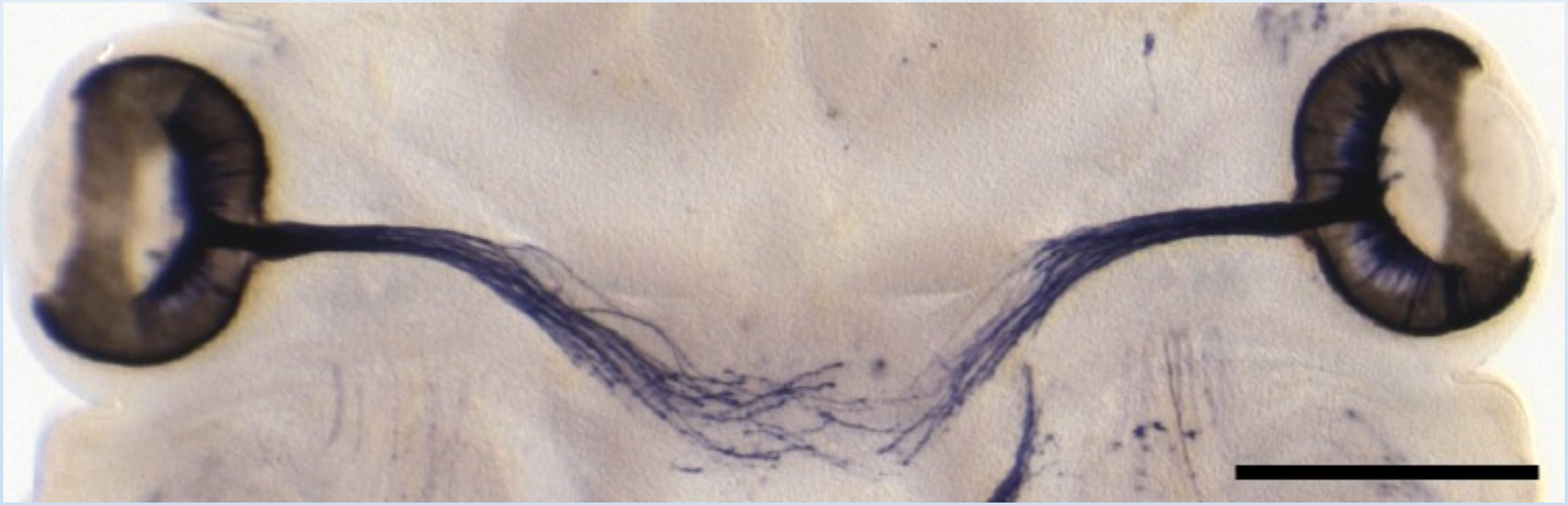
Manipulări Genetice in studiul Celulelor Retinale Ganglionare

- de la mecanisme moleculare la circuite neuronale-



Tudor C. Badea,

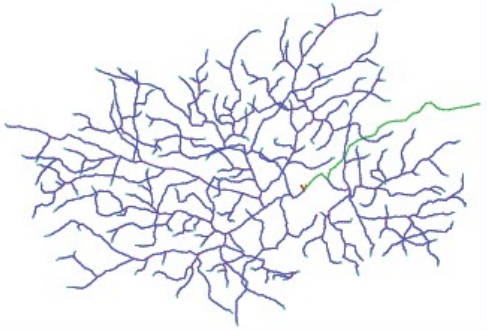
Cercetator Stiintific II,

Institutul de Cercetare – Dezvoltare,

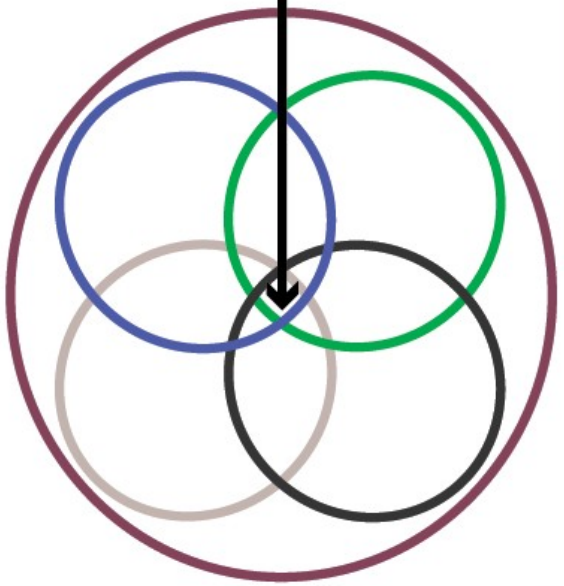
Facultatea de Medicina, Universitatea Transilvania

What is a neuronal cell type ?

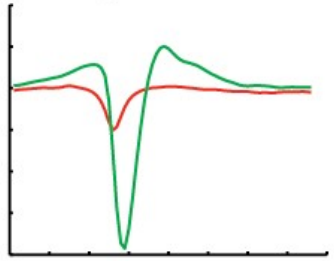
Arbor Morphology



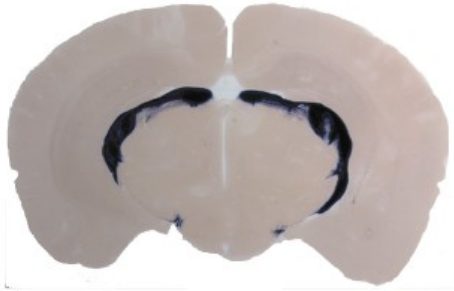
Cell type



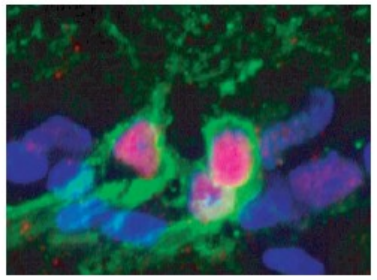
Physiological Properties



Connectivity

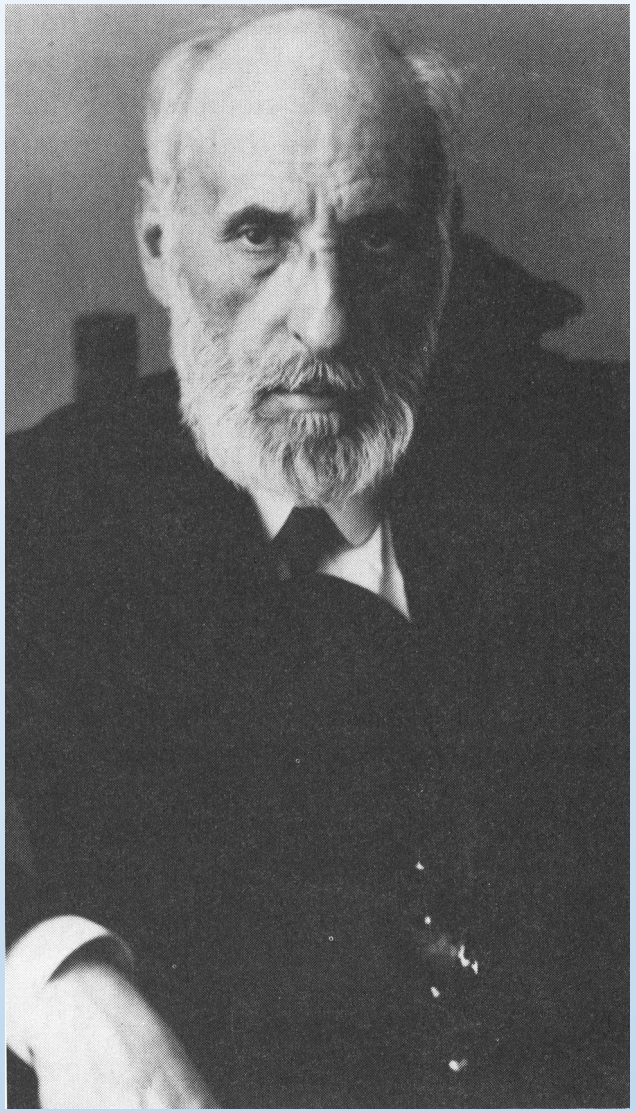


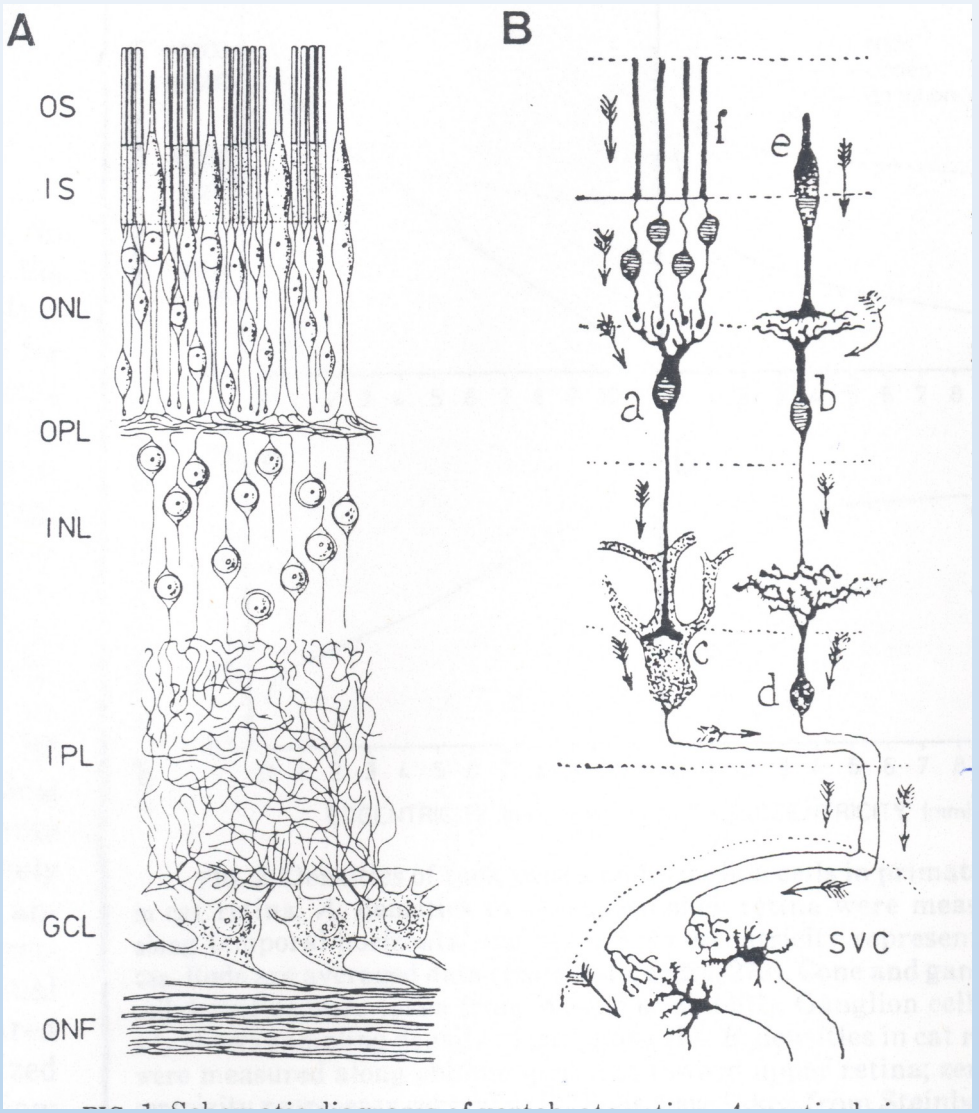
Molecular determinants



Function

Morfologia arborilor neuronali este un instrument important in clasificarea tipurilor neuronale





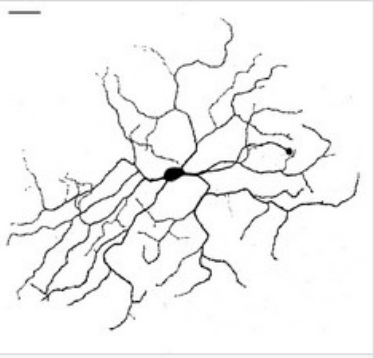
Dendritic Arbor Morphology Correlates With RGC Physiological Type

Retinal Ganglion Cell Typology Project Menu

Cell Type	EyeWire Type	Functional Class	filter	# Cells
ON alpha	8w	ON sustained		101

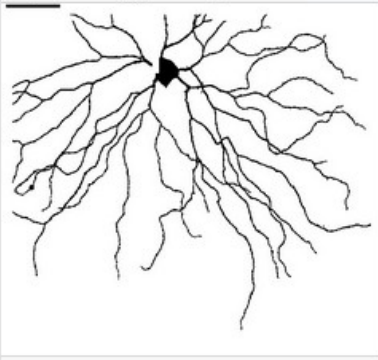
Expand Table

Light Microscopy



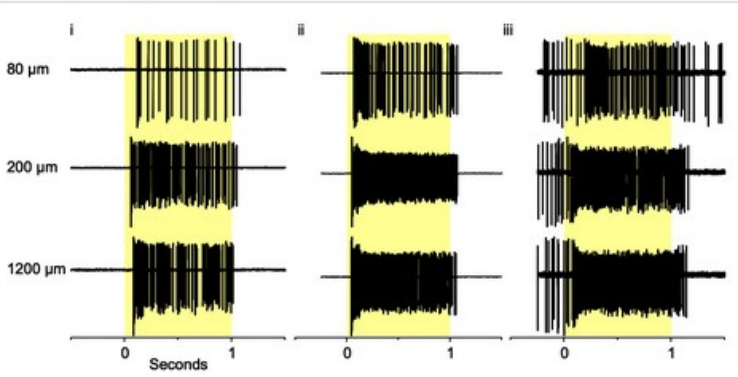
Scale bar = 50 µm

EyeWire (type 8w)

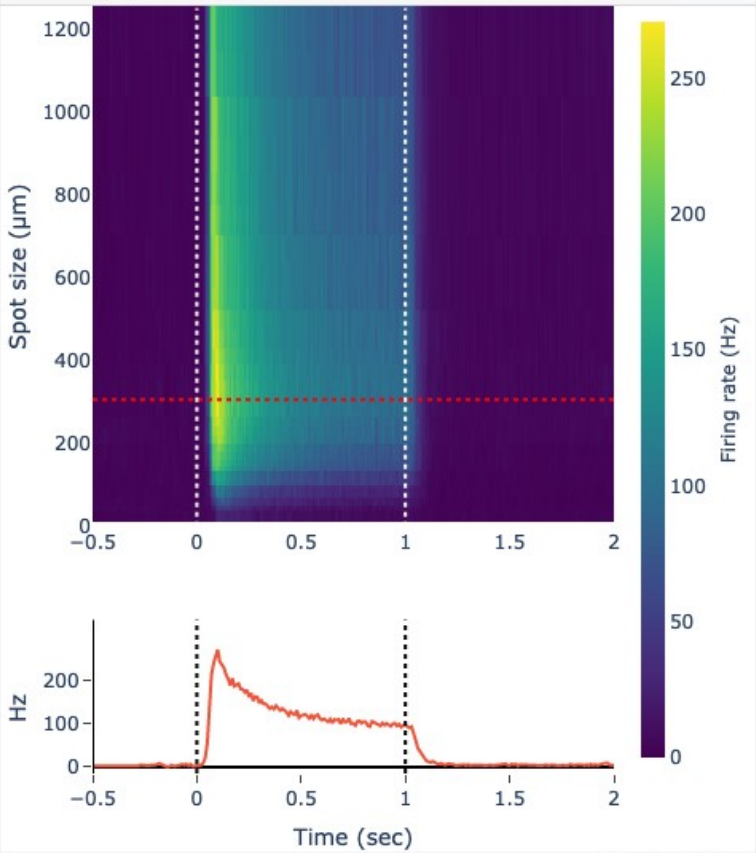


Scale bar = 50 µm

Example Light Response Traces

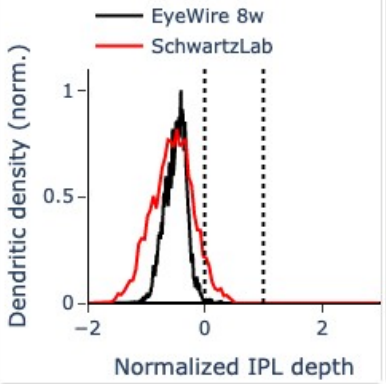


PSTH By Spot Size

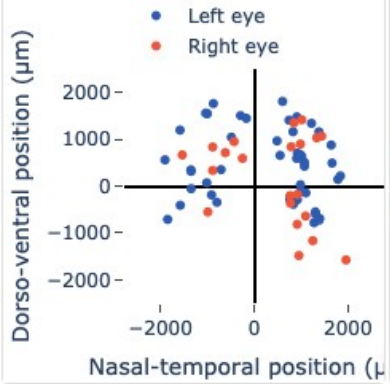


Spot size: 306 µm

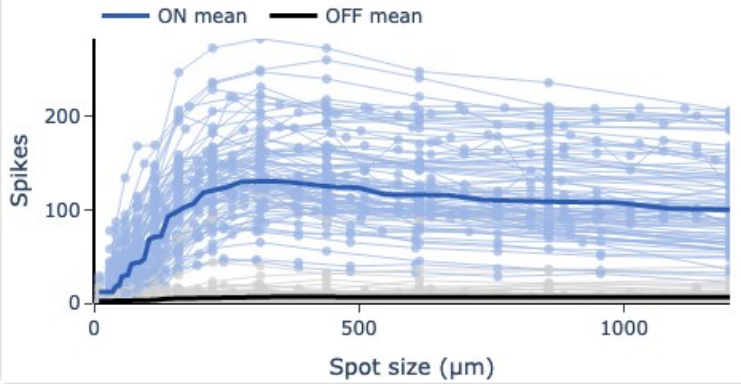
Stratification Profile



Cell Positions

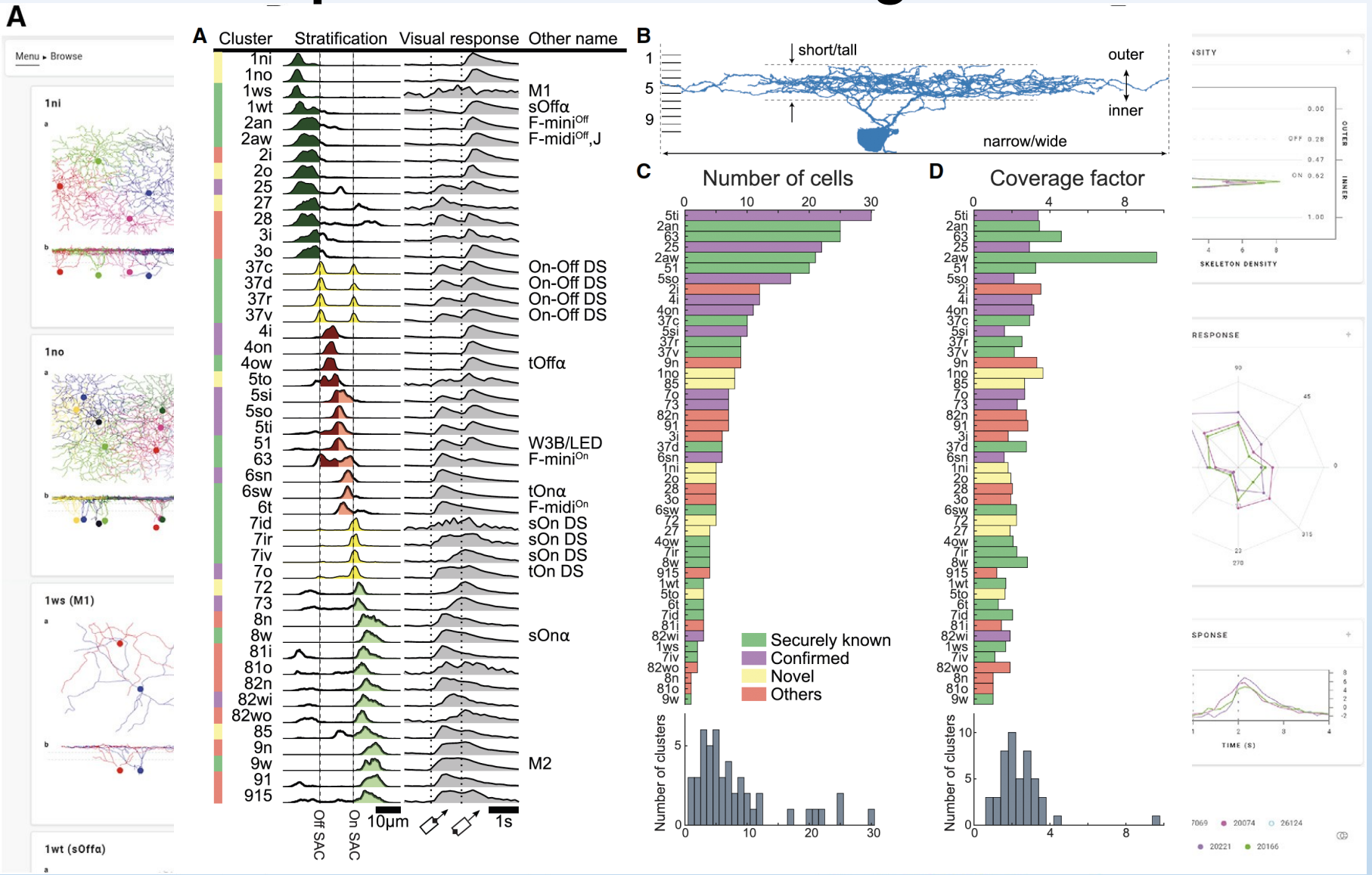


Spike Count vs. Spot Size



Schwartz Lab – RGCTYPES.ORG

Clasificări Anatomic-Funcționale bazate pe Microscopie Electronica si Imagistica de Calciu

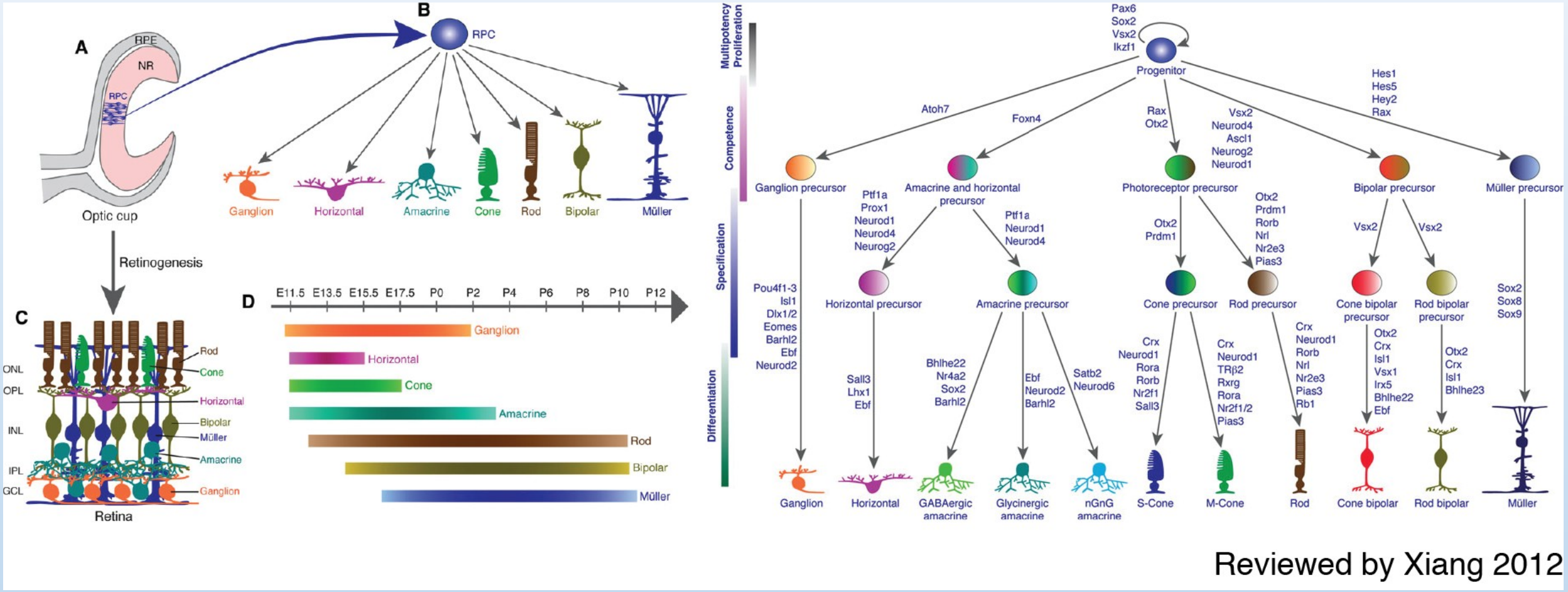


Helmstaedter 2013 Bae 2018

Clasificări Moleculare bazate pe secventarea ARN din celule izolate

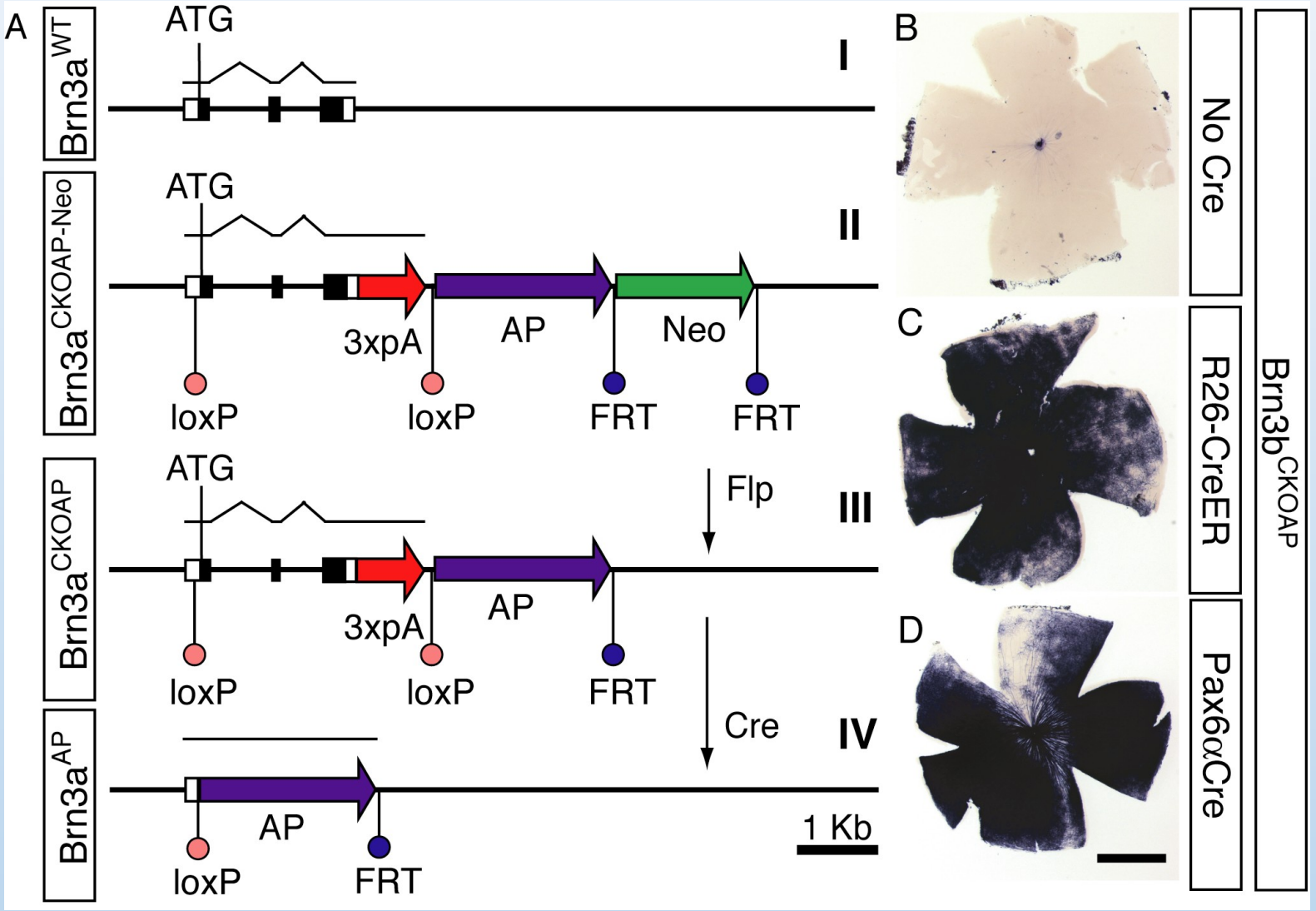
Rheaume 2018, Tran 2019

Neuronii din Retina sânt specificați prin cascade transcriptionale aranjate cronologic.



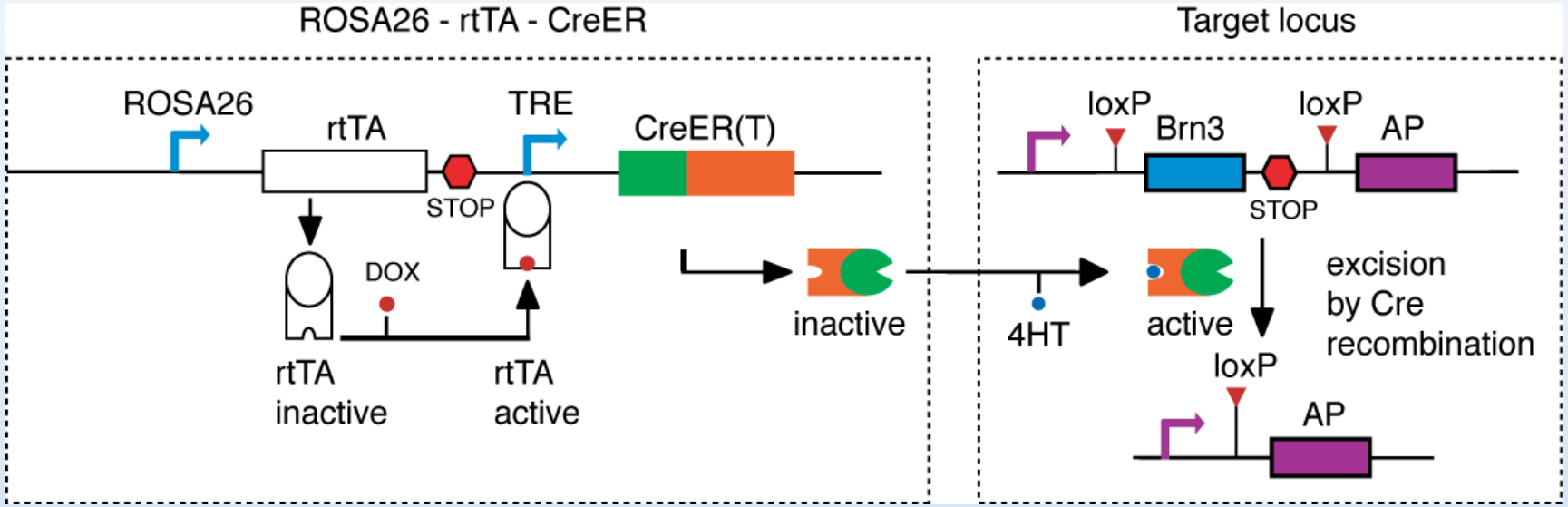
Reviewed by Xiang 2012

Alele knock-out condiționale cuplate cu gene indicator permit vizualizarea neuronilor care exprima factorii de transcripție din clasa Pou4f: Brn3a, Brn3b si Brn3c.

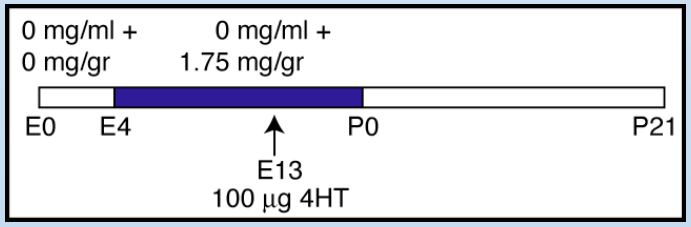


Badea et al 2009

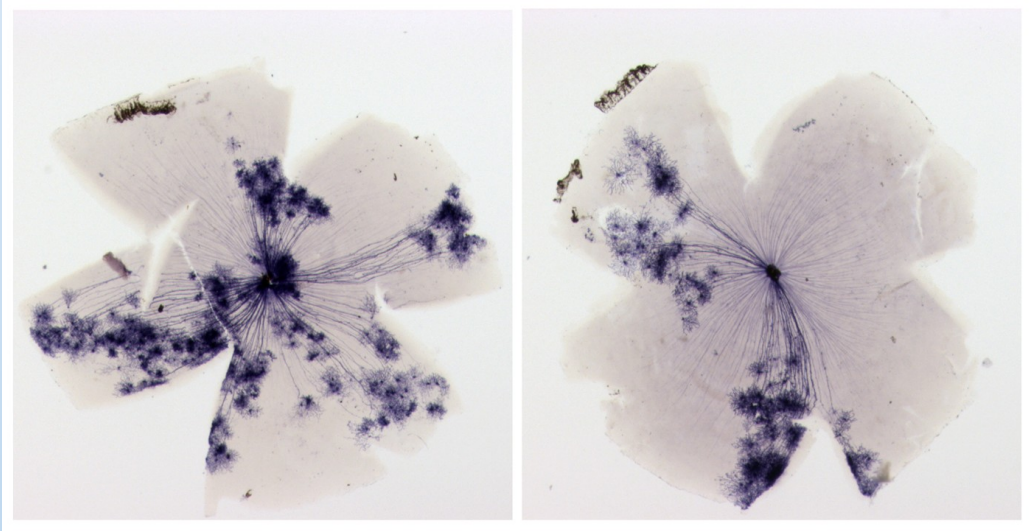
Recombinare Cre – loxP indusa prin control farmacologic dual (tamoxifen + Doxyciclina) marchează celule RGC izolate



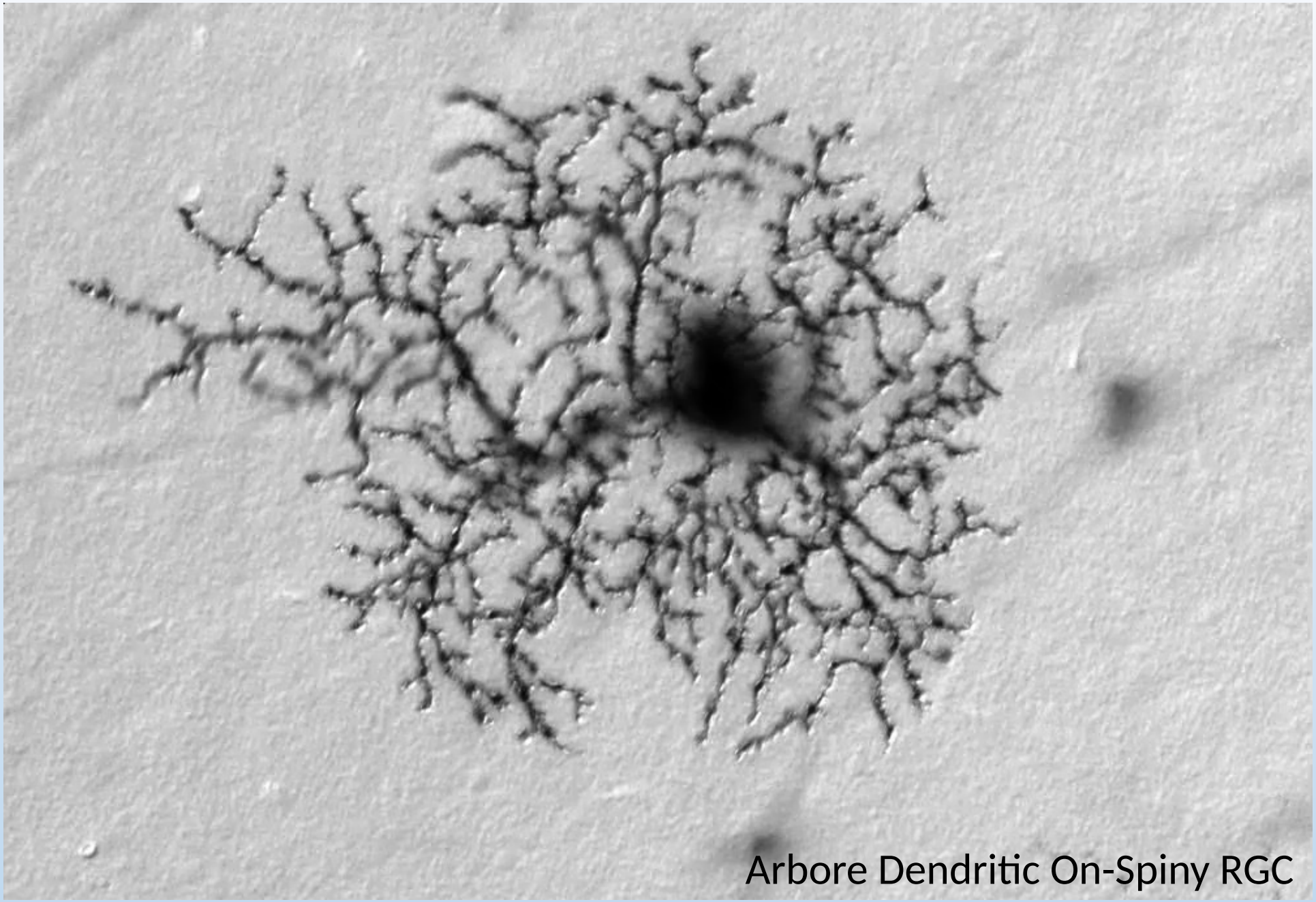
Example of DOX (blue bar) and 4HT administration protocol



R26TC - Brn3a$^{CKOAP/+}$ R26TC - Brn3a$^{CKOAP/-}$

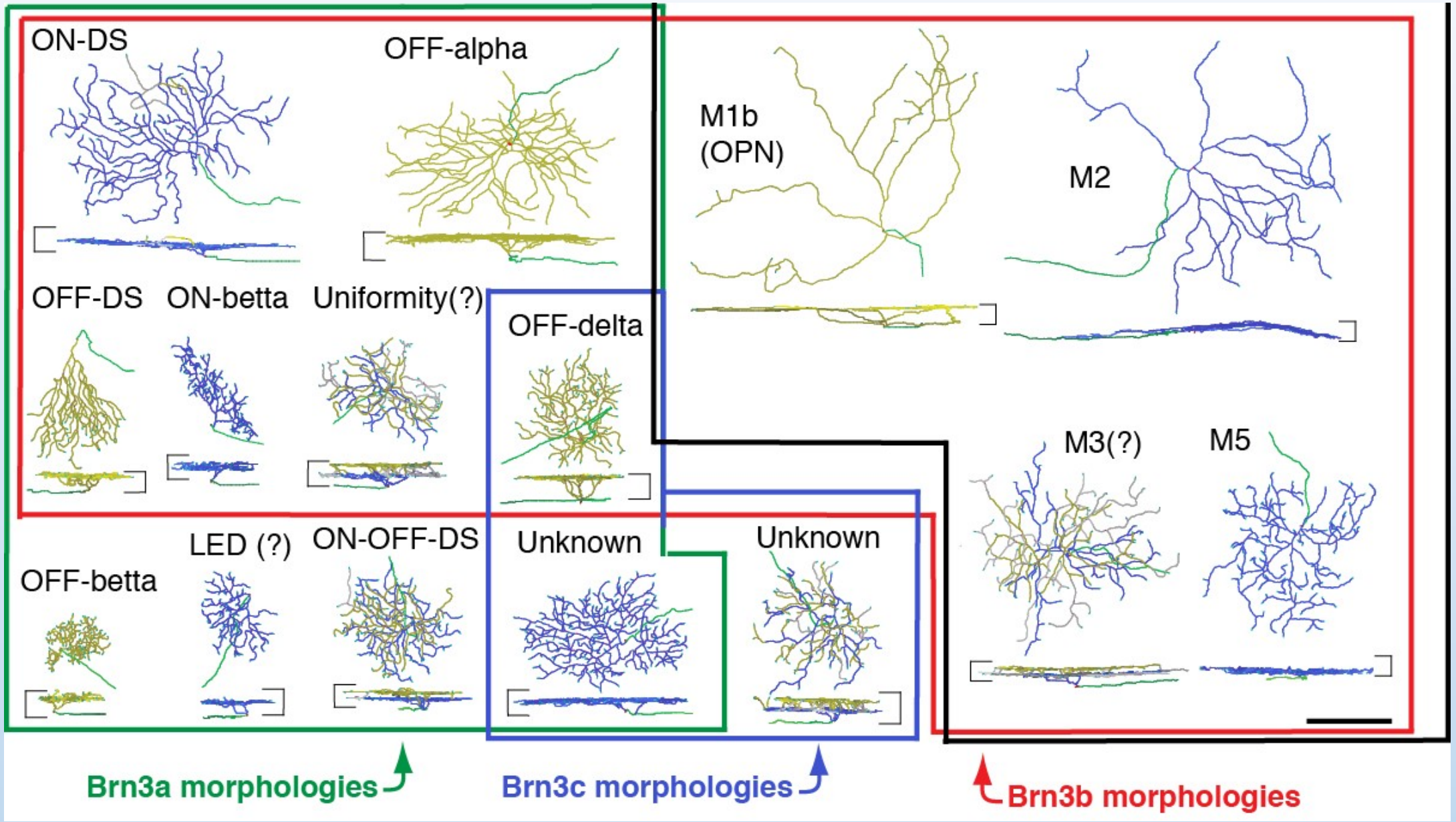


Badea et al PLOSone 2009 and Vision research 2011



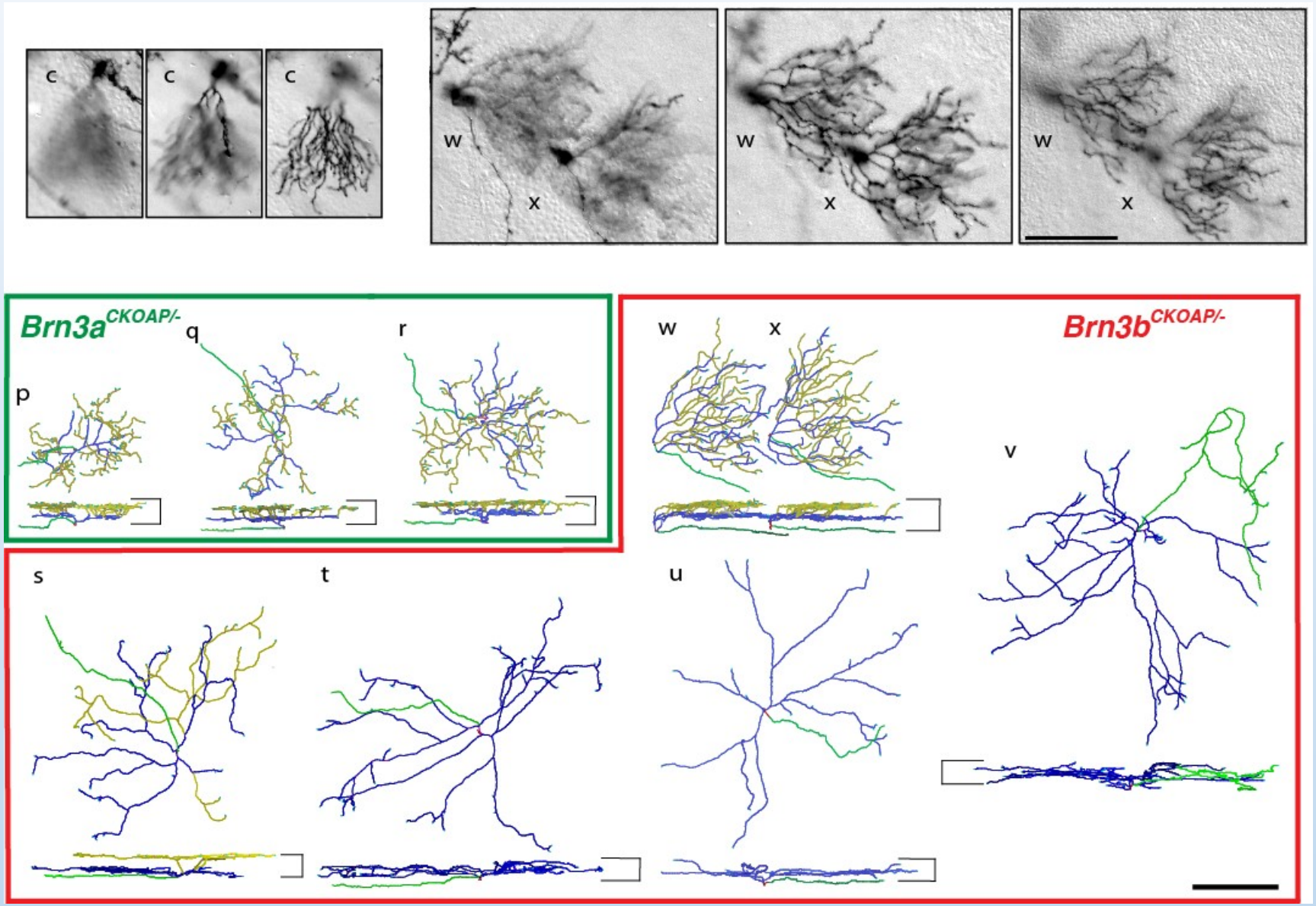
Arbore Dendritic On-Spiny RGC

Tipurile celulare RGC sant specificate printr-un Cod Transcriptional Combinatorial



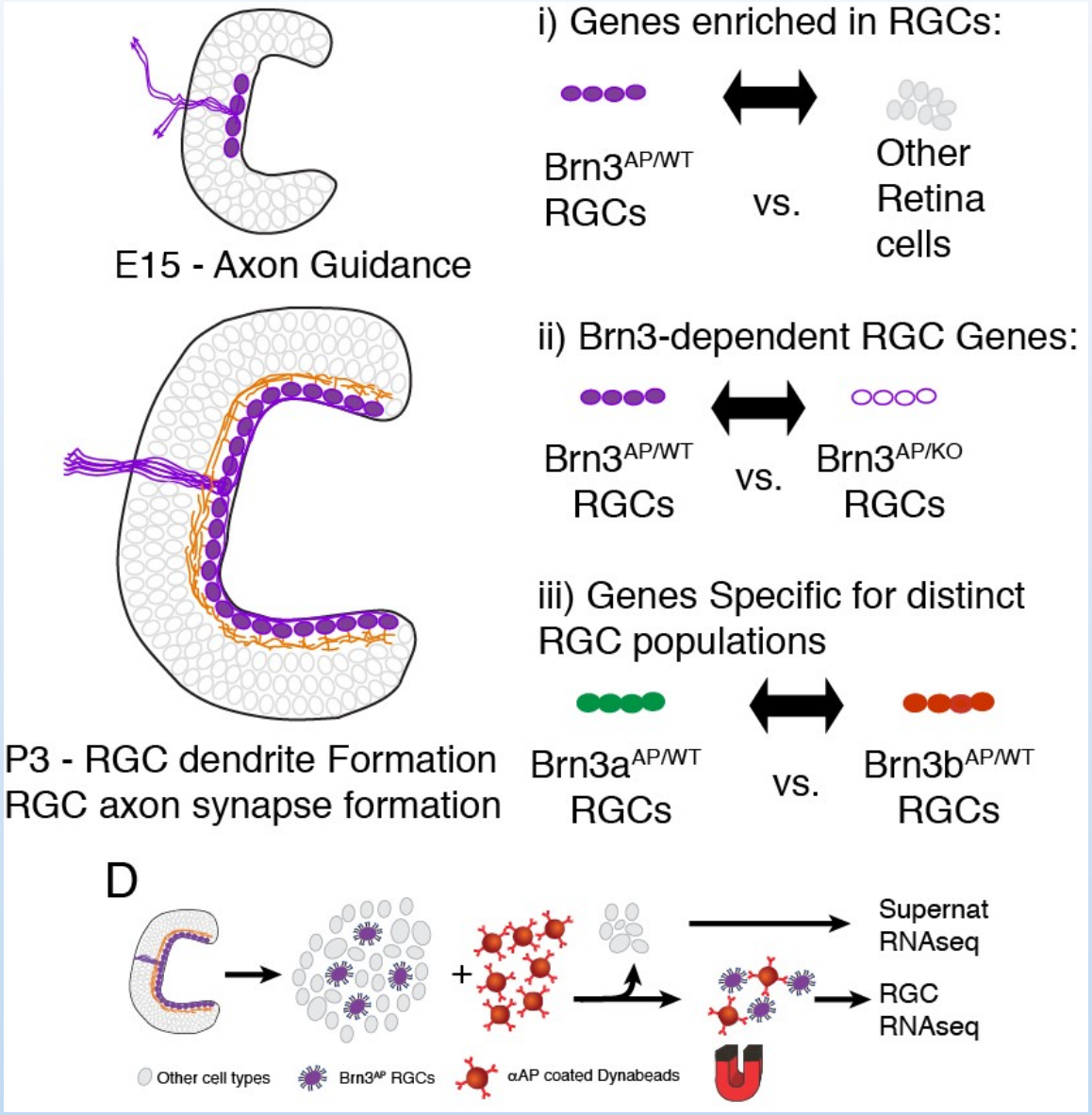
Badea 2011 & 2012, Chen 2011, Shi 2013, Parmhans 2018

Dendritele celulelor RGC suferă modificări semnificative in mutanții de Brn3a si Brn3b



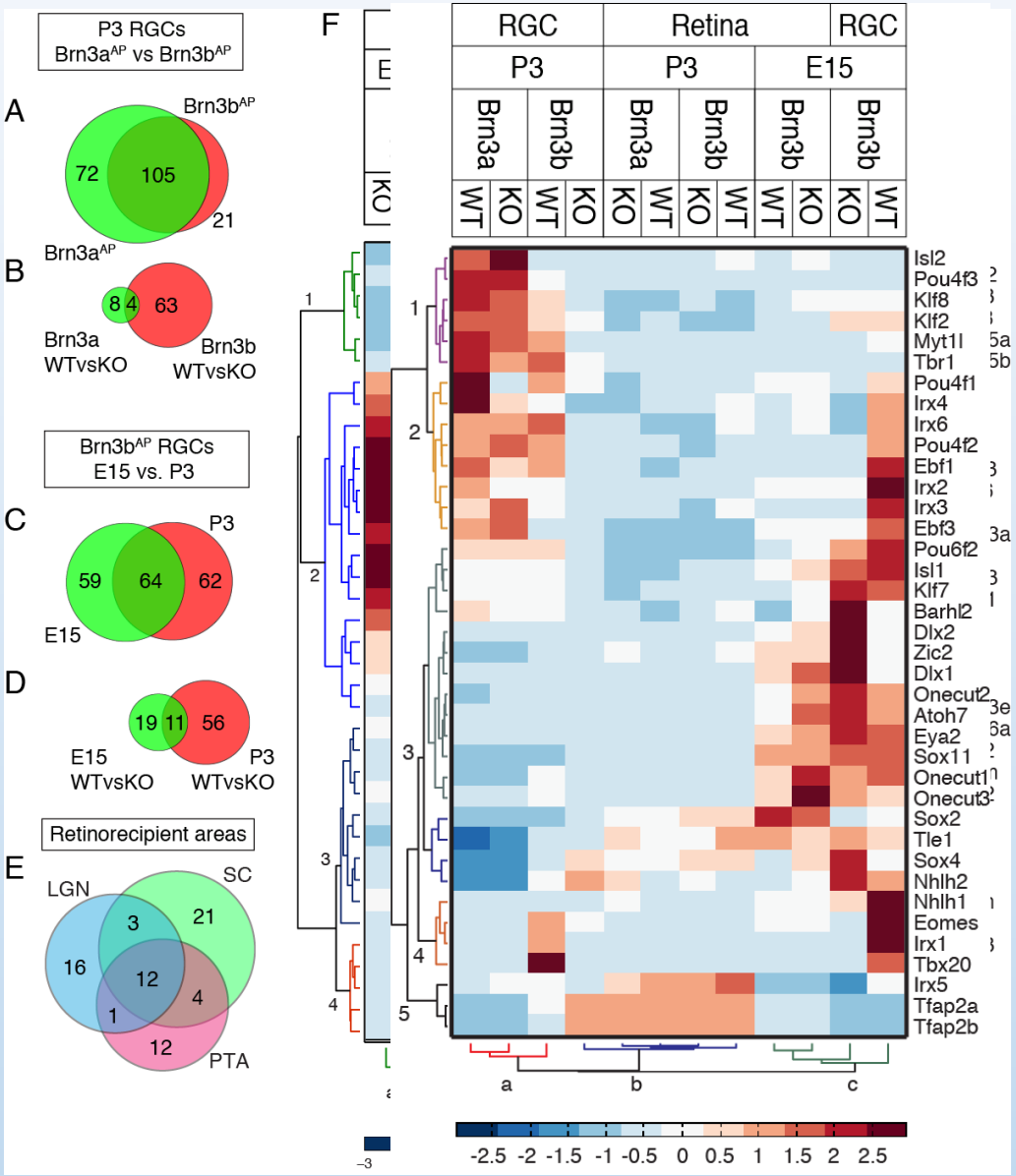
Badea and Nathans 2011

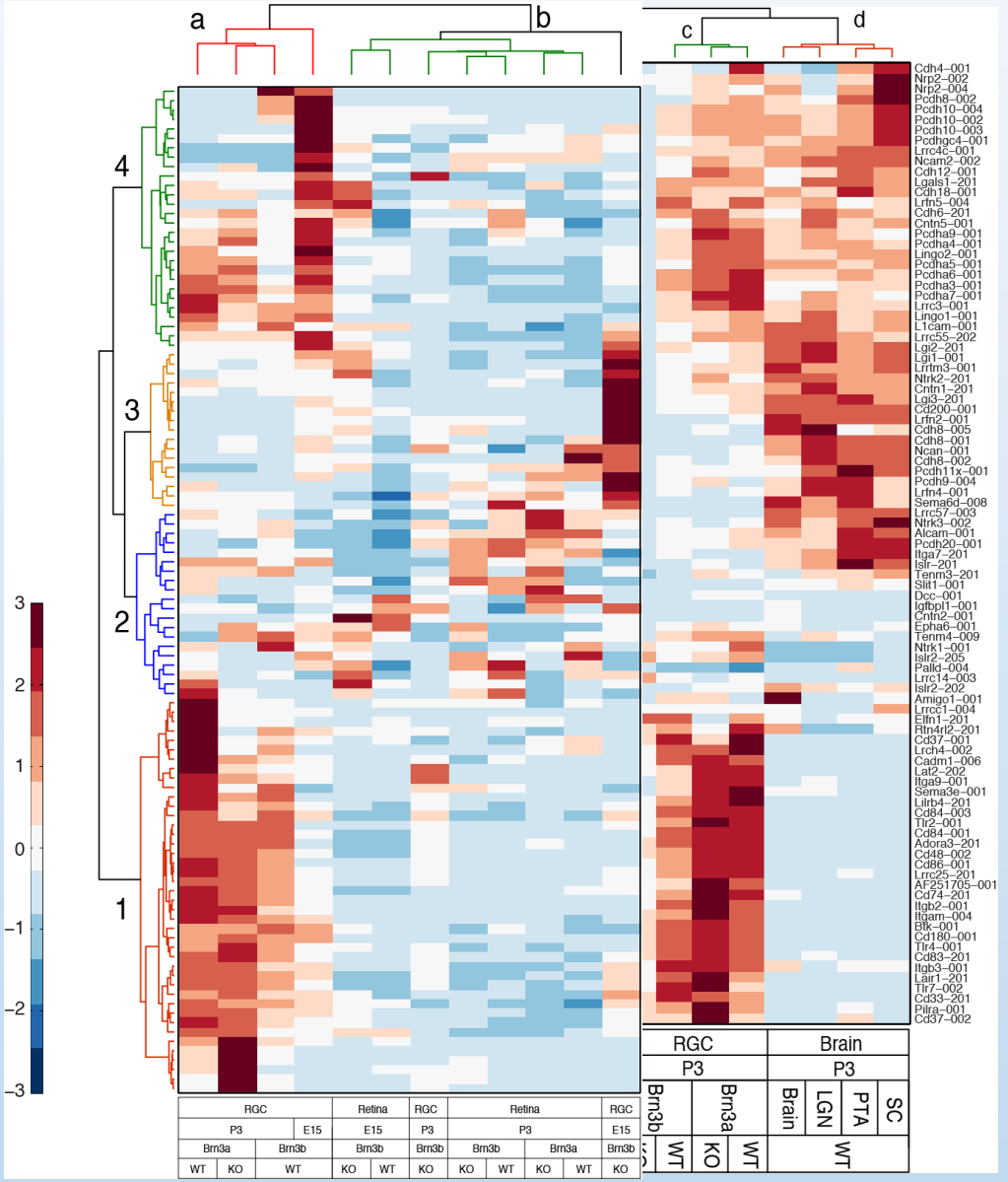
Purificare Imunomagnetica si RNAseq din celule RGC Brn3^{AP}



Szilard Sajgo, Miruna Ghinia, Matthew Brooks 2017

Transcription Factor Combinatorial Code

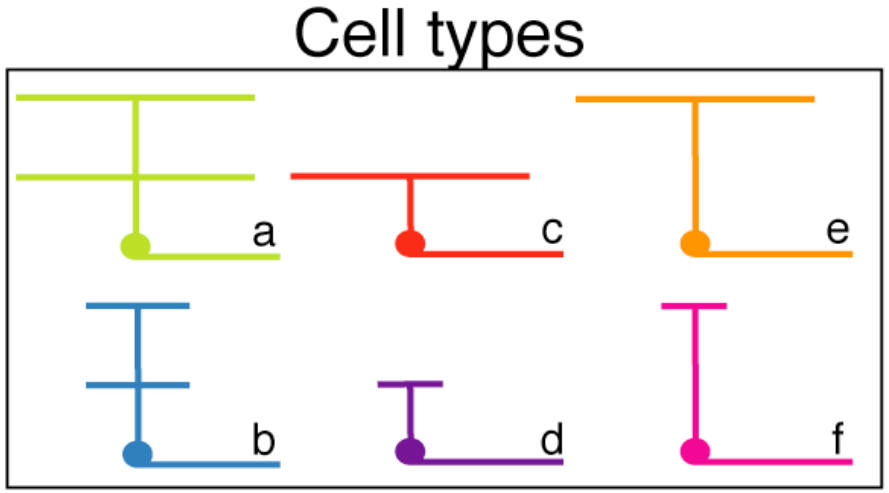




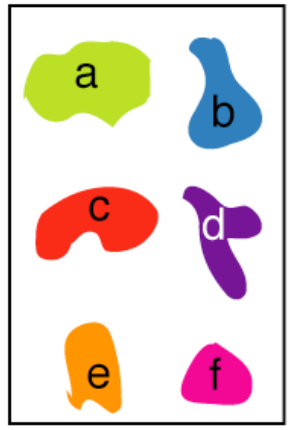
Szilard Sajgo, Miruna Ghinia, Matthew Brooks 2017

Cum funcționează codurile de transcripție ?

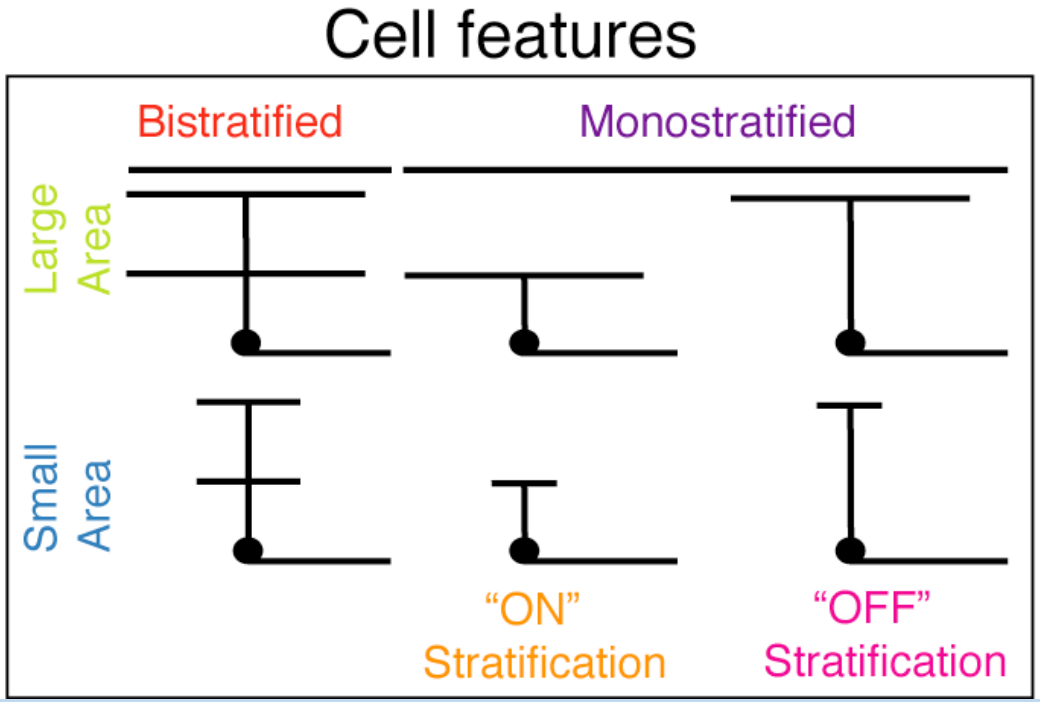
Strategy 1:
Transcription factors regulate individual cell types (specifying all necessary cell features).



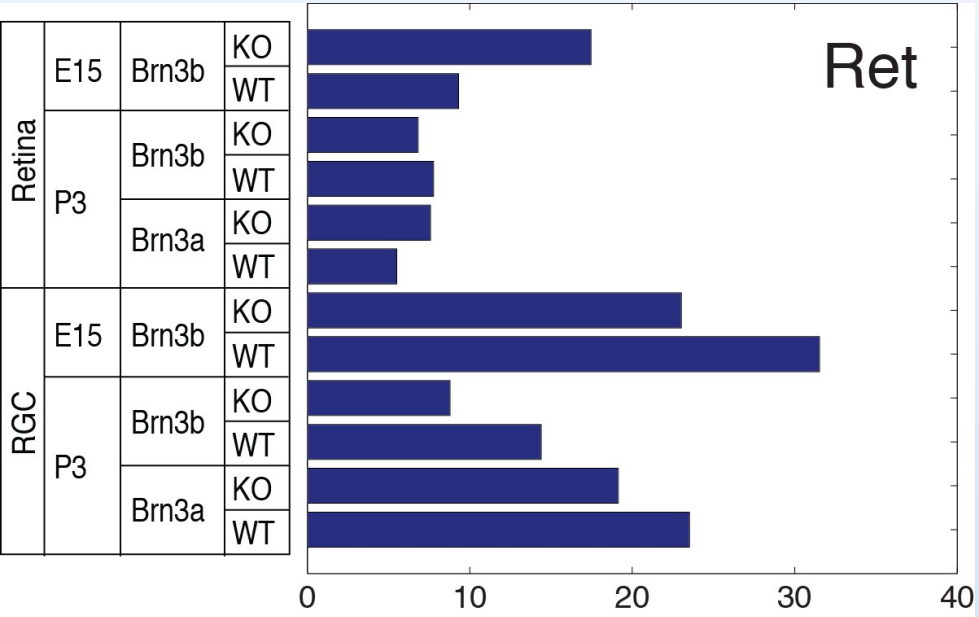
Transcription factors

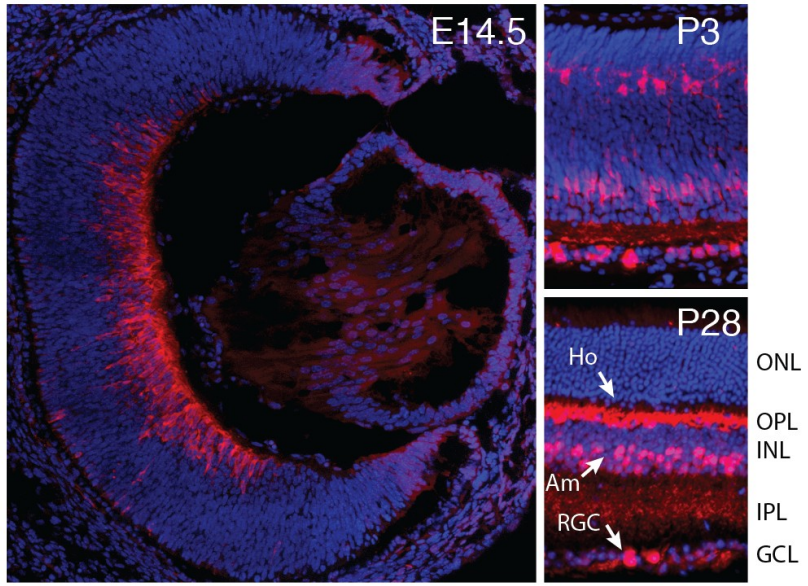


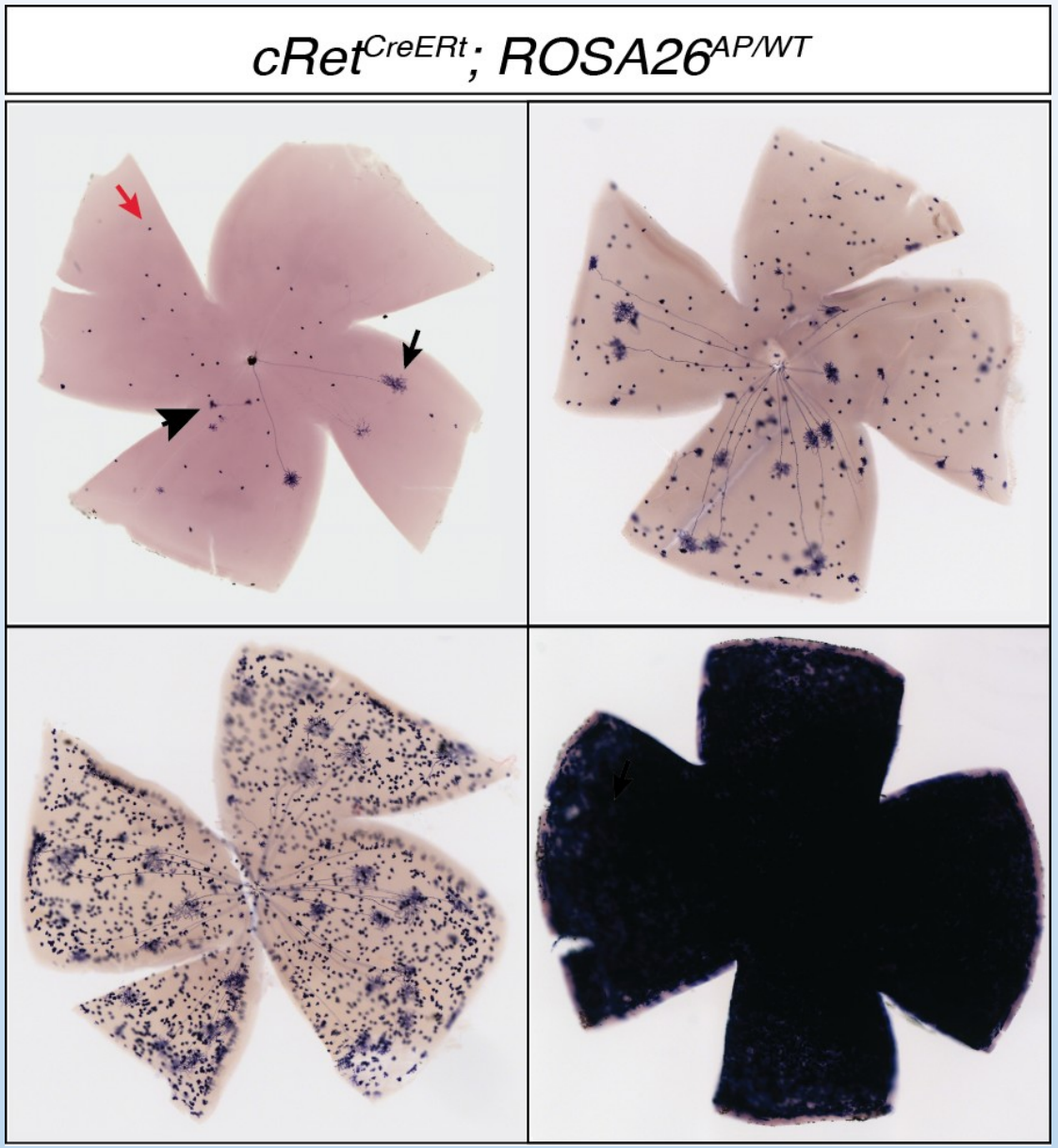
Strategy 2:
Transcription factors regulate cell features. Acquisition of pertinent cell features results in cell type identity.



Receptorul de GDNF, Ret, este exprimat dinamic in RGC, Celule Orizontale si Amacrine

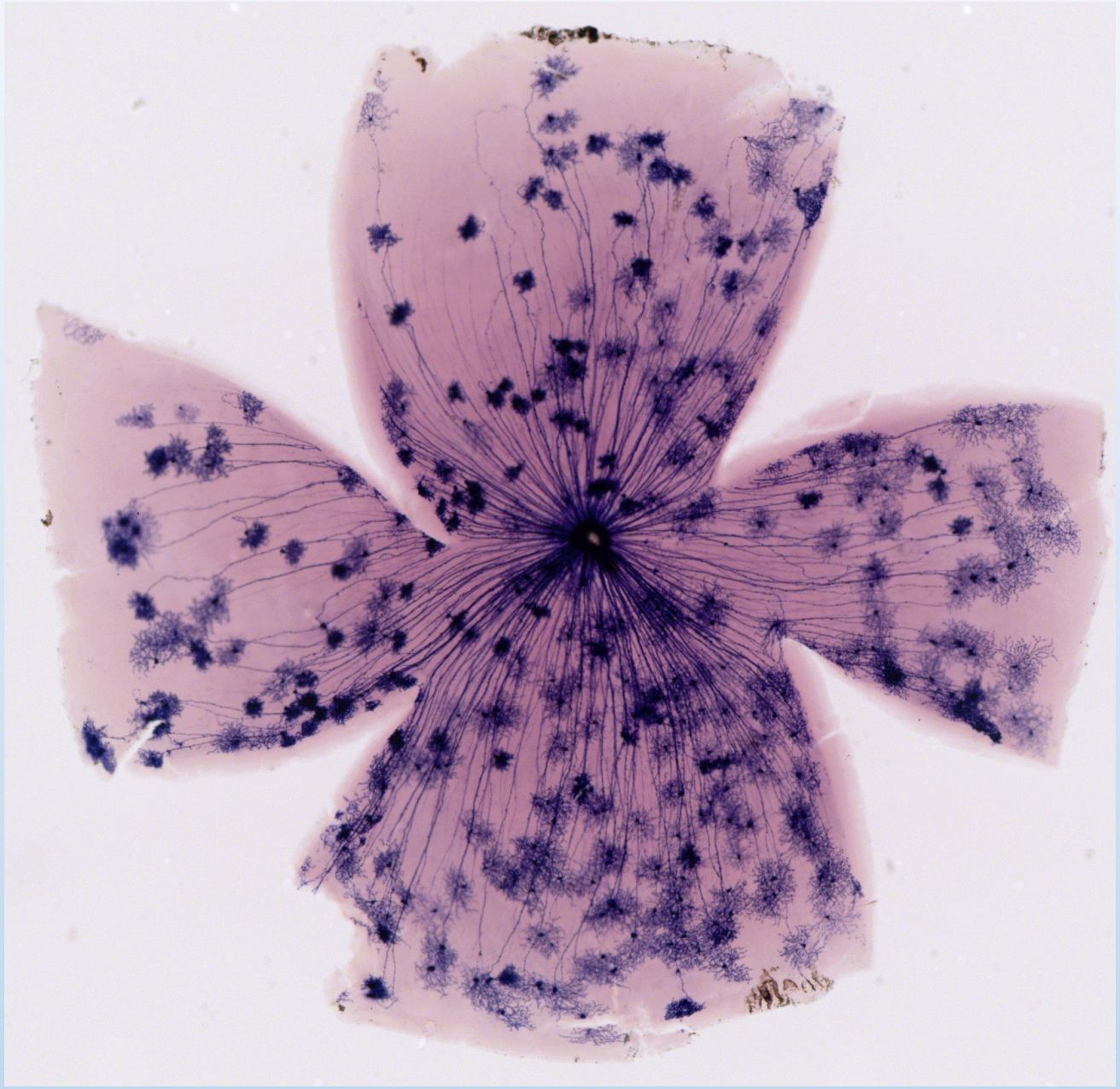


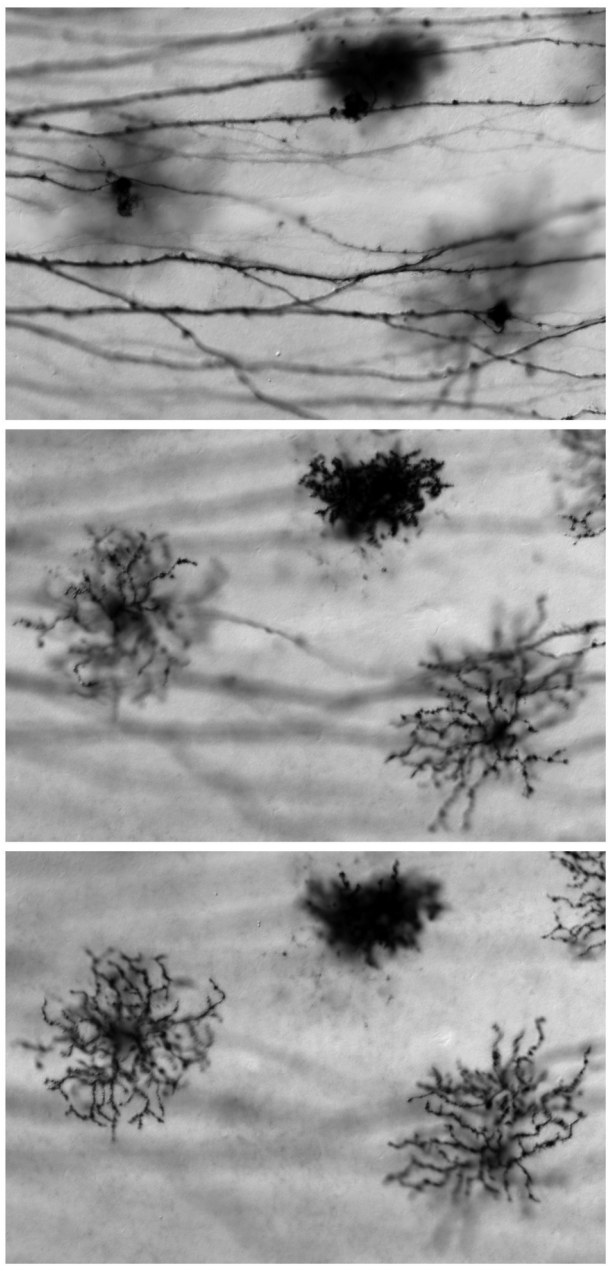




Parmhans & Sajgo

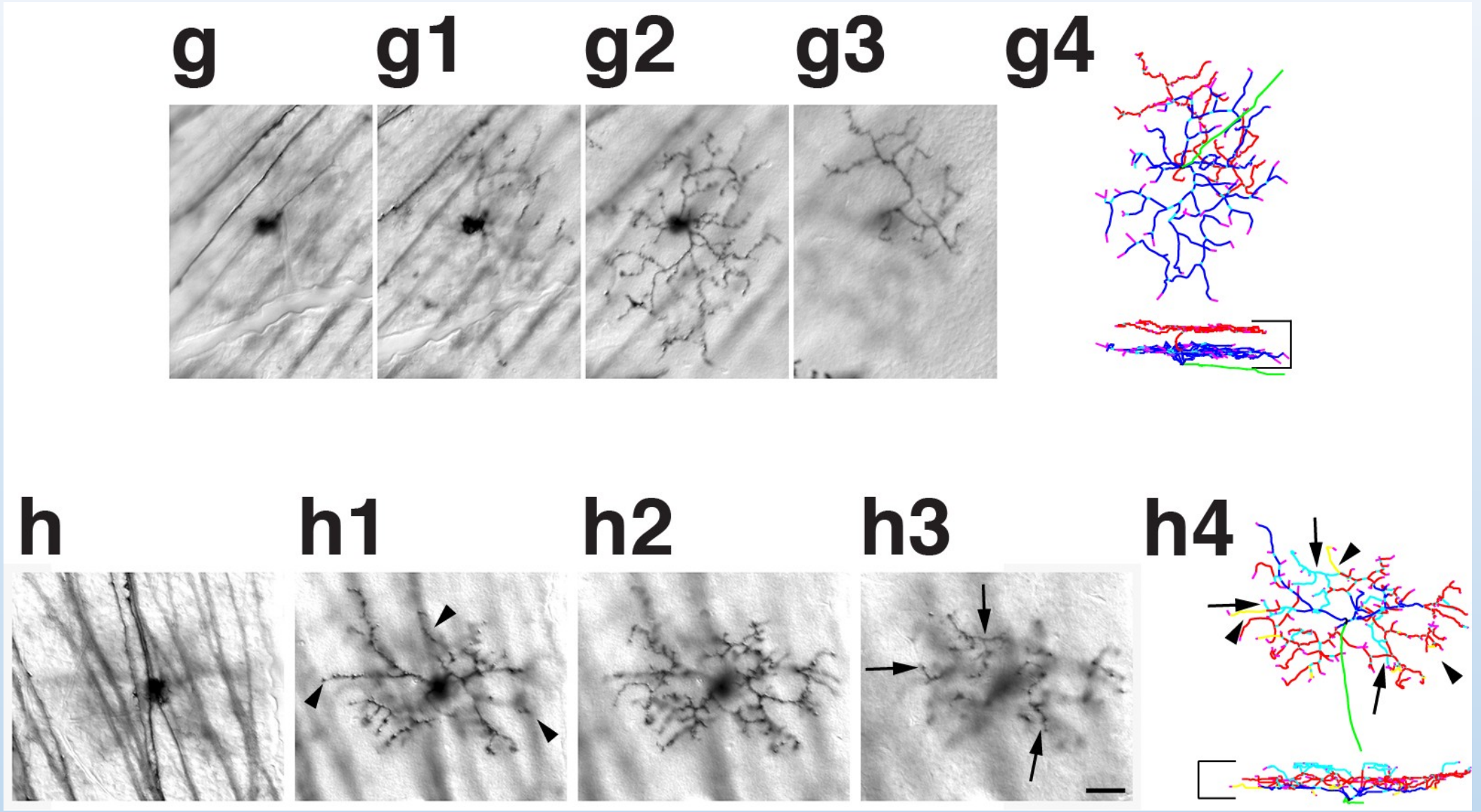
Intersectia Ret^{CreERt}; $Brn3a^{CKOAP}$ = ON-OFF-DS si On/OFFbetta RGCs





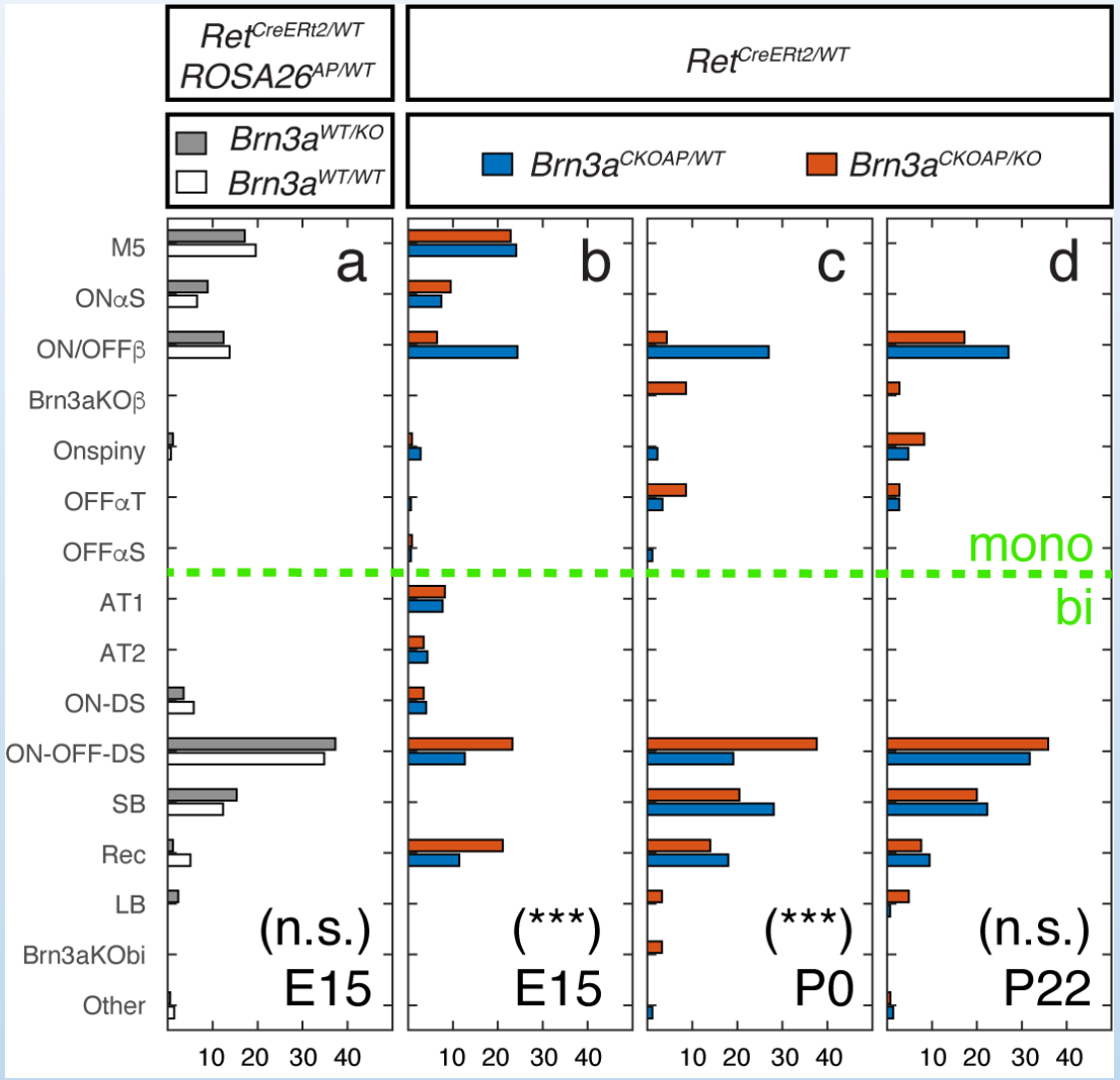
Parmhans 2018
Muzyka 2021

RGC Bistratificate anormale in mozaicism embrionar dublu heterozigot ($Ret^{+/-}$; $Brn3a^{+/-}$).



Muzyka 2021

Sumarul modificarilor de tipuri celulare RGC in Mosaicismul Dublu Heterozigot Embrionar ($Ret^{+/-}$; $Brn3a^{+/-}$)



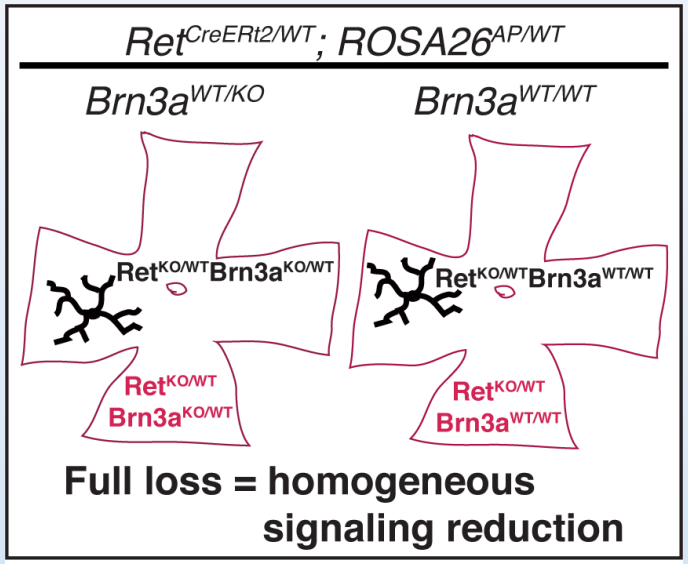
Tip celular (Procente relative la intreaga populatie) Muzyka 2021, Neural Development

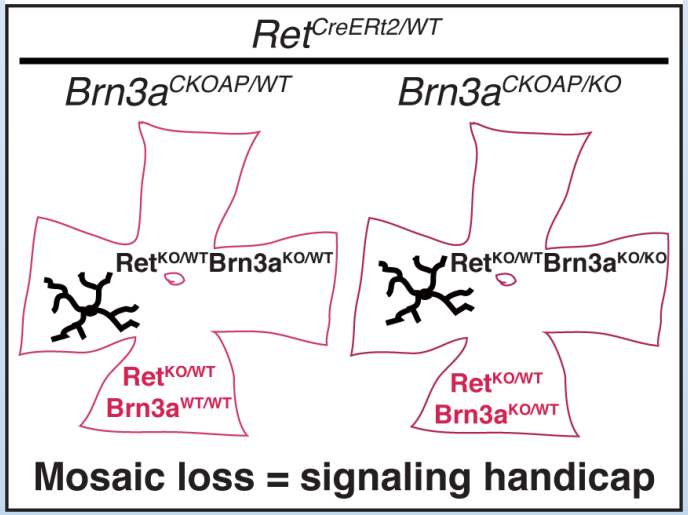
Deficite de receptori neurotrofici in RGC cu mutatii in Brn3a

Brn3a$^{AP/WT}$ RGCs Neurotrophin receptor expression	
NGF family receptors: TrkA TrkB TrkC	GDNF family receptors Ret GFRα1 GFRα2 GFRα3

Brn3a$^{AP/KO}$ RGCs Cell autonomous regulation	
NGF family receptors: TrkA ↘ TrkB = TrkC ↘	GDNF family receptors Ret ↘ GFRα1 ↘ GFRα2 = GFRα3 ↘

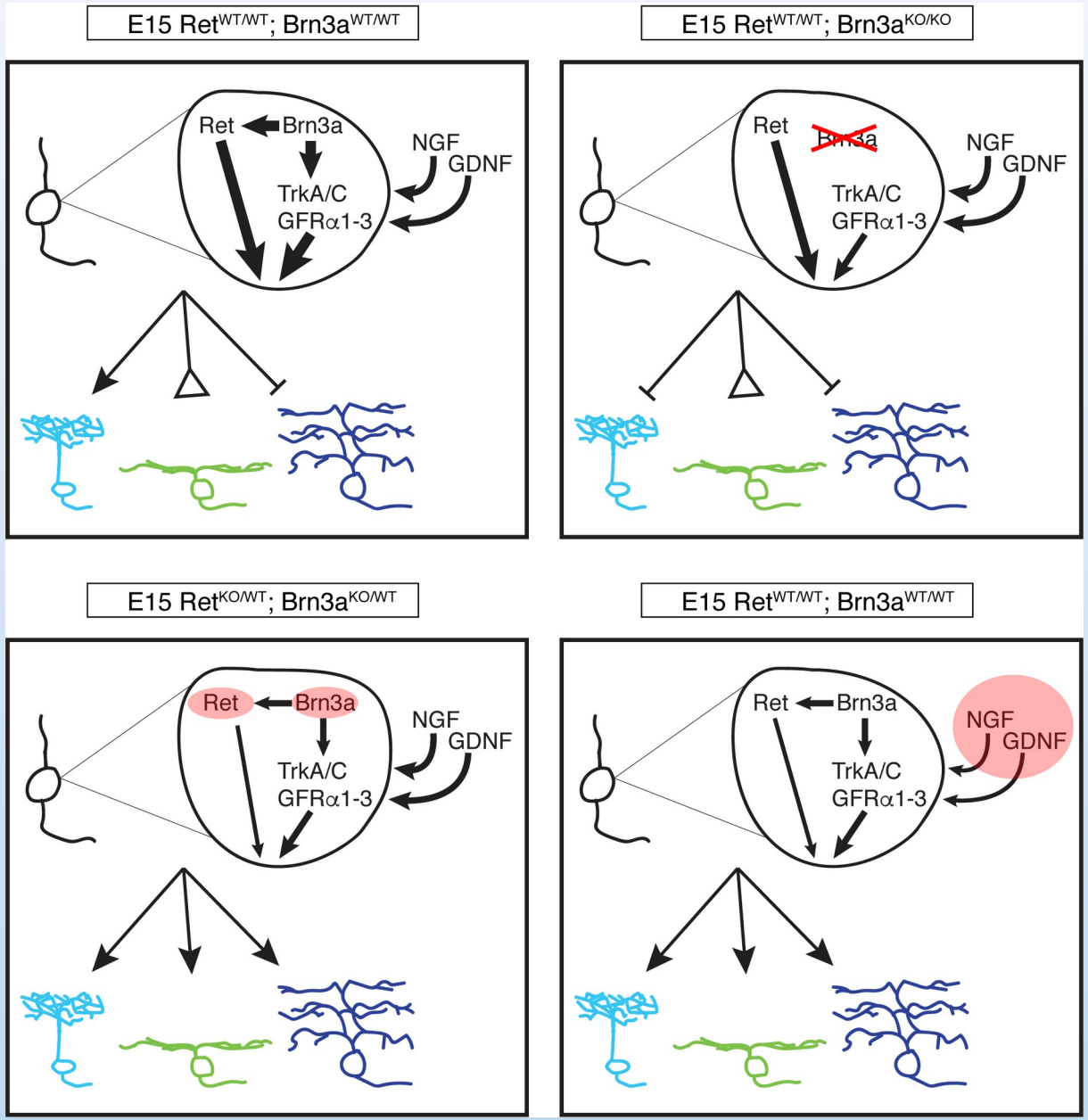
Other RGCs in Brn3a$^{AP/KO}$ retina Non-Cell autonomous regulation	
NGF family receptors: TrkA ↗ TrkB = TrkC ↗	GDNF family receptors Ret ↗↗ GFRα1 ↗↗ GFRα2 ↗↗ GFRα3 ↗↗





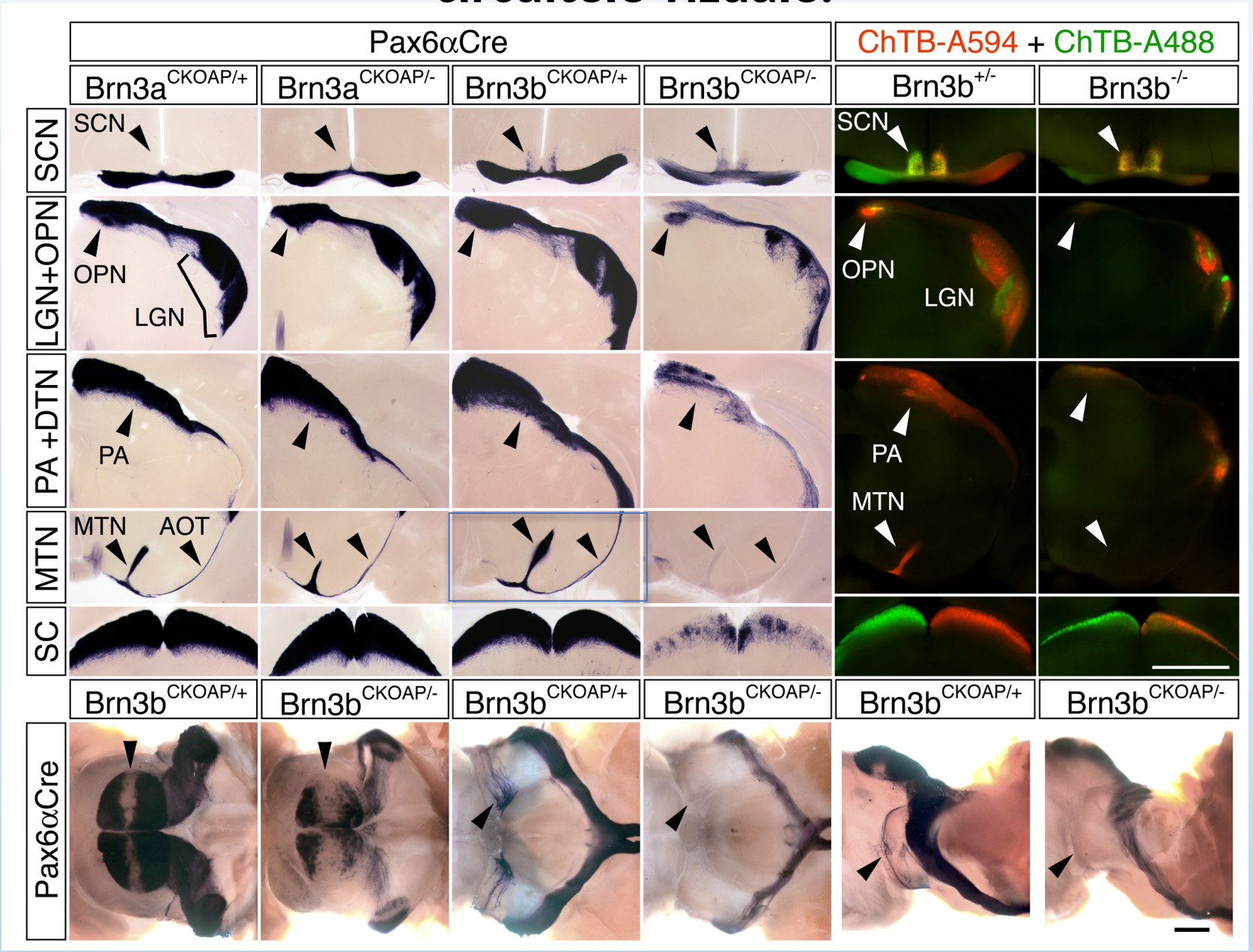
Muzyka 2021, Neural Development

Semnalizare Neurotrofica si Transcriptie in specificarea RGC

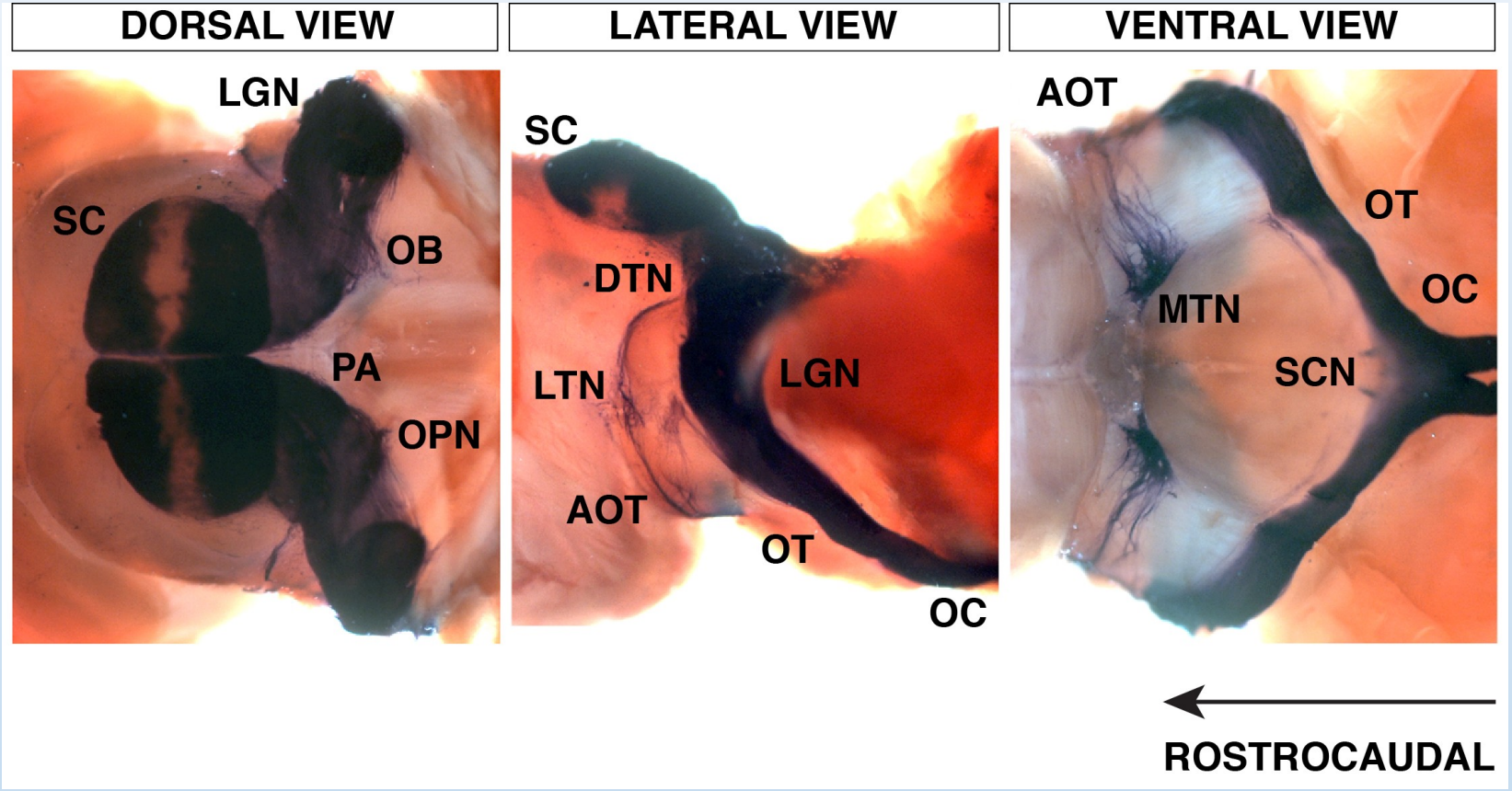


Muzyka 2021,
Neural Development

Deficitele de Inervare a Nucleilor Retinorecipienti Sugerează Funcțiile RGC in circuitele vizuale.



Mutantii de Brn3b pierd ~75 % din RGC si au deficite vizuale functionale bine delimitate.



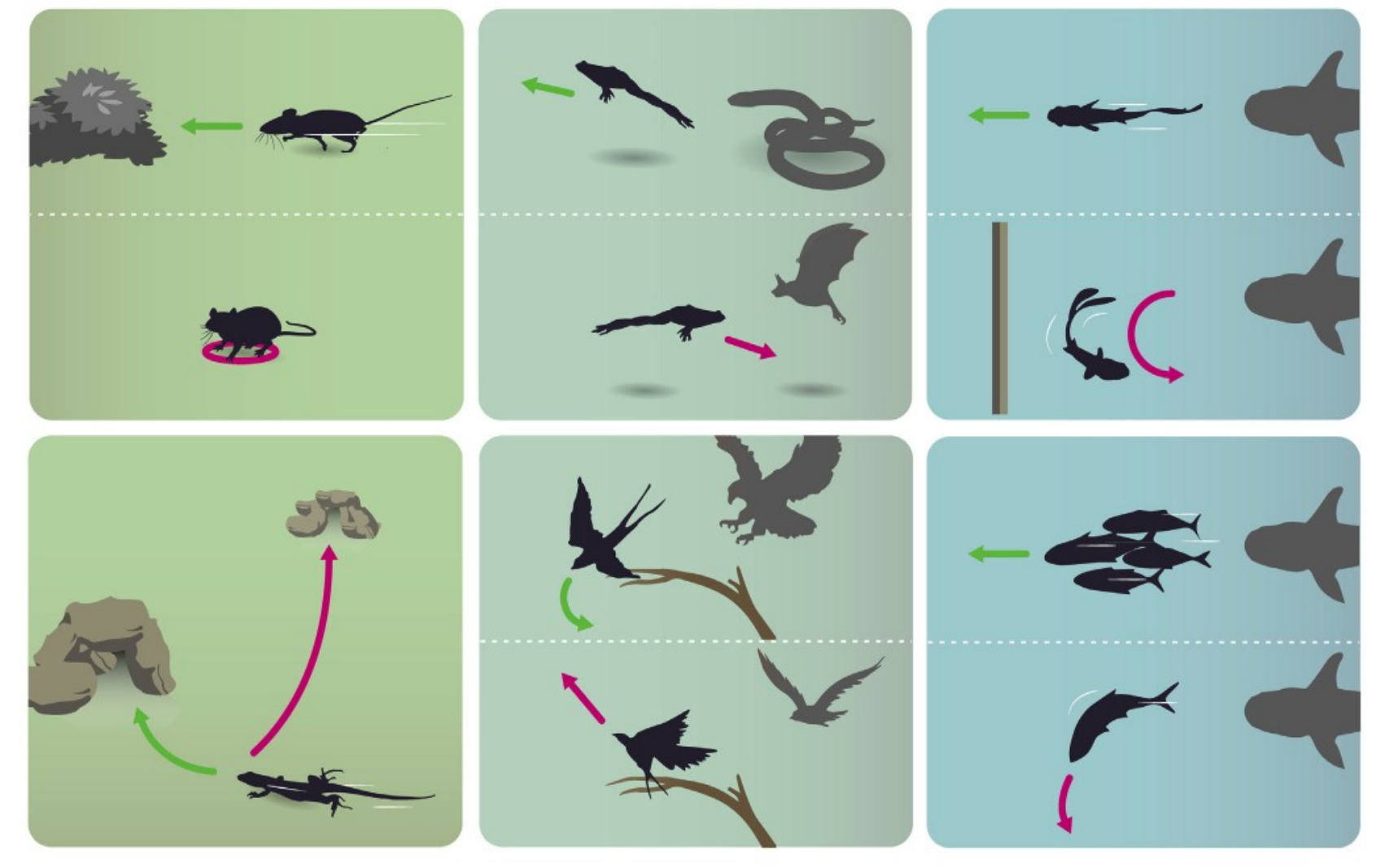
TRACTS
OC = Optic Chiasm
OT = Optic Tract
AOT = Accessory Optic Tract
OB = Optic Brachium

NUCLEI
SCN = Suprachiasmatic nucleus
-> Circadian Photoentrainment
MTN + LTN +DTN = Medial, Lateral and Dorsal Terminal Nuclei
-> Vestibulo-Ocular Coordination (OKR)
LGN = Lateral Geniculate Nucleus
-> Relay to cortex, object color, shape and motion, conscious vision, etc...
OPN = Ollivary Pretectal Nucleus
-> Pupillary Light Reflex (PLR)
SC = Superior Colliculus
->"Ancestral Visual Brain", integration of body head and eye movement, perhaps all other functions

Badea 2009, Chen 2011, Kretschmer 2015, 2017

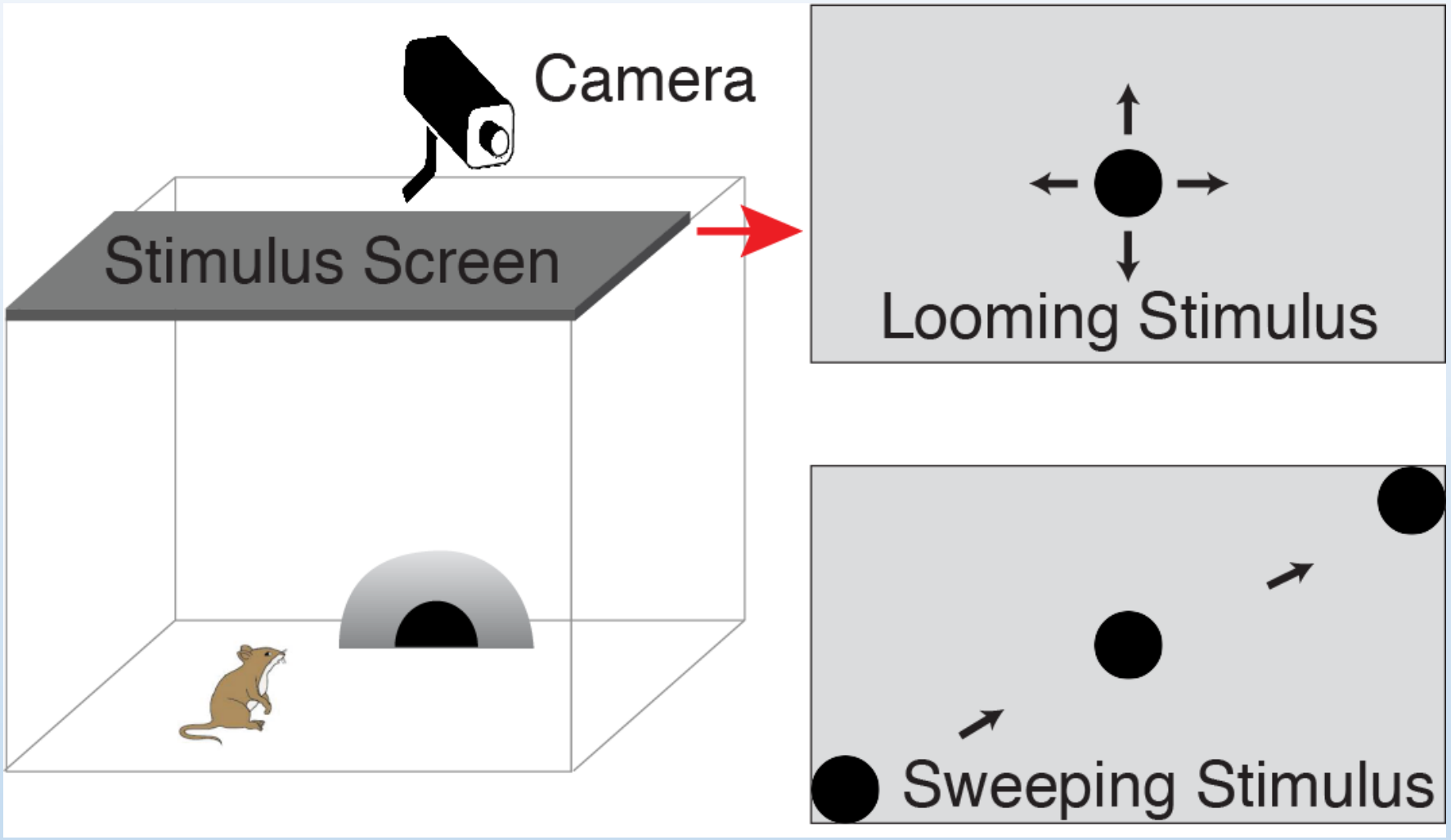
Comportamente defensive evocate visual

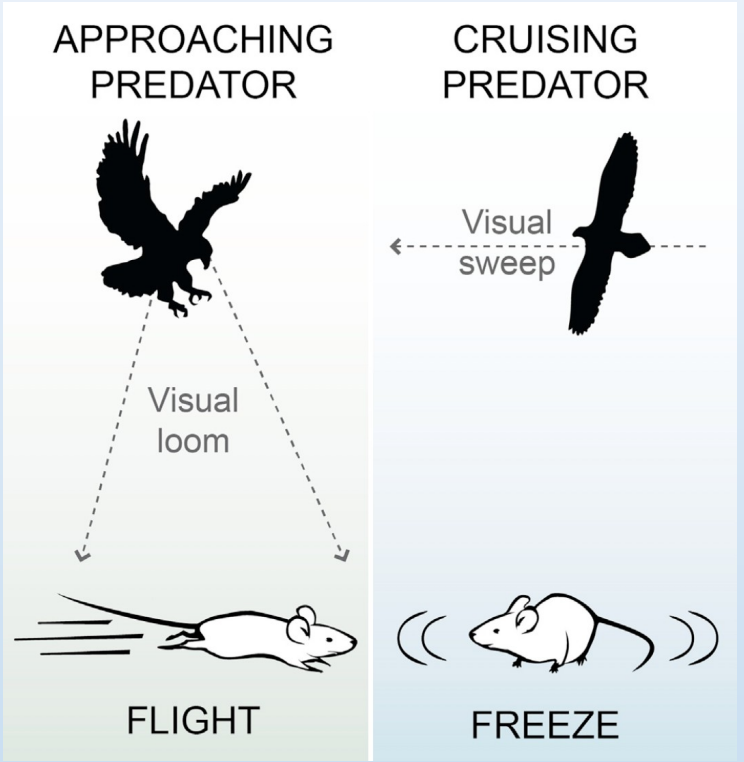
Stimuli/Context au un rol determinant



Evans 2019

Reactie de fuga sau "inghetare" in soareci Brn3b-KO

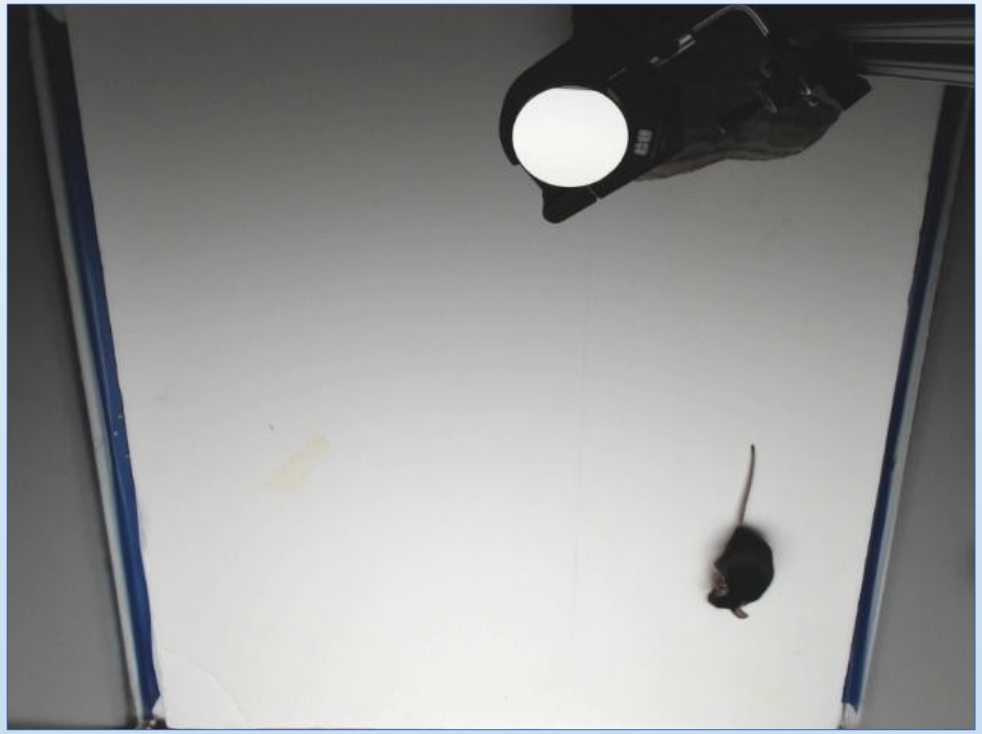




Yilmaz & Meister 2013
DeFranceschi 2016

Mutantii Brn3b ingheata in loc sa fuga de stimuli "de apropiere"

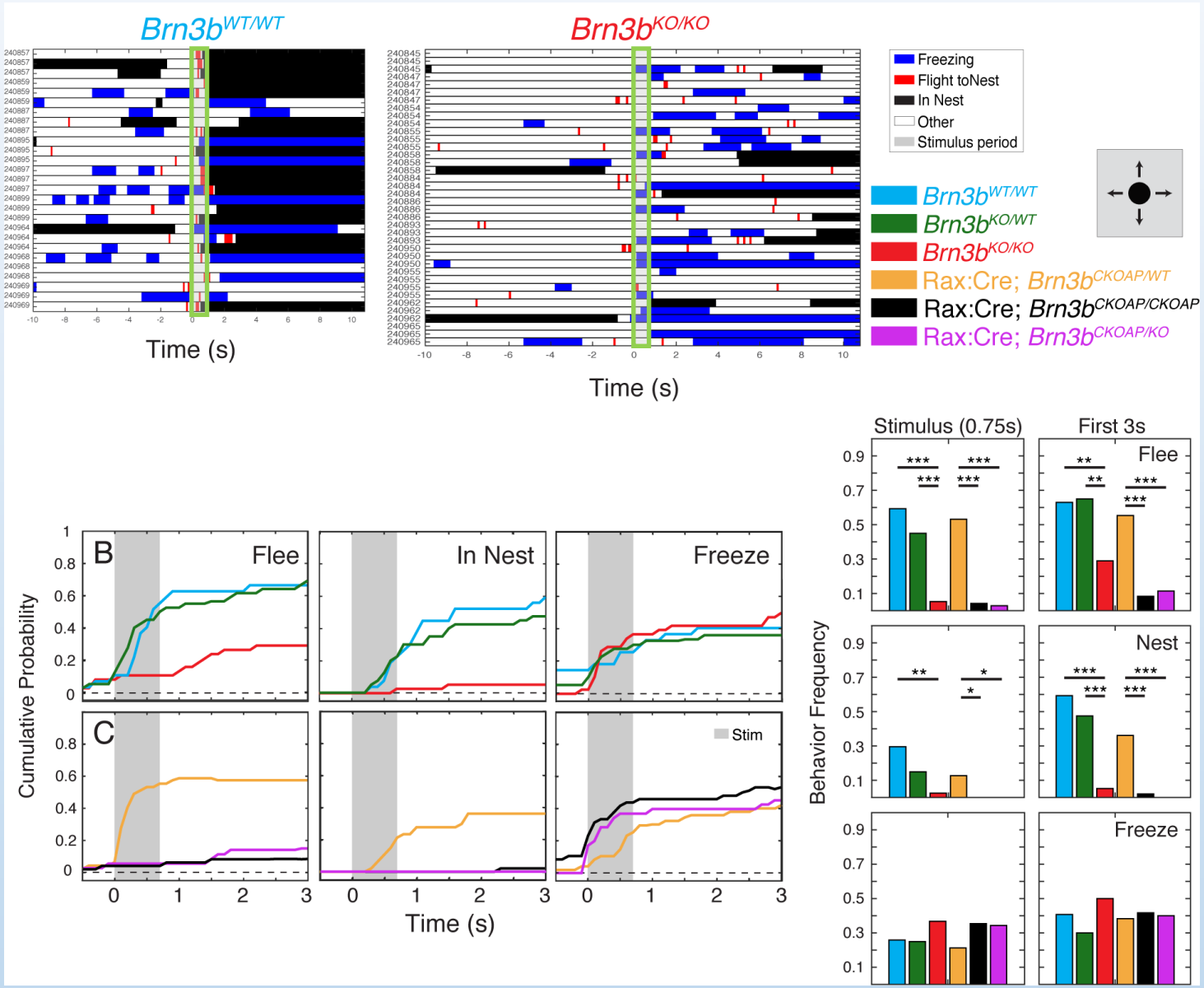
Fleeing - *Brn3b*$^{WT/WT}$



Freezing - *Brn3b*$^{KO/KO}$

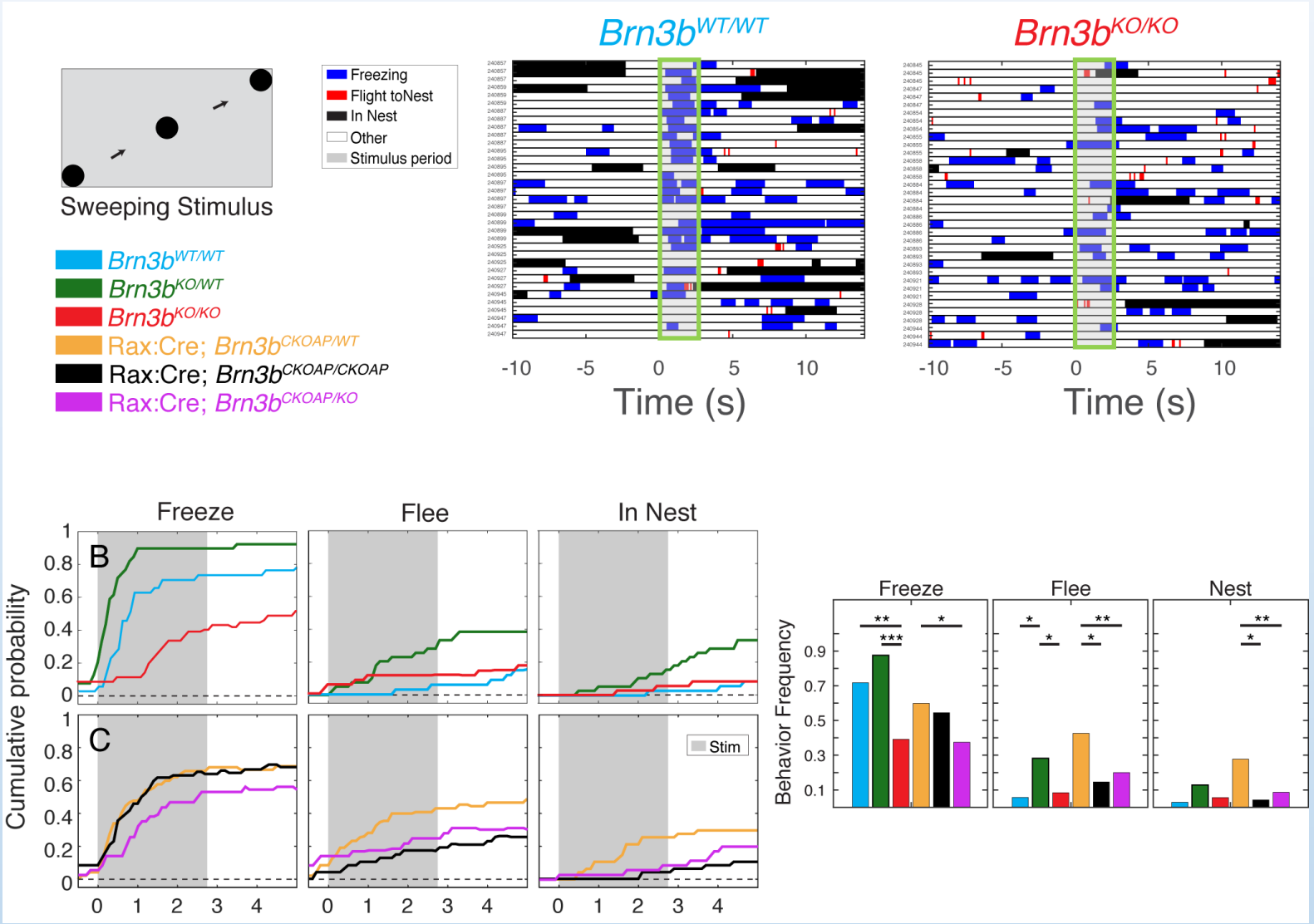
Rebecca Lees

Mutantii Brn3b ingheata in loc sa fuga de stimuli "de apropiere"



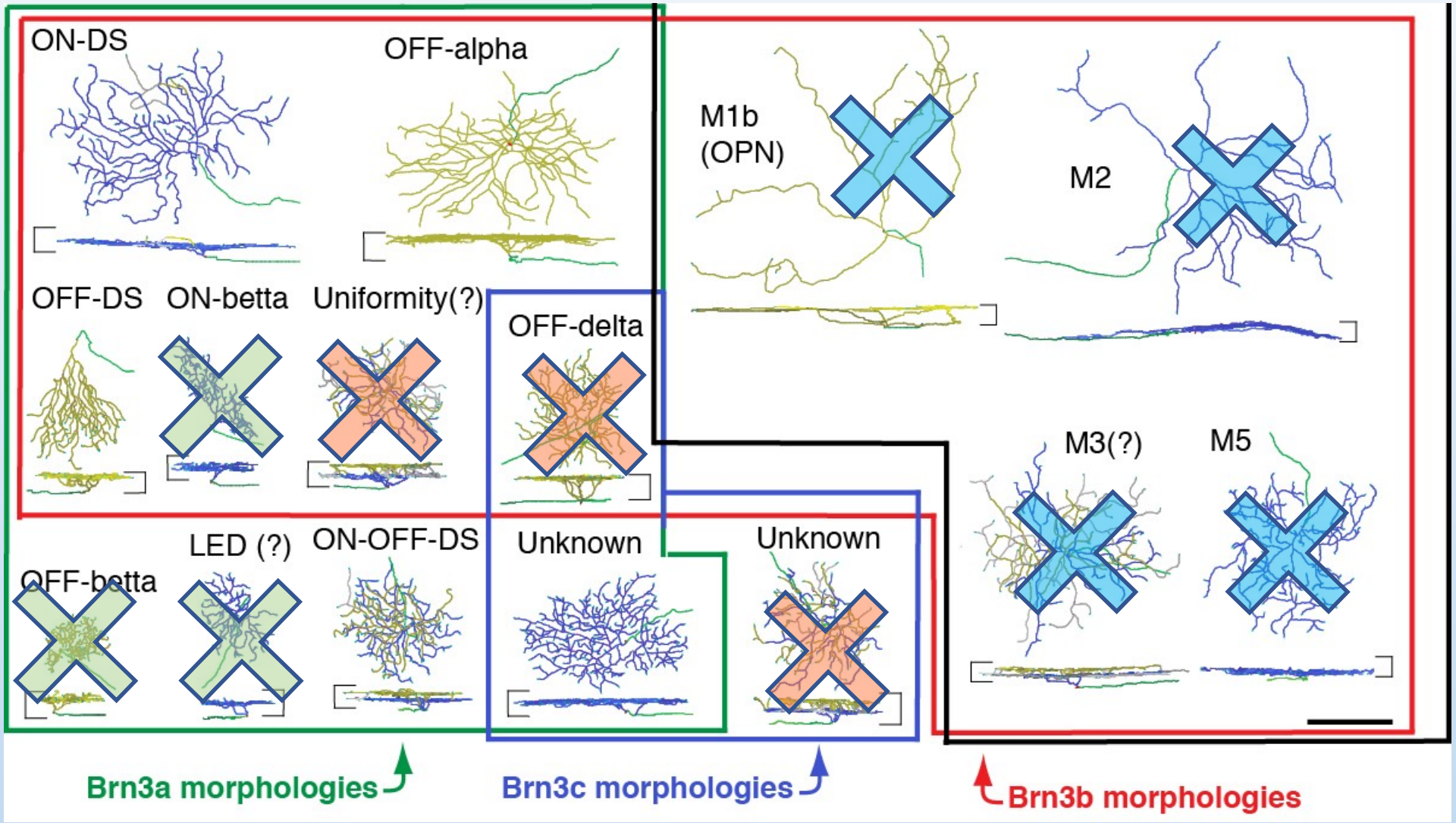
Rebecca Lees & Armaan Akbar

Mutantii Brn3b au reactii de inghetare diminuate la stimuli "de survolare"



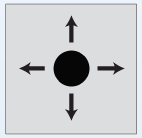
Rebecca Lees & Armaan Akbar

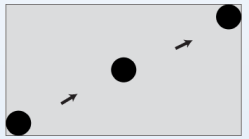
Cateva subpopulatii distincte de RGC sant necesare pentru reactia de fuga de stimulii "de apropiere"

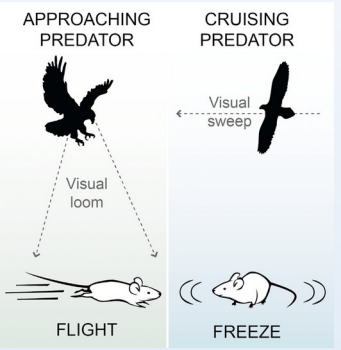


Badea 2011 & 2012, Chen 2011, Shi 2013, Parmhans 2018 Lees&Akbar 2020

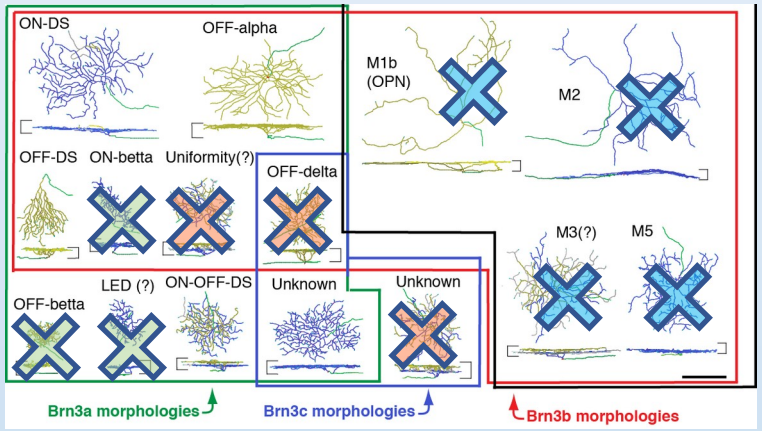
Cateva subpopulatii distincte de RGC sant necesare pentru reactia de fuga de stimulii "de apropiere"







	Looming		Sweeping		
Genotype	Flight	Freeze	Freeze	Flight	RGCs affected
Brn3b$^{WT/WT}$	0.59	0.26	0.71	0.06	none
Brn3b$^{KO/WT}$	0.45	0.25	0.87	↑ 0.28	None described
Brn3b$^{KO/KO}$	↓↓ 0.05	0.37	↓ 0.39	0.08	75 % loss
Rax:Cre; Brn3b$^{CKOAP/WT}$	0.53	0.21	0.60	0.43	None described
Rax:Cre; Brn3b$^{CKOAP/CKOAP}$	↓↓ 0.04	0.35	0.54	↓ 0.15	75 % loss
Rax:Cre; Brn3b$^{CKOAP/KO}$	↓↓ 0.03	0.34	↓ 0.37	↓ 0.20	75 % loss
Rax:Cre; Brn3a$^{CKOAP/WT}$	0.61	0.15	0.80	0.27	None described
Rax:Cre; Brn3a$^{CKOAP/KO}$	↓ 0.32	0.27	0.70	↓ 0.03	20 % =~ "betta RGCs"
Brn3b$^{cDTA/WT}$	0.53	0.22	0.64	0.22	None described
Opn4$^{Cre/WT}$	0.50	0.11	0.69	0.36	None described
Opn4$^{Cre/WT}$; Brn3b$^{cDTA/WT}$	↓ 0.25	0.32	0.80	↓ 0.16	ipRGCs except M1-SCN
Brn3c$^{Cre/WT}$	0.25	0.11	0.61	0.32	None described
Brn3c$^{Cre/WT}$; Brn3b$^{cDTA/WT}$	↓ 0.10	0.28	0.88	↓ 0.13	OFF widefield



Becca Lees & Armaan Akbar

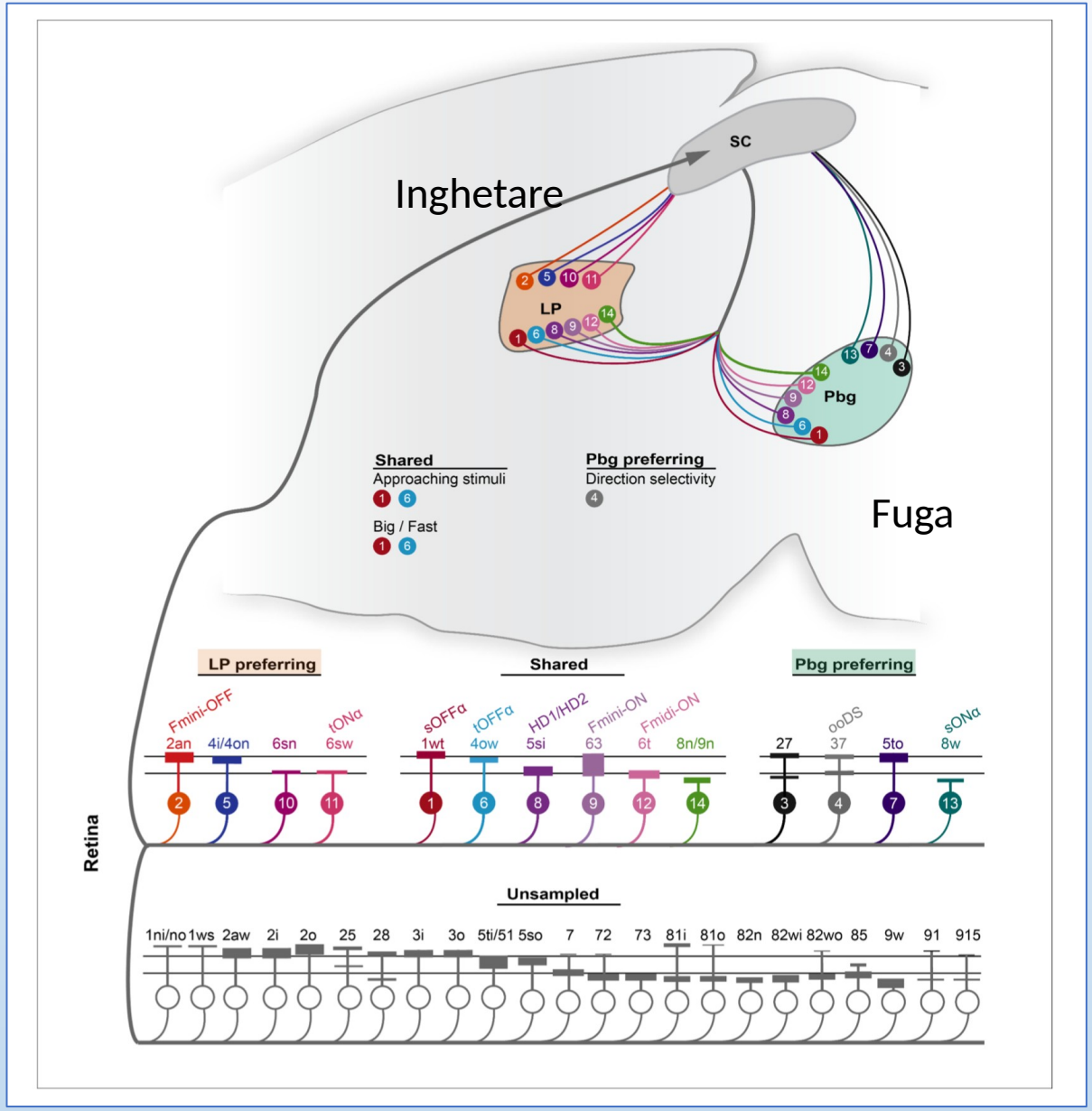
Concluzii

"Fuga de pradatori" are nevoie de cateva tipuri de RGC distincte

- Proiectii multiple in SC -> calcularea stimulului
- Reinhard 2019: RGC conexiuni diferentiale catre nuclei secundari
- Manipularile noastre dezactiveaza RGC din toate supgrupurile

"Inghetarea de expectativa"

- este mult mai greu de inlaturat
- orice miscare declanseaza inghetarea
- Nu am gasit populatia RGC specifica
- (ON-OFF DS?)



Reinhard et al, *eLIFE*. 2019

Group members:

Postdocs
Friedrich Kretschmer
Alireza Ghahari
Manvi Goel
Vladimir Muzyka
Raluca Pascalau

Graduate Students
Miruna Ghinia
Szilard Sajgo
Walid Chatila
Momina Tariq

Committee Member (UMBC)
Preethy Somasundaram
Alexis Paige Nobleman

PostBacs
Melody Shi
Sumit Kumar
Oluwaseyi Motajo
Katherine Chuang
Eileen Nguyen
Rebecca Lees
Annie Fuller
Armaan Akbar

Undergrads
Nadia Parmhans
Beverly Wu
Esika Savsani
Tyger Lin

Contractor
Seid Ali

Collaborators:
Wenqin Luo & Jingwen Niu – U. Penn - cRet
Xuqian Mu – SUNY Buffallo – Isl1
Samer Hattar / Phylis Robinson – Melanopsin
Chai-An Mao – U Texas – RGC TFs
Tiansen Li – N-NRL – Cell Biology of Copines
Horea Rus – U. Maryland – RGC-32
Marius Pachitariu – Janelia Farms - Kilosort
Greg Schwartz – Northwestern – Brn3c RGC physiology

Zhijian Wu , Peter Colosi, Hiriyanna Suja – virus design
Michael Cashel Yuri Sergeev – roxP

Many thanks to:
Anand Swaroop
Matthew Brooks – RNA sequencing
Jacob Nellissery
Linn Gieser

Many thanks to almost every lab in the NEI for assistance, discussions and help with various experiments and protocols.

Parcurs Profesional: Educatie & Pozitii de cercetator

- M.D.; 1988 - 1994; Universitatea de Medicina si Farmacie “Iuliu Hatieganu”, Cluj-Napoca, Romania, Medicina generala
- Instructor; 1994 - 1995; – Divizia de of Immunopatologie si Resident Laborator Clinic, Clinica medicala Nr.1, UMF Cluj-Napoca, Romania
- Research Fellow; 1995 - 1998; Department of Pathology, Medical School, University of Maryland at Baltimore (Mentori Moon L. Shin, Horea Rus and Florin Niculescu) – Colaborare inca in curs
- M.A.; 1998 - 1999; Columbia University, New York (Mentori Rafael Yuste and Darcy Kelley), Graduate Student, Major Biology
- Ph.D.; 1999 - 2005; Johns Hopkins University, Baltimore, Graduate Student, Biochemistry, Cell and Molecular Biology School of Medicine, (Mentor Jeremy Nathans)
- Postdoctoral Fellow; 2005 - 2010; Postdoctoral Fellow, Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, School of Medicine, Johns Hopkins University, Baltimore, (Mentor Jeremy Nathans)
- Investigator and Unit Head, 2010 - 2021 ; Retinal Circuits Development and Genetics Unit, N-NRL/NEI/NIH
- Cercetător Stiintific II din Septembrie 2021 ; ICDT / Facultatea de Medicina / Universitatea Transilvania

Realizări si Interese Științifice

Manipulări genetice in Șoareci

- Recombinare genetica aleatorie rara cuplata cu marcarea morfologica in neuroni ("Colorație Golgi" genetica)
- Alele knock-in condiționale cuplate cu gena indicator pentru studiul efectelor cu autonomie celulara produse de mutații genetice si analize de mozaicism.
- Instrumentar nou (secvențe genetice sintetice noi, vectori de expresie in bacterii si celule eucariote, linii celulare HEK293, linii de șoareci modificați genetic) pentru experimentele de genetica combinatorica utilizând trei locații cromozomiale si doua recombinase (Cre si Dre).

Realizări si Interese Științifice

Clasificarea tipurilor neuronale in Retina

Anatomie – Dezvoltare – Funcție in Circuit - Patologie

- Produs prima descriere globala a tipurilor morfologice de celule din retina de șoarece.
- Demonstrat existenta unui cod combinatoric bazat pe factorii Pou4f/Brn3 pentru controlul transcriptional al dezvoltării celulelor RGC.
- Descoperit coduri transcriptionale Brn3 echivalente in alți neuroni senzoriali de proiecție (Auz, Sistem vestibular, Sistem Somatosenzorial).
- Descoperit transcriptoamele controlate de Brn3a si Brn3b in RGC prin tehnici de Deep Sequencing
- Demonstrat funcțiile in circuitul vizual pentru mai multe tipuri de RGC folosind experimente de comportament vizual in șoareci modificați genetic.
- Design si Construcție de echipament pentru studiul comportamentelor/reflexelor vizuale la animale de experiment (unul comercializat)

Realizări si Interese Științifice

Reacții regenerative tisulare inițiate prin stimuli inflamatorii

(Colaborare cu H. Rus la Universitatea Maryland, Facultatea de Medicina)

- Clonat RGC-32 \ Rgcc (response gene to complement-32 = Regulator of Cell Cycle), ca gena indusa prin atacul sublitic al sistemului complement asupra oligodendrocitelor in cultura.
- RGC-32 este activat prin stimuli inflamatori într-o varietate de țesuturi, incluzând celule endoteliale, astrocite, si de asemenea in tranziția Epiteliala – Mezenchim ala – indusa de TGF-beta.
- RGC-32 este de asemenea indus in țesuturi in regenerare si anumite forme de cancer.
- Construit alele de Knock-out (KO) si Knock-out condițional (CKO) pentru RGC-32, cu care H&V Rus au demonstrat rolul RGC32 in Experimental Autoimmune Encephalomyelitis, un model pe animal pentru Scleroza Multipla.
- RGC-32 a fost de curând descoperit ca gena indusa in vasculatura coroida a pacientilor cu AMD (Degenerescenta Maculara legata de varsta)

Parcurs Profesional: Sumar

- M.D., M.A., Ph.D.
- Co-Autor 61 lucrari in extenso (59 ISI), incluzand 3 Nature, 1 Cell, 2 Neuron, 3 PNAS,1 Cell reports, 2 Nature Communications, 1 Science advances, 5 J. Neuroscience, 1 J. Immunology, 1 eLife, 6 Journal of Comparative Neurology, 2 Journal of Neurophysiology.
- Citari = 4585 – Google Scholar / 3284 – Scopus / 3218 WoS. H index 30/28/28
- 2 capitole de carte
- 1 Patent.
- Construit aproximativ 30 linii de soareci modificate genetic, majoritatea distribuite prin Jax Laboratories.
- Mentor ~ 20 Students (5 Postdoctoral Fellows, 4 PhD, 2 Msc, 8 Postbaccalaureate Students
- Ultimii 10 ani finantati prin NEI intramural research project
- De la intoarcerea in tara 1 proiect PCE

Project Title	Department	IC	Project Number	Project Leader	Organization Name	Organization City	Country	FY	FY Total Cost
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-01	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2011	1442239
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-02	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2012	900981
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-03	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2013	1158320
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-04	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2014	1321373
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-05	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2015	1100071
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-06	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2016	1422176
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-07	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2017	1811467
RETINAL CIRCUIT DEVELOPMENT & GENETICS	HHS	NEI	1ZIAEY000504-08	BADEA, TUDOR	NATIONAL INSTITUTES OF HEALTH	BEHTESDA, MD	UNITED STATES	2018	1586247

Parcurs Profesional: Sumar

a) Membru in societăți științifice: American Association for The Advancement of Science (AAAS), Society for Neuroscience, Association for Research in Vision and Ophthalmology, International Society for Eye Research, American Physiological Society, *Genetics* Society of America.

b) Referent pentru reviste științifice: Acta Histochemica; Biochimica et Biophysica Acta; BMC Biology; BMC Molecular Brain; BMC Molecular Medicine; Cell Death and Disease; Developmental Dynamics; Developmental Biology; eNeuro; FEBS Letters; Genesis; Graefe's Archiv; Journal of Comparative Neurology; Journal of Neuroscience; Journal of Neurophysiology; Molecular Vision; Molecular Cellular Neuroscience; Nature Communications; Neuroscience; Pigment Cell and Melanoma Research; PLOS One; Proceedings of the National Academy of Sciences (USA); Proceedings of the Royal Society (B)

Editor: PLOS One

c) Comitete științifice si administrative NIH:

- Stadtmann Tenure Track Investigator Recruitment Committee – 2012 – 2013

- Animal Care and Use Committee - NEI – din 2012

d) Referent pentru concursuri de proiecte de cercetare:

- Agencie Nationale de la Recherche – Franta

- Wellcome Trust – „Sir Henry Dale Fellowship" - Marea Britanie

- Association Retina - Franta

- Dutch Research Council – Veni program – Olanda

e) Referent pentru promovari in poziția de conferențiar (Tenure and promotion to Asssociate Proffessor)

- University of Virginia, Charlottesville, VA, USA

- Baylor College of Medicine, Houston, TX, USA

Proiecte de Viitor

II. Planul de Cercetare/Dezvoltare ca membru al ICDT

II.1 Direcții Științifice

- **Embriologia RGC in șoareci modificați genetic (in paralel pe țesuturi umane in măsura disponibilității)**
- **Relația Transcripție – Neurotrofine in Dezvoltare si Neuroprotectie**
- **Metodologii Genetice de trasare – manipulare neuronala**
- **Analiza de circuite neuronale folosind liniile noastre modificate genetic si aparatura de electrofiziologie/comportament**
- **Analiza funcțiilor moleculare al genelor implicate in dezvoltarea RGC, prin tehnici de biochimie, biologie celulara, si apoi in animal experimental**

II.2 Dezvoltare Infrastructura

- **Capabilități de genomică (analiza pe calculator, preparare de librarii), single cell**
- **Culturi Celulare, Immunofluorescenta, analiza de proteine (western blot, spectrometrie de masa)**
- **Histologie pe țesuturi de la animale: criostat, vibrotom, Microscop cu fluorescenta, Confocal**
- **Electrofiziologie : Multielectrozi si Patch Clamp**
- **Comportament: Echipament de analiza comportamentului vizual**

II. Planul de Cercetare/Dezvoltare ca membru al ICDT

II. 3 Rețea de Colaboratori

- **Facultatea de Medicina – UTB**
- **Celelalte Centre de Cercetare ale UTB si ICDT**
- **Alte Institute si Universități din tara.**
- **Creat un acord de colaborare cu NIH/USA – expertiza, schimb de experiența, proiecte, finanțări**
- **Contacte in Uniunea Europeana, Marea Britanie, China, Japonia (colaborări in curs convertibile in proiecte de finanțare)**

II.4 Plan de Finanțare

- **Proiecte de colaborare – vezi mai sus**
- **Fonduri de Infrastructura si de Reintegrare UE.**
- **Fonduri la Nivel National**
- **Preliminarii – Date încă nepublicate sau lucrări pe cale de publicare (6 sânt in curs de derulare in acest moment)**

III. Plan academic/didactic

III.1 Plan academic

- **Obținerea gradului de cercetător științific I**
- **Rețea de Proiecte si colaborări in cadrul ICDT**
- **Participare la Conferințe Naționale/Internaționale**
- **Organizarea unei Conferințe de neurostiinte vizuale cu participare internaționala**

III.2 Plan didactic

- **Educarea in metodologii de Biologie Moleculara/Celulara, Histologie, Neurostiinte Fiziologie Comportament a oricăror studenți interesați – colegiu – facultățile UTB, apoi master si doctoranzi in co-tutela.**
- **După abilitare, si studenți proprii – emfaza pe calitate, nu cantitate**
- **Predarea la cursuri de Master (Brașov, Bucuresti, Cluj?)**
- **Participarea la procesul de predare (sau dezvoltarea cursurilor practice) in disciplinele relevante mie: anatomie, embriologie, fiziologie, biochimie biologie celulara, genetica.**

IV. Integrarea in Strategia UTB / Facultății de Medicina Brașov.

- **Facultatea de Medicina: Dezvoltarea unei Infrastructuri integrate la dispoziția tuturor cercetătorilor/cadrelor didactice interesate din facultate**
- **Dezvoltarea de proiecte comune, care țin cont de expertiza si interesele științifice ale fiecăruia**
- **La nivel UTB/ICDT sânt posibilități de sinergie cu grupurile interesate de genetica si genomica, cu grupurile interesate de informatica, inginerie si mecanica fina / electronica, cu care putem dezvolta aparatura pentru investigații / aplicații biomedicale.**
- **Organizarea de conferințe si colaborări internaționale/naționale care sa crească vizibilitatea UTB**

V. Interacțiuni la nivel National

- **Rețea de colaborari cu alte grupuri din țara – în neurostiinte, genetica moleculara și regenerare.**