., Swygart, D. I., Wienbar, S. R., Upton, B. A., Zhang, K. X., Mackin, R. D., Casasent, A. K., Samuel, M. A., Schwartz, G. W., & Lang, R. A. (2021). Retinal patterns and the cellular repertoire of neuropsin (Opn5) retinal ganglion cells. *The Journal of Comparative Neurology*. https://doi.org/10.1002/cne.25272
Elshatory, Y., Deng, M., Xie, X., & Gan, L. (2007). Expression of the LIM-homeodomain protein Isl1 in the developing and mature mouse retina. *The Journal of Comparative Neurology*, *503*(1), 182–197. https://doi.org/10.1002/cne.21390

Elshatory, Y., & Gan, L. (2008). The LIM-homeobox gene Islet-1 is required for the development of restricted forebrain cholinergic neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(13), 3291–3297. https://doi.org/10.1523/JNEUROSCI.5730-07.2008
Erkman, L., McEvilly, R. J., Luo, L., Ryan, A. K., Hooshmand, F., O'Connell, S. M., Keithley, E. M., Rapaport, D. H., Ryan, A. F., & Rosenfeld, M. G. (1996). Role of transcription factors Brn-3.1 and Brn-3.2 in auditory and visual system development. *Nature*, *381*(6583), 603–606. https://doi.org/10.1038/381603a0
Evans, D. A., Stempel, A. V., Vale, R., & Branco, T. (2019). Cognitive Control of Escape Behaviour. *Trends in Cognitive Sciences*, *23*(4), 334–348. https://doi.org/10.1016/j.tics.2019.01.012
Evans, D. A., Stempel, A. V., Vale, R., Ruehle, S., Lefler, Y., & Branco, T. (2018). A synaptic threshold mechanism for computing escape decisions. *Nature*, *558*(7711), 590–594. https://doi.org/10.1038/s41586-018-0244-6
Feller, M. B., Wellis, D. P., Stellwagen, D., Werblin, F. S., & Shatz, C. J. (1996). Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science*, *272*(5265), 1182–1187.
Field, G. D., & Chichilnisky, E. J. (2007). Information processing in the primate retina: Circuitry and coding. *Annu Rev Neurosci*, *30*, 1--30. https://doi.org/10.1146/annurev.neuro.30.051606.094252
Gan, L., Wang, S. W., Huang, Z., & Klein, W. H. (1999). POU domain factor Brn-3b is essential for retinal ganglion cell differentiation and survival but not for initial cell fate specification. *Dev Biol*, *210*(2), 469–480. https://doi.org/10.1006/dbio.1999.9280
Gan, L., Xiang, M., Zhou, L., Wagner, D. S., Klein, W. H., & Nathans, J. (1996). POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. *Proc Natl Acad Sci U S A*, *93*(9), 3920–3925.
Garrett, A. M., & Burgess, R. W. (2011). Candidate molecular mechanisms for establishing cell identity in the developing retina. *Developmental Neurobiology*, *71*(12), 1258–1272. https://doi.org/10.1002/dneu.20926
Ghosh, K. K., Bujan, S., Haverkamp, S., Feigenspan, A., & Wassle, H. (2004). Types of bipolar cells in the mouse retina. *J Comp Neurol*, *469*(1), 70--82. https://doi.org/10.1002/cne.10985
Goetz, J., Jessen, Z. F., Jacobi, A., Mani, A., Cooler, S., Greer, D., Kadri, S., Segal, J., Shekhar, K., Sanes, J., & Schwartz, G. W. (2021). Unified classification of mouse retinal ganglion cells using function, morphology, and gene expression. *BioRxiv*, 2021.06.10.447922. https://doi.org/10.1101/2021.06.10.447922
Graw, J. (1996). Genetic aspects of embryonic eye development in vertebrates. *Developmental Genetics*, *18*(3), 181–197. https://doi.org/10.1002/(SICI)1520-6408(1996)18:3<181::AID-DVG1>3.0.CO;2-5
Grindley, N. D. F., Whiteson, K. L., & Rice, P. A. (2006). Mechanisms of site-specific recombination. *Annual Review of Biochemistry*, *75*, 567–605. https://doi.org/10.1146/annurev.biochem.73.011303.073908
Grünert, U., & Martin, P. R. (2020). Cell types and cell circuits in human and non-human primate retina. *Progress in Retinal and Eye Research*, *78*, 100844. https://doi.org/10.1016/j.preteyeres.2020.100844
Grunert, U., Martin, P. R., & Wassle, H. (1994). Immunocytochemical analysis of bipolar cells in the macaque monkey retina. *J Comp Neurol*, *348*(4), 607--627. https://doi.org/10.1002/cne.903480410
Guler, A. D., Ecker, J. L., Lall, G. S., Haq, S., Altimus, C. M., Liao, H.-W., Barnard, A. R., Cahill, H., Badea, T. C., Zhao, H., Hankins, M. W., Berson, D. M., Lucas, R. J., Yau, K.-W., & Hattar, S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*, *453*(7191), 102--105. https://doi.org/10.1038/nature06829
Hattar, S., Liao, H. W., Takao, M., Berson, D. M., & Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science*, *295*(5557), 1065--1070. https://doi.org/10.1126/science.1069609
Hattar, S., Lucas, R. J., Mrosovsky, N., Thompson, S., Douglas, R. H., Hankins, M. W., Lem, J., Biel, M., Hofmann, F., Foster, R. G., & Yau, K.-W. (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*, *424*(6944), 76–81. https://doi.org/10.1038/nature01761
Helmstaedter, M., Briggman, K. L., Turaga, S. C., Jain, V., Seung, H. S., & Denk, W. (2013). Connectomic reconstruction of the inner plexiform layer in the mouse retina. *Nature*, *500*(7461), 168–174. https://doi.org/10.1038/nature12346
Herrera, E., Erskine, L., & Morenilla-Palao, C. (2019). Guidance of retinal axons in mammals. *Seminars in Cell & Developmental Biology*, *85*, 48–59. https://doi.org/10.1016/j.semcdb.2017.11.027

Hobert, O. (2011). Regulation of terminal differentiation programs in the nervous system. *Annu Rev Cell Dev Biol*, *27*, 681–696. https://doi.org/10.1146/annurev-cellbio-092910-154226
Hong, Y. K., Kim, I. J., & Sanes, J. R. (2011). Stereotyped axonal arbors of retinal ganglion cell subsets in the mouse superior colliculus. *J Comp Neurol*, *519*(9), 1691–1711. https://doi.org/10.1002/cne.22595
Huberman, A. D., Wei, W., Elstrott, J., Stafford, B. K., Feller, M. B., & Barres, B. A. (2009). Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron*, *62*(3), 327--334. https://doi.org/10.1016/j.neuron.2009.04.014
Jacobs, G. H., Williams, G. A., Cahill, H., & Nathans, J. (2007). Emergence of novel color vision in mice engineered to express a human cone photopigment. *Science*, *315*(5819), 1723–1725. https://doi.org/10.1126/science.1138838
Jacoby, J., & Schwartz, G. W. (2017). Three Small-Receptive-Field Ganglion Cells in the Mouse Retina Are Distinctly Tuned to Size, Speed, and Object Motion. *J Neurosci*, *37*(3), 610–625. https://doi.org/10.1523/JNEUROSCI.2804-16.2016
Jacoby, J., & Schwartz, G. W. (2018). Typology and Circuitry of Suppressed-by-Contrast Retinal Ganglion Cells. *Frontiers in Cellular Neuroscience*, *12*, 269. https://doi.org/10.3389/fncel.2018.00269
Jefferis, G. S., & Livet, J. (2012). Sparse and combinatorial neuron labelling. *Current Opinion in Neurobiology*, *22*(1), 101–110. https://doi.org/10.1016/j.conb.2011.09.010
Jensen, P., & Dymecki, S. M. (2014). Essentials of recombinase-based genetic fate mapping in mice. *Methods in Molecular Biology (Clifton, N.J.)*, *1092*, 437–454. https://doi.org/10.1007/978-1-60327-292-6_26
Jessell, T. M. (2000). Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. *Nat Rev Genet*, *1*(1), 20–29. https://doi.org/10.1038/35049541
Joesch, M., & Meister, M. (2016). A neuronal circuit for colour vision based on rod-cone opponency. *Nature*, *532*(7598), 236–239. https://doi.org/10.1038/nature17158
Johnson, K. P., Zhao, L., & Kerschensteiner, D. (2018). A Pixel-Encoder Retinal Ganglion Cell with Spatially Offset Excitatory and Inhibitory Receptive Fields. *Cell Rep*, *22*(6), 1462–1472. https://doi.org/10.1016/j.celrep.2018.01.037
Kawai, S., Maekawajiri, S., & Yamane, A. (1993). A Simple Method of Detecting Amplified DNA with Immobilized Probes on Microtiter Wells. *Analytical Biochemistry*, *209*(1), 63–69. https://doi.org/10.1006/abio.1993.1083
Kay, J. N., De la Huerta, I., Kim, I. J., Zhang, Y., Yamagata, M., Chu, M. W., Meister, M., & Sanes, J. R. (2011). Retinal ganglion cells with distinct directional preferences differ in molecular identity, structure, and central projections. *J Neurosci*, *31*(21), 7753–7762. https://doi.org/10.1523/JNEUROSCI.0907-11.2011
Komiyama, T., & Luo, L. (2006). Development of wiring specificity in the olfactory system. *Curr Opin Neurobiol*, *16*(1), 67–73. https://doi.org/S0959-4388(05)00184-4 [pii] 10.1016/j.conb.2005.12.002
Lee, T., & Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron*, *22*(3), 451–461.
Levick, W. R. (1975). Form and function of cat retinal ganglion cells. *Nature*, *254*(5502), 659--662.
Lin, B., Wang, S. W., & Masland, R. H. (2004). Retinal ganglion cell type, size, and spacing can be specified independent of homotypic dendritic contacts. *Neuron*, *43*(4), 475–485. https://doi.org/10.1016/j.neuron.2004.08.002 S0896627304004891 [pii]
Liu, W., Khare, S. L., Liang, X., Peters, M. A., Liu, X., Cepko, C. L., & Xiang, M. (2000). All Brn3 genes can promote retinal ganglion cell differentiation in the chick. *Development*, *127*(15), 3237–3247.
Liu, W., Mo, Z., & Xiang, M. (2001). The Ath5 proneural genes function upstream of Brn3 POU domain transcription factor genes to promote retinal ganglion cell development. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(4), 1649–1654. https://doi.org/10.1073/pnas.98.4.1649
Luo, W., Enomoto, H., Rice, F. L., Milbrandt, J., & Ginty, D. D. (2009). Molecular identification of rapidly adapting mechanoreceptors and their developmental dependence on ret signaling. *Neuron*, *64*(6), 841–856. https://doi.org/10.1016/j.neuron.2009.11.003
Luo, W., Wickramasinghe, S. R., Savitt, J. M., Griffin, J. W., Dawson, T. M., & Ginty, D. D. (2007). A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. *Neuron*, *54*(5), 739–754. https://doi.org/10.1016/j.neuron.2007.04.027

Lyu, J., & Mu, X. (2021). Genetic control of retinal ganglion cell genesis. *Cellular and Molecular Life Sciences: CMLS*, *78*(9), 4417–4433. https://doi.org/10.1007/s00018-021-03814-w
MacNeil, M. A., & Masland, R. H. (1998). Extreme Diversity among Amacrine Cells: Implications for Function. *Neuron*, *20*(5), 971–982. https://doi.org/10.1016/S0896-6273(00)80478-X
Macosko, E. Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A. R., Kamitaki, N., Martersteck, E. M., Trombetta, J. J., Weitz, D. A., Sanes, J. R., Shalek, A. K., Regev, A., & McCarroll, S. A. (2015). Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell*, *161*(5), 1202–1214. https://doi.org/10.1016/j.cell.2015.05.002
Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., Berka, J., Braverman, M. S., Chen, Y.-J., Chen, Z., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V., Goodwin, B. C., He, W., Helgesen, S., Ho, C. H., Irzyk, G. P., … Rothberg, J. M. (2005). Genome Sequencing in Open Microfabricated High Density Picoliter Reactors. *Nature*, *437*(7057), 376–380. https://doi.org/10.1038/nature03959
Mariani, A. P. (1990). Amacrine cells of the rhesus monkey retina. *Journal of Comparative Neurology*, *301*(3), 382–400. https://doi.org/10.1002/cne.903010305
Marquardt, T., & Gruss, P. (2002). Generating neuronal diversity in the retina: One for nearly all. *Trends Neurosci*, *25*(1), 32–38.
Martersteck, E. M., Hirokawa, K. E., Evarts, M., Bernard, A., Duan, X., Li, Y., Ng, L., Oh, S. W., Ouellette, B., Royall, J. J., Stoecklin, M., Wang, Q., Zeng, H., Sanes, J. R., & Harris, J. A. (2017). Diverse Central Projection Patterns of Retinal Ganglion Cells. *Cell Rep*, *18*(8), 2058–2072. https://doi.org/10.1016/j.celrep.2017.01.075
Masland, R. H. (2001). The fundamental plan of the retina. *Nat Neurosci*, *4*(9), 877--886. https://doi.org/10.1038/nn0901-877
Masland, R. H. (2012). The neuronal organization of the retina. *Neuron*, *76*(2), 266–280. https://doi.org/S0896-6273(12)00883-5 [pii] 10.1016/j.neuron.2012.10.002
Mason, C., & Slavi, N. (2020). Retinal Ganglion Cell Axon Wiring Establishing the Binocular Circuit. *Annual Review of Vision Science*, *6*(1), 215–236. https://doi.org/10.1146/annurev-vision-091517-034306
Masri, R. A., Percival, K. A., Koizumi, A., Martin, P. R., & Grünert, U. (2019). Survey of retinal ganglion cell morphology in marmoset. *The Journal of Comparative Neurology*, *527*(1), 236–258. https://doi.org/10.1002/cne.24157
Masseck, O. A., & Hoffmann, K.-P. (2009). Comparative neurobiology of the optokinetic reflex. *Annals of the New York Academy of Sciences*, *1164*, 430–439. https://doi.org/10.1111/j.1749-6632.2009.03854.x
McEvilly, R. J., Erkman, L., Luo, L., Sawchenko, P. E., Ryan, A. F., & Rosenfeld, M. G. (1996). Requirement for Brn-3.0 in differentiation and survival of sensory and motor neurons. *Nature*, *384*(6609), 574–577. https://doi.org/10.1038/384574a0
Meister, M., Pine, J., & Baylor, D. A. (1994). Multi-neuronal signals from the retina: Acquisition and analysis. *J Neurosci Methods*, *51*(1), 95–106.
Meister, M., Wong, R. O., Baylor, D. A., & Shatz, C. J. (1991). Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science*, *252*(5008), 939–943.
Missaire, M., & Hindges, R. (2015). The role of cell adhesion molecules in visual circuit formation: From neurite outgrowth to maps and synaptic specificity. *Developmental Neurobiology*, *75*(6), 569–583. https://doi.org/10.1002/dneu.22267
Molecular Cloning: A Laboratory Manual (Fourth Edition). (n.d.). Retrieved November 15, 2021, from https://cshlpress.com/default.tpl?action=full&src=pdf&--eqskudatarq=934
Mu, X., Fu, X., Beremand, P. D., Thomas, T. L., & Klein, W. H. (2008). Gene regulation logic in retinal ganglion cell development: Isl1 defines a critical branch distinct from but overlapping with Pou4f2. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(19), 6942–6947. https://doi.org/10.1073/pnas.0802627105
Nath, A., & Schwartz, G. W. (2016). Cardinal Orientation Selectivity Is Represented by Two Distinct Ganglion Cell Types in Mouse Retina. *Journal of Neuroscience*, *36*(11), 3208–3221. https://doi.org/10.1523/JNEUROSCI.4554-15.2016

Nguyen-Ba-Charvet, K. T., & Rebsam, A. (2020). Neurogenesis and Specification of Retinal Ganglion Cells. *International Journal of Molecular Sciences*, *21*(2), E451. https://doi.org/10.3390/ijms21020451
Pan, L., Deng, M., Xie, X., & Gan, L. (2008). ISL1 and BRN3B co-regulate the differentiation of murine retinal ganglion cells. *Development*, *135*(11), 1981–1990. https://doi.org/10.1242/dev.010751
Pan, L., Yang, Z., Feng, L., & Gan, L. (2005). Functional equivalence of Brn3 POU-domain transcription factors in mouse retinal neurogenesis. *Development*, *132*(4), 703–712. https://doi.org/10.1242/dev.01646
Peichl, L., & Gonzalez-Soriano, J. (1994). Morphological types of horizontal cell in rodent retinae: A comparison of rat, mouse, gerbil, and guinea pig. *Vis Neurosci*, *11*(3), 501–517.
Peng, Y. R., Shekhar, K., Yan, W., Herrmann, D., Sappington, A., Bryman, G. S., van Zyl, T., Do, M. T. H., Regev, A., & Sanes, J. R. (2019). Molecular Classification and Comparative Taxonomics of Foveal and Peripheral Cells in Primate Retina. *Cell*, *176*(5), 1222-1237 e22. https://doi.org/10.1016/j.cell.2019.01.004
Polyak, S. L. (1941). *The retina: The anatomy and the histology of the retina in man, ape, and monkey, including the consideration of visual functions, the history of physiological optics, and the histological laboratory technique*. Chicago.
Prigge, C. L., & Kay, J. N. (2018). Dendrite morphogenesis from birth to adulthood. *Current Opinion in Neurobiology*, *53*, 139–145. https://doi.org/10.1016/j.conb.2018.07.007
Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., & Rollag, M. D. (2000). A novel human opsin in the inner retina. *J Neurosci*, *20*(2), 600–605.
Ramón y Cajal, S. (1972). *The structure of the retina*. C.C. Thomas.
Rheaume, B. A., Jereen, A., Bolisetty, M., Sajid, M. S., Yang, Y., Renna, K., Sun, L., Robson, P., & Trakhtenberg, E. F. (2018). Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat Commun*, *9*(1), 2759. https://doi.org/10.1038/s41467-018-05134-3
Rivlin-Etzion, M., Zhou, K., Wei, W., Elstrott, J., Nguyen, P. L., Barres, B. A., Huberman, A. D., & Feller, M. B. (2011). Transgenic mice reveal unexpected diversity of on-off direction-selective retinal ganglion cell subtypes and brain structures involved in motion processing. *J Neurosci*, *31*(24), 8760–8769. https://doi.org/10.1523/JNEUROSCI.0564-11.2011
Rockhill, R. L., Daly, F. J., MacNeil, M. A., Brown, S. P., & Masland, R. H. (2002). The diversity of ganglion cells in a mammalian retina. *J Neurosci*, *22*(9), 3831–3843. https://doi.org/20026369 22/9/3831 [pii]
Rodieck, R. W. (1998). *The first steps in seeing*. Sinauer Associates.
Rodieck, R. W., & Watanabe, M. (1993). Survey of the morphology of macaque retinal ganglion cells that project to the pretectum, superior colliculus, and parvicellular laminae of the lateral geniculate nucleus. *J Comp Neurol*, *338*(2), 289--303. https://doi.org/10.1002/cne.903380211
Rousso, D. L., Qiao, M., Kagan, R. D., Yamagata, M., Palmiter, R. D., & Sanes, J. R. (2016). Two Pairs of ON and OFF Retinal Ganglion Cells Are Defined by Intersectional Patterns of Transcription Factor Expression. *Cell Rep*, *15*(9), 1930–1944. https://doi.org/10.1016/j.celrep.2016.04.069
Rucci, M., & Victor, J. D. (2015). The unsteady eye: An information-processing stage, not a bug. *Trends in Neurosciences*, *38*(4), 195–206. https://doi.org/10.1016/j.tins.2015.01.005
Rupprecht, P., Carta, S., Hoffmann, A., Echizen, M., Blot, A., Kwan, A. C., Dan, Y., Hofer, S. B., Kitamura, K., Helmchen, F., & Friedrich, R. W. (2021). A database and deep learning toolbox for noise-optimized, generalized spike inference from calcium imaging. *Nature Neuroscience*, *24*(9), 1324–1337. https://doi.org/10.1038/s41593-021-00895-5
Salay, L. D., & Huberman, A. D. (2021). Divergent outputs of the ventral lateral geniculate nucleus mediate visually evoked defensive behaviors. *Cell Reports*, *37*(1), 109792. https://doi.org/10.1016/j.celrep.2021.109792
Salay, L. D., Ishiko, N., & Huberman, A. D. (2018). A midline thalamic circuit determines reactions to visual threat. *Nature*, *557*(7704), 183–189. https://doi.org/10.1038/s41586-018-0078-2
Sanes, J. R., & Zipursky, S. L. (2020). Synaptic Specificity, Recognition Molecules, and Assembly of Neural Circuits. *Cell*, *181*(3), 536–556. https://doi.org/10.1016/j.cell.2020.04.008

Sauer, B., & Henderson, N. (1988). Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5166–5170.
Sauer, B., & McDermott, J. (2004). DNA recombination with a heterospecific Cre homolog identified from comparison of the pac-c1 regions of P1-related phages. *Nucleic Acids Res*, *32*(20), 6086–6095. https://doi.org/10.1093/nar/gkh941
Schuster, S. C. (2008). Next-generation sequencing transforms today's biology. *Nature Methods*, *5*(1), 16–18. https://doi.org/10.1038/nmeth1156
Simpson, J. I. (1984). The accessory optic system. *Annu Rev Neurosci*, *7*, 13–41. https://doi.org/10.1146/annurev.ne.07.030184.000305
Sitko, A. A., & Goodrich, L. V. (2021). Making sense of neural development by comparing wiring strategies for seeing and hearing. *Science (New York, N.Y.)*, *371*(6525), eaaz6317. https://doi.org/10.1126/science.aaz6317
Smallwood, P. M., Olveczky, B. P., Williams, G. L., Jacobs, G. H., Reese, B. E., Meister, M., & Nathans, J. (2003). Genetically engineered mice with an additional class of cone photoreceptors: Implications for the evolution of color vision. *Proc Natl Acad Sci U S A*, *100*(20), 11706–11711. https://doi.org/10.1073/pnas.1934712100
Spering, M., & Carrasco, M. (2015). Acting without seeing: Eye movements reveal visual processing without awareness. *Trends in Neurosciences*, *38*(4), 247–258. https://doi.org/10.1016/j.tins.2015.02.002
Stahl, J. S. (2004). Using eye movements to assess brain function in mice. *Vision Research*, *44*(28), 3401–3410. https://doi.org/10.1016/j.visres.2004.09.011
Sun, W., Li, N., & He, S. (2002a). Large-scale morophological survey of rat retinal ganglion cells. *Vis Neurosci*, *19*(4), 483–493.
Sun, W., Li, N., & He, S. (2002b). Large-scale morphological survey of mouse retinal ganglion cells. *J Comp Neurol*, *451*(2), 115–126. https://doi.org/10.1002/cne.10323
Tank, D. W., Sugimori, M., Connor, J. A., & Llinás, R. R. (1988). Spatially resolved calcium dynamics of mammalian Purkinje cells in cerebellar slice. *Science (New York, N.Y.)*, *242*(4879), 773–777. https://doi.org/10.1126/science.2847315
Taub, F., E., DeLEO, J. M., & Thompson, E. B. (1983). Sequential Comparative Hybridizations Analyzed by Computerized Image Processing Can Identify and Quantitate Regulated RNAs. *DNA*, *2*(4), 309–327. https://doi.org/10.1089/dna.1983.2.309
Thoreson, W. B., & Dacey, D. M. (2019). Diverse Cell Types, Circuits, and Mechanisms for Color Vision in the Vertebrate Retina. *Physiological Reviews*, *99*(3), 1527–1573. https://doi.org/10.1152/physrev.00027.2018
Tien, N. W., Pearson, J. T., Heller, C. R., Demas, J., & Kerschensteiner, D. (2015). Genetically Identified Suppressed-by-Contrast Retinal Ganglion Cells Reliably Signal Self-Generated Visual Stimuli. *J Neurosci*, *35*(30), 10815–10820. https://doi.org/10.1523/JNEUROSCI.1521-15.2015
Tran, N. M., Shekhar, K., Whitney, I. E., Jacobi, A., Benhar, I., Hong, G., Yan, W., Adiconis, X., Arnold, M. E., Lee, J. M., Levin, J. Z., Lin, D., Wang, C., Lieber, C. M., Regev, A., He, Z., & Sanes, J. R. (2019). Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes. *Neuron*, *104*(6), 1039-1055 e12. https://doi.org/10.1016/j.neuron.2019.11.006
Troy, J. B., & Shou, T. (2002). The receptive fields of cat retinal ganglion cells in physiological and pathological states: Where we are after half a century of research. *Progress in Retinal and Eye Research*, *21*(3), 263–302. https://doi.org/10.1016/s1350-9462(02)00002-2
Turan, S., Zehe, C., Kuehle, J., Qiao, J., & Bode, J. (2013). Recombinase-mediated cassette exchange (RMCE)—A rapidly-expanding toolbox for targeted genomic modifications. *Gene*, *515*(1), 1–27. https://doi.org/10.1016/j.gene.2012.11.016
Velculescu, V. E., Zhang, L., Vogelstein, B., & Kinzler, K. W. (1995). Serial analysis of gene expression. *Science (New York, N.Y.)*, *270*(5235), 484–487. https://doi.org/10.1126/science.270.5235.484
Vooijs, M., Jonkers, J., & Berns, A. (2001). A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. *EMBO Reports*, *2*(4), 292–297. https://doi.org/10.1093/embo-reports/kve064

Wang, S. W., Kim, B. S., Ding, K., Wang, H., Sun, D., Johnson, R. L., Klein, W. H., & Gan, L. (2001). Requirement for math5 in the development of retinal ganglion cells. *Genes Dev*, *15*(1), 24–29.
Wang, S. W., Mu, X., Bowers, W. J., Kim, D. S., Plas, D. J., Crair, M. C., Federoff, H. J., Gan, L., & Klein, W. H. (2002). Brn3b/Brn3c double knockout mice reveal an unsuspected role for Brn3c in retinal ganglion cell axon outgrowth. *Development*, *129*(2), 467–477.
Wassle, H. (2004). Parallel processing in the mammalian retina. *Nat Rev Neurosci*, *5*(10), 747--757. https://doi.org/10.1038/nrn1497
Watanabe, M., & Rodieck, R. W. (1989). Parasol and midget ganglion cells of the primate retina. *J Comp Neurol*, *289*(3), 434--454. https://doi.org/10.1002/cne.902890308
Wilson, S. W., & Houart, C. (2004). Early steps in the development of the forebrain. *Developmental Cell*, *6*(2), 167–181. https://doi.org/10.1016/s1534-5807(04)00027-9
Wu, F., Kaczynski, T. J., Sethuramanujam, S., Li, R., Jain, V., Slaughter, M., & Mu, X. (2015). Two transcription factors, Pou4f2 and Isl1, are sufficient to specify the retinal ganglion cell fate. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(13), E1559-68. https://doi.org/10.1073/pnas.1421535112
Xiang, M., Gan, L., Li, D., Chen, Z. Y., Zhou, L., O'Malley, J., Klein, W., & Nathans, J. (1997). Essential role of POU-domain factor Brn-3c in auditory and vestibular hair cell development. *Proc Natl Acad Sci U S A*, *94*(17), 9445–9450.
Xiang, M., Gan, L., Li, D., Zhou, L., Chen, Z. Y., Wagner, D., O'Malley, J., Klein, W., & Nathans, J. (1997). Role of the Brn-3 family of POU-domain genes in the development of the auditory/vestibular, somatosensory, and visual systems. *Cold Spring Harb Symp Quant Biol*, *62*, 325–336.
Xiang, M., Gan, L., Zhou, L., Klein, W. H., & Nathans, J. (1996). Targeted deletion of the mouse POU domain gene Brn-3a causes selective loss of neurons in the brainstem and trigeminal ganglion, uncoordinated limb movement, and impaired suckling. *Proc Natl Acad Sci U S A*, *93*(21), 11950–11955.
Xiang, M., Zhou, L., Macke, J. P., Yoshioka, T., Hendry, S. H., Eddy, R. L., Shows, T. B., & Nathans, J. (1995). The Brn-3 family of POU-domain factors: Primary structure, binding specificity, and expression in subsets of retinal ganglion cells and somatosensory neurons. *J Neurosci*, *15*(7 Pt 1), 4762–4785.
Xiang, M., Zhou, L., Peng, Y. W., Eddy, R. L., Shows, T. B., & Nathans, J. (1993). Brn-3b: A POU domain gene expressed in a subset of retinal ganglion cells. *Neuron*, *11*(4), 689–701.
Yan, W., Peng, Y. R., van Zyl, T., Regev, A., Shekhar, K., Juric, D., & Sanes, J. R. (2020). Cell Atlas of The Human Fovea and Peripheral Retina. *Sci Rep*, *10*(1), 9802. https://doi.org/10.1038/s41598-020-66092-9
Yilmaz, M., & Meister, M. (2013). Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology: CB*, *23*(20), 2011–2015. https://doi.org/10.1016/j.cub.2013.08.015
Yonehara, K., Ishikane, H., Sakuta, H., Shintani, T., Nakamura-Yonehara, K., Kamiji, N. L., Usui, S., & Noda, M. (2009). Identification of retinal ganglion cells and their projections involved in central transmission of information about upward and downward image motion. *PLoS ONE*, *4*(1), e4320. https://doi.org/10.1371/journal.pone.0004320
Yonehara, K., Shintani, T., Suzuki, R., Sakuta, H., Takeuchi, Y., Nakamura-Yonehara, K., & Noda, M. (2008). Expression of SPIG1 reveals development of a retinal ganglion cell subtype projecting to the medial terminal nucleus in the mouse. *PLoS ONE*, *3*(2), e1533. https://doi.org/10.1371/journal.pone.0001533
Yuste, R., & Katz, L. C. (1991). Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron*, *6*(3), 333–344. https://doi.org/10.1016/0896-6273(91)90243-s
Zhang, Y., Kim, I. J., Sanes, J. R., & Meister, M. (2012). The most numerous ganglion cell type of the mouse retina is a selective feature detector. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(36), E2391-8. https://doi.org/10.1073/pnas.1211547109