NS201B
Lecture part 2
November, 2016

How neural activity and molecular cues guide formation of maps and connections between visual areas

Michael P Stryker

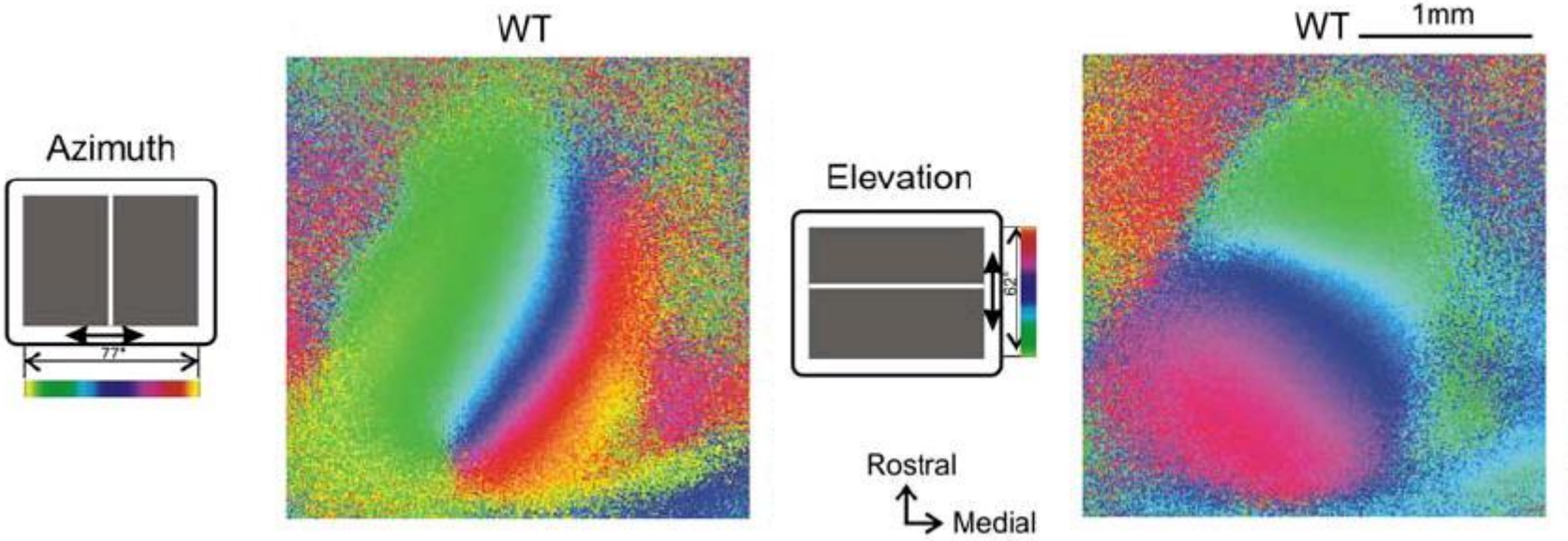
How do we study mapping?

- **Tracers**
  - Reveal the details anatomical connections
  - Two types: anterograde and retrograde
  - Cannot reveal neuronal responses
- **Electrophysiology**
- **Intrinsic Signal Imaging**
- **Calcium Imaging**

Mapping the visual field:

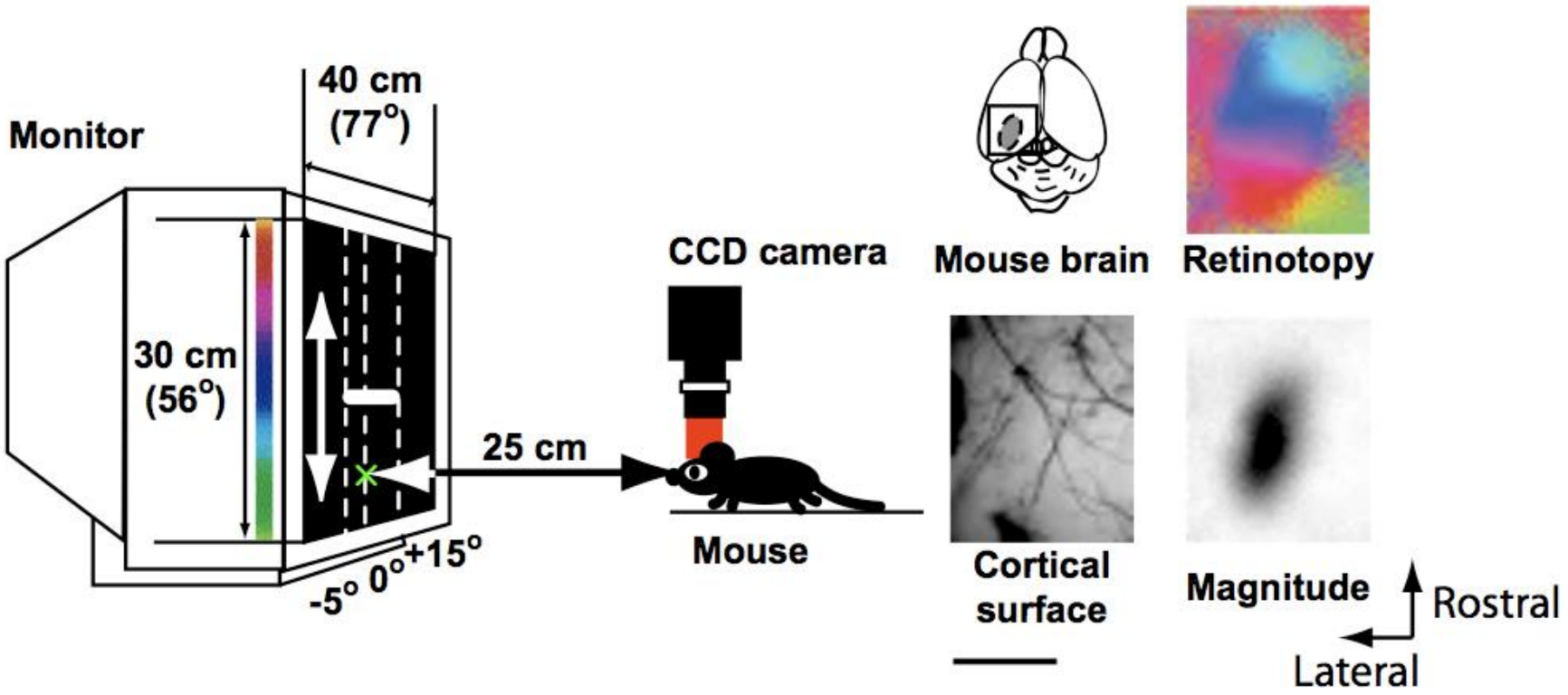
- The visual system has two axes

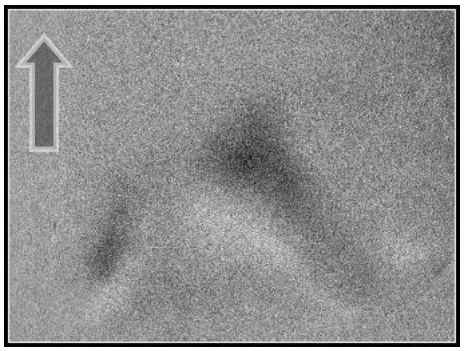
Dorsoventral = **Elevation**
Nasotemporal = **Azimuth**

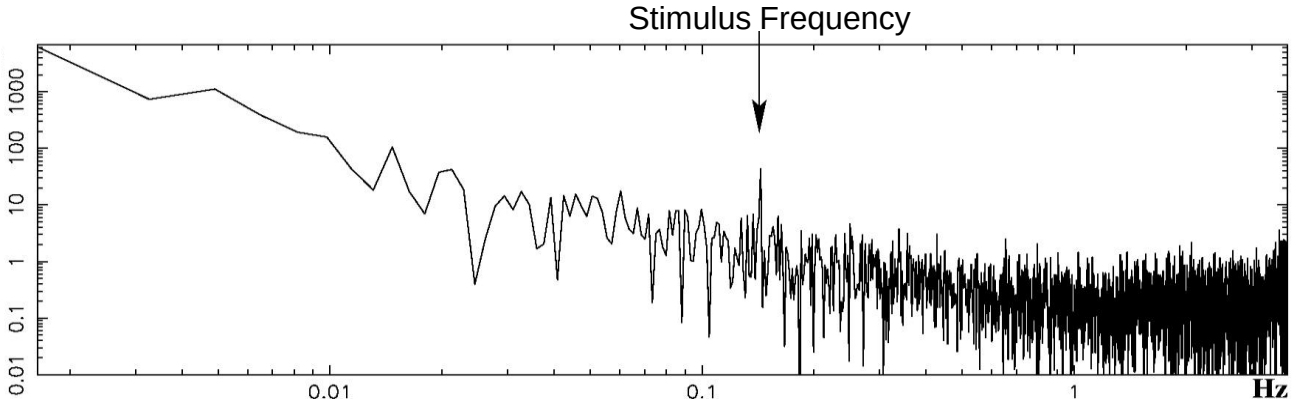


Cang *et al*. 2005

Fourier Intrinsic Signal Imaging using phase encoding





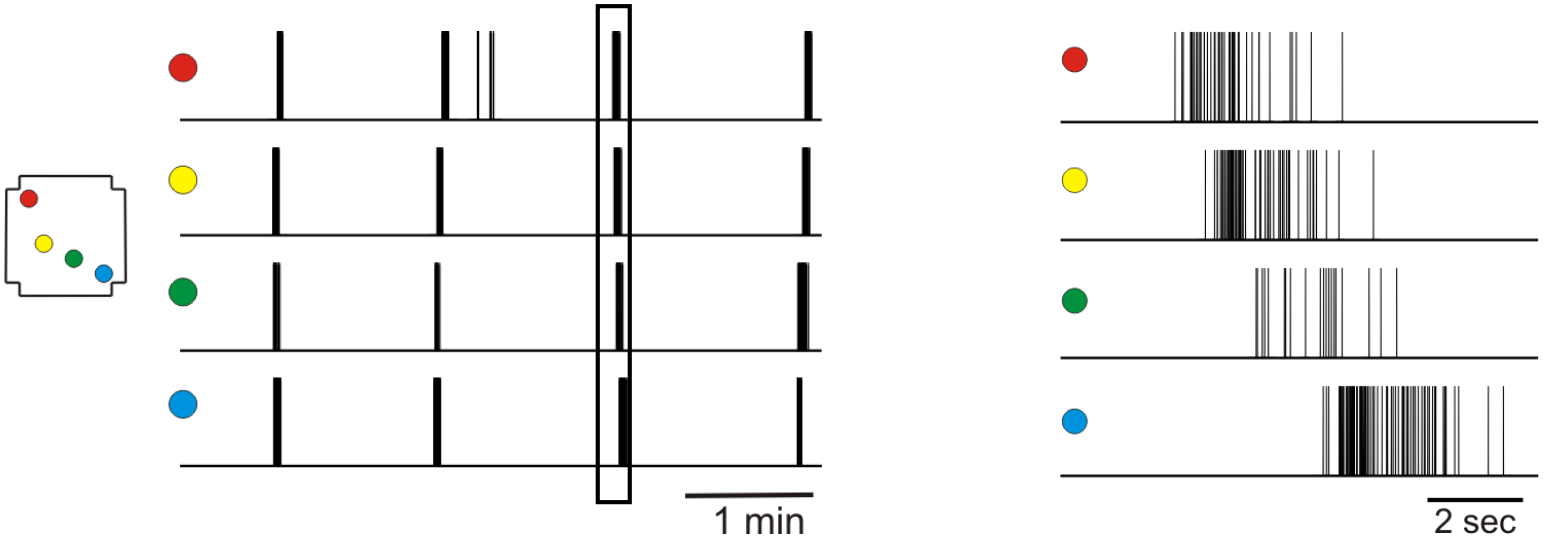


Kalatsky & Stryker, *Neuron* 2003

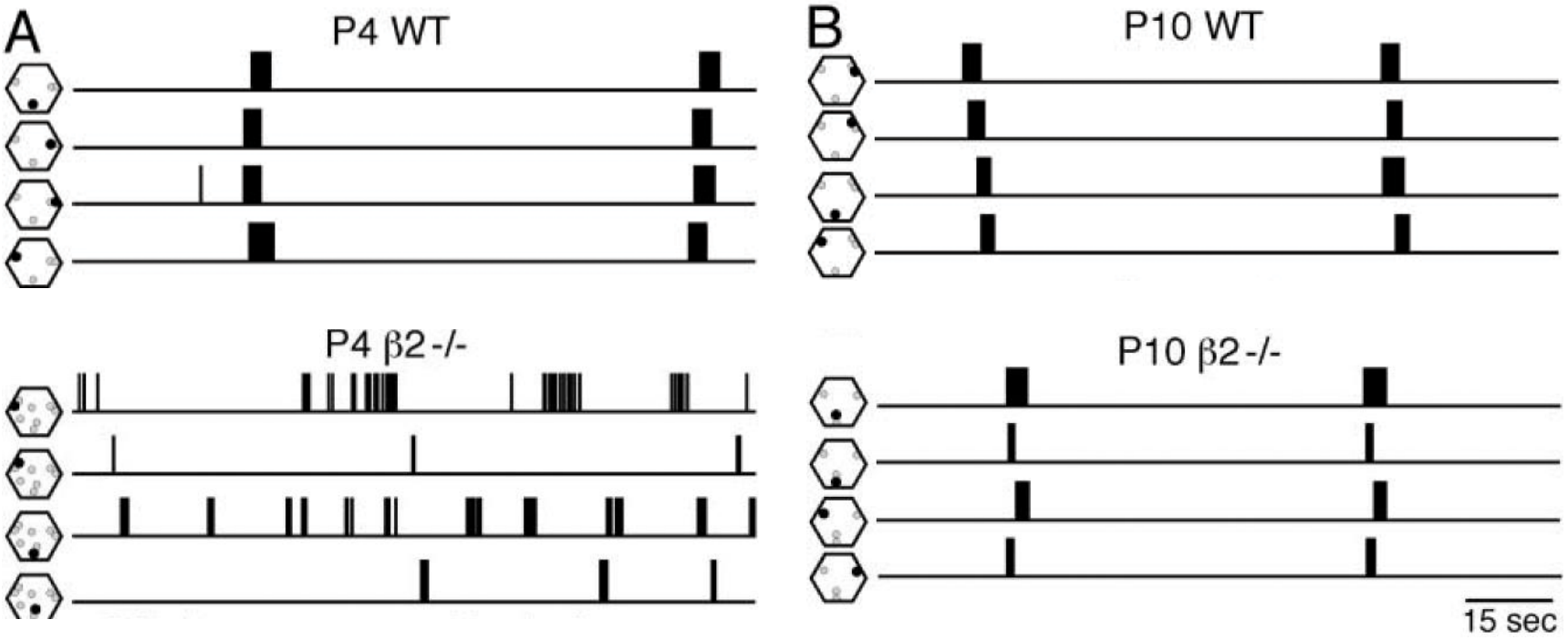
We sought to determine how topographic maps in the visual system were created.

- Does neural activity play a role?
- What are the molecular cues?

Does neural activity play a role in constructing visual maps: Retinal Waves

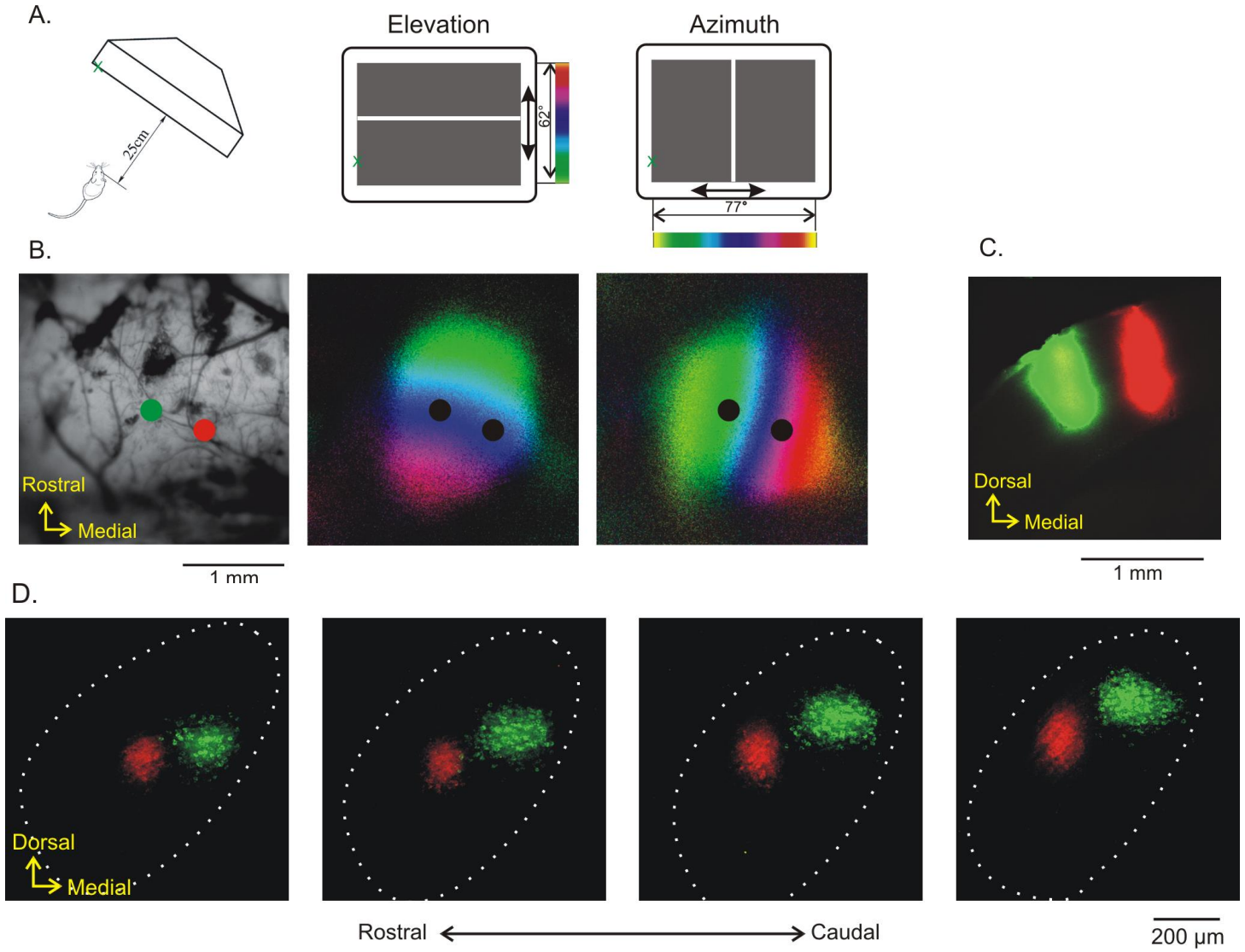


Mice lacking the β 2 subunit of nAChR have uncorrelated retinal activity during the first postnatal week

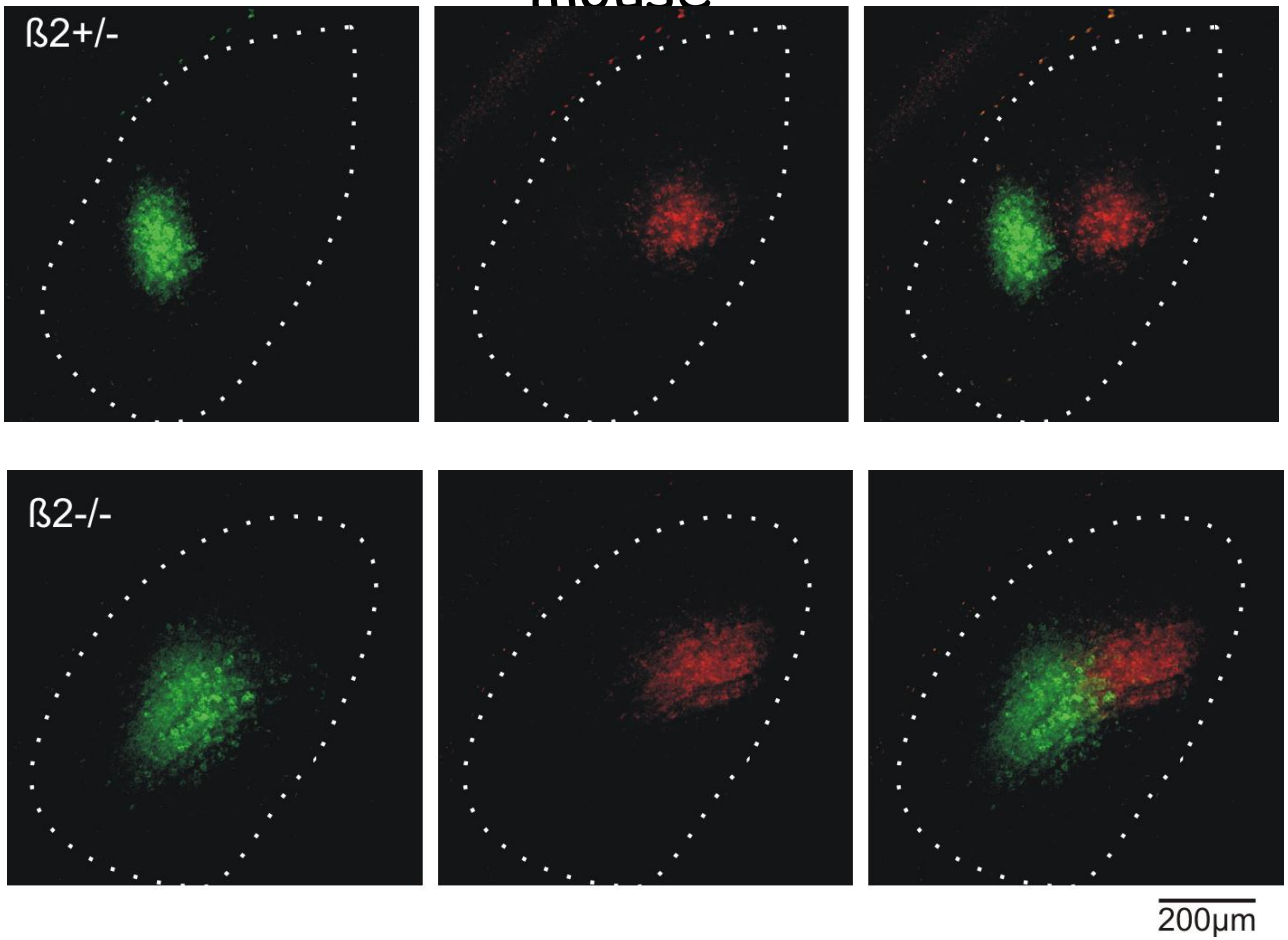


McLaughlin et al., 2003

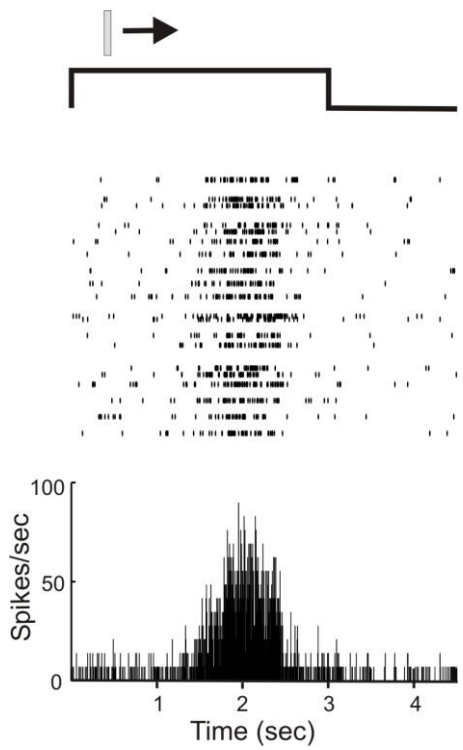
Anatomical tracing of Geniculocortical Projections

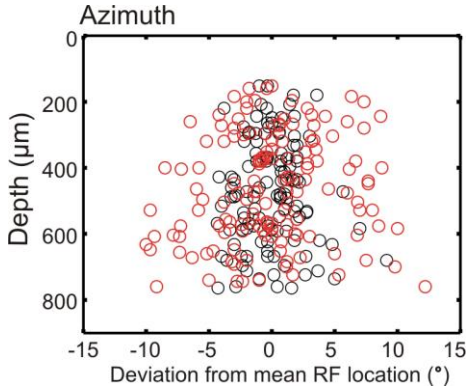


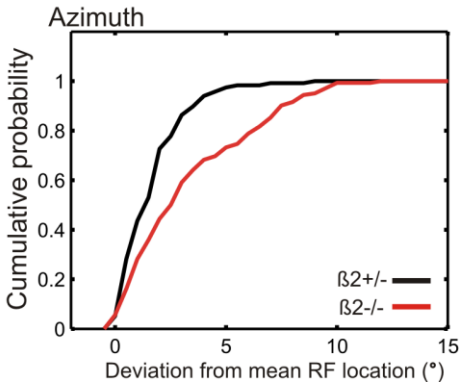
Retrograde labeling shows imprecise mapping of geniculocortical afferents in homozygous nAChR-β 2 mouse

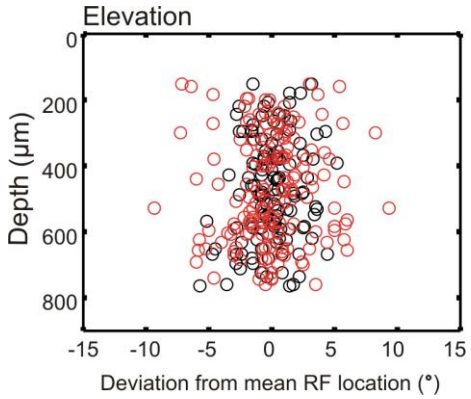


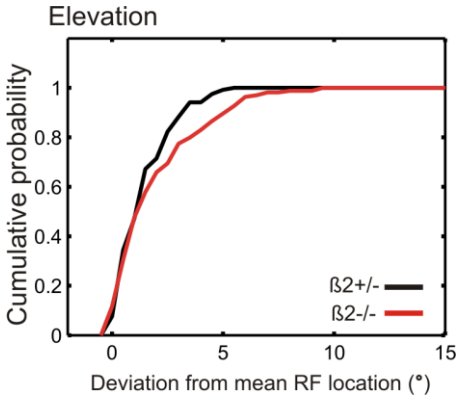
Cortical receptive fields are much more scattered in β 2 KO



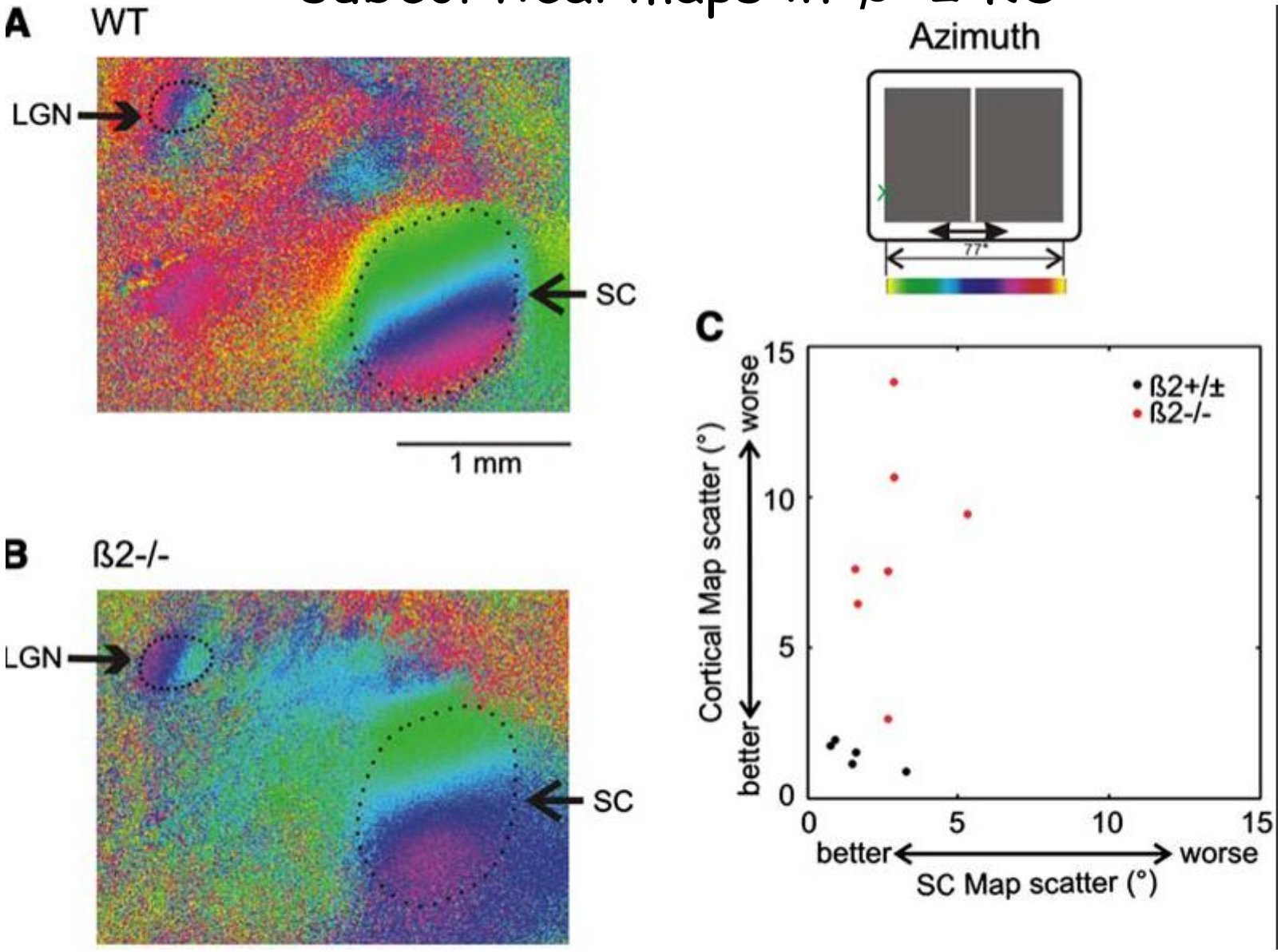








Cortical retinotopic maps are more defective than subcortical maps in β 2 KO

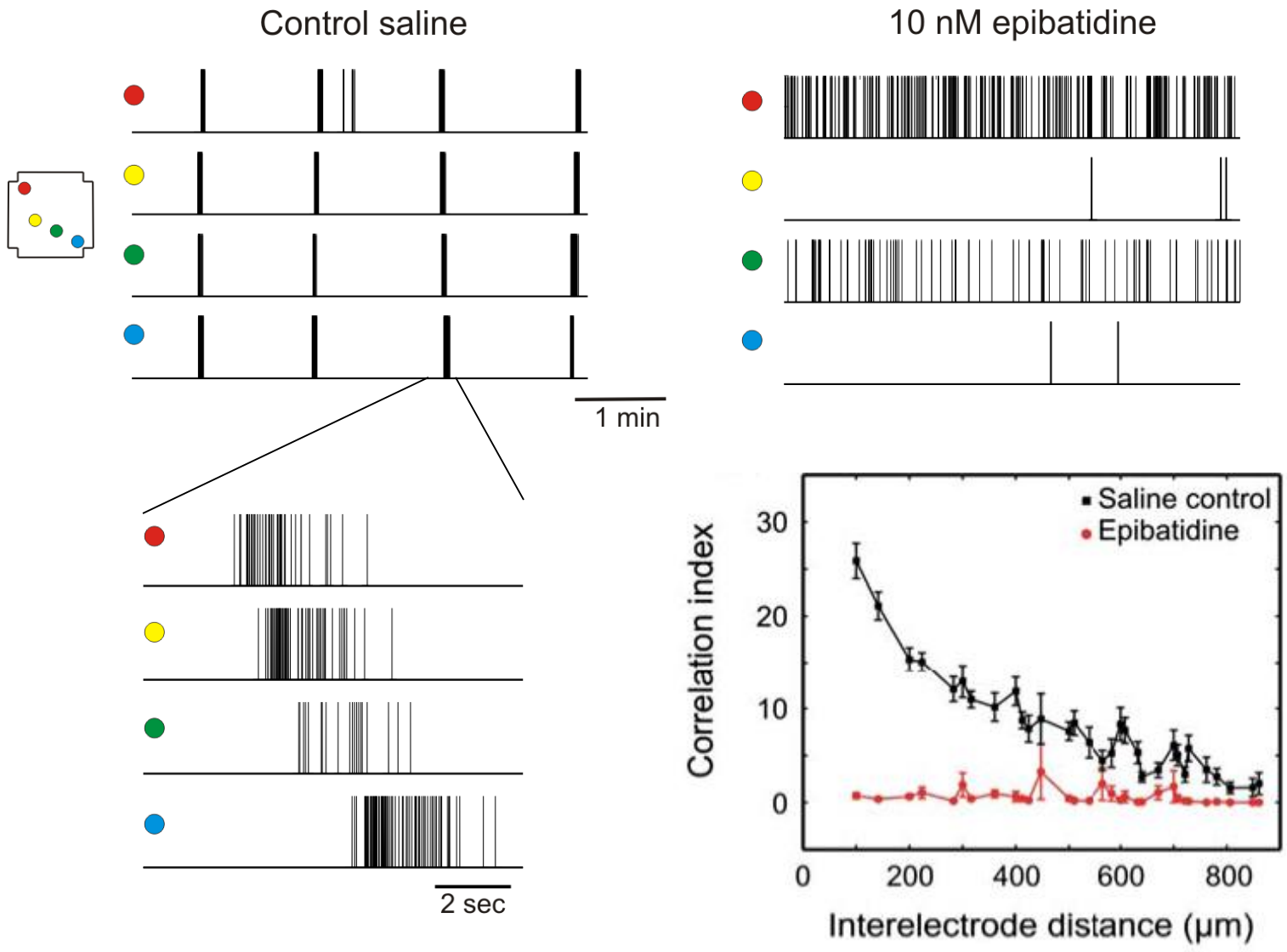


Results above demonstrate that geniculocortical map is defective in nAChR-$\beta 2^{-/-}$ mouse.

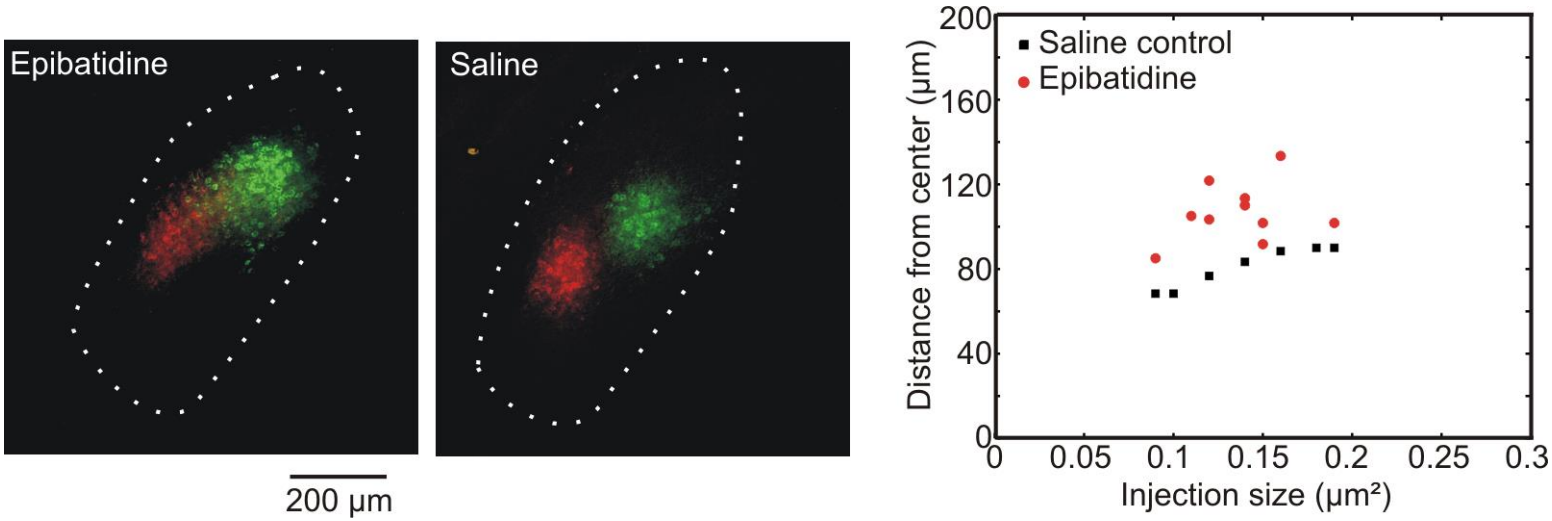
The genetic defect is present throughout the nervous system, not just in the eyes.

Does this mapping defect result from the disruption in **retinal** activity during the first week of life?

Nicotinic agonist epibatidine eliminates retinal waves during first week of life in mouse



Pharmacological disruption of retinal waves in WT mouse during the first week phenocopies nAChR-β 2 KO in the targeting of geniculocortical afferents.



0.3-1 nM epibatidine (or saline) was injected into both eyes of WT mice at P1, P3, P5 and P7, examined at P40.

Conclusion about Neural Activity:

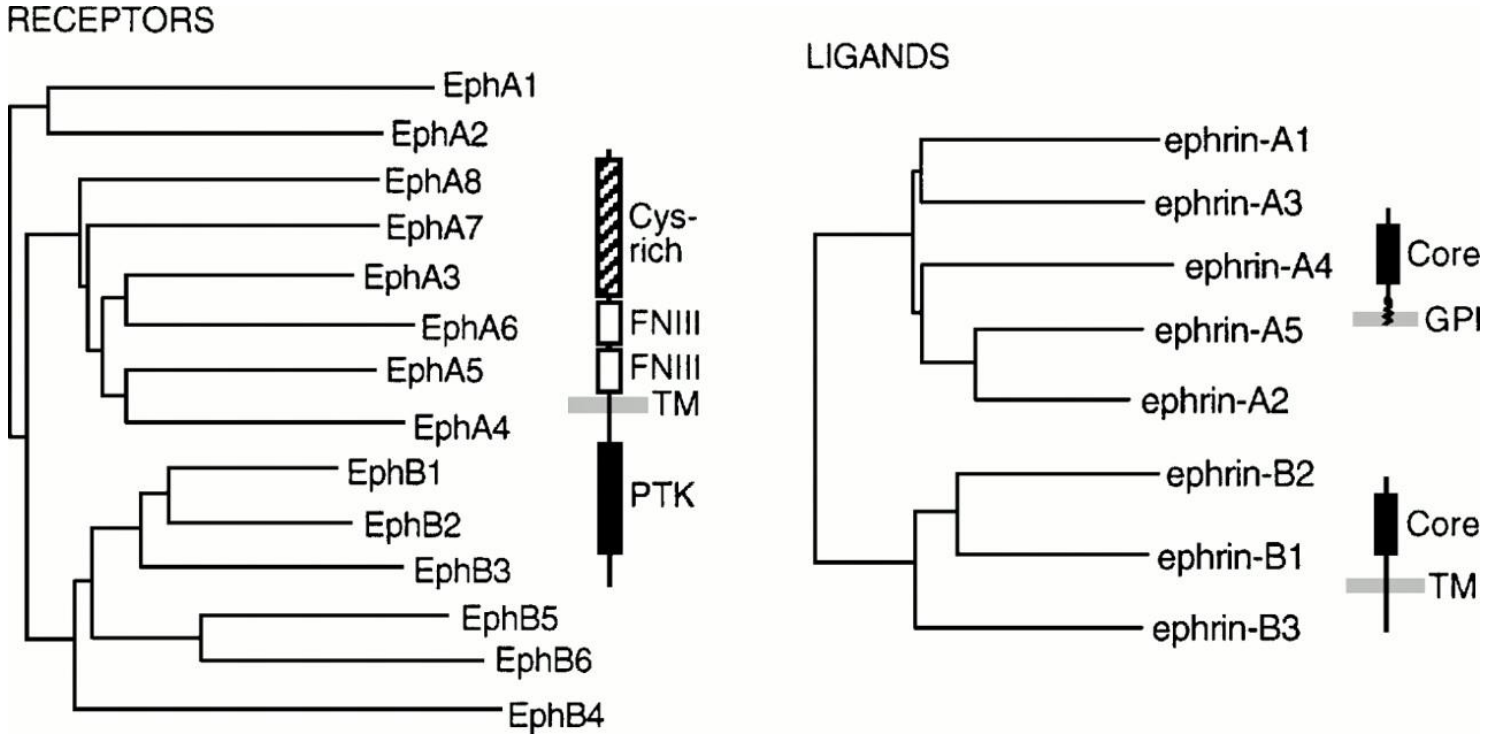
Neuronal activity originating in the periphery of the developing visual system during the first week of life guides the refinement of thalamocortical maps. This provides support for the models of Butts & Rokhsar (2001) on the information content of retinal waves.

Map refinement, and not ocular dominance plasticity, is the phenomenon that resembles the plasticity of the whisker barrels during a similar critical period in the first week.

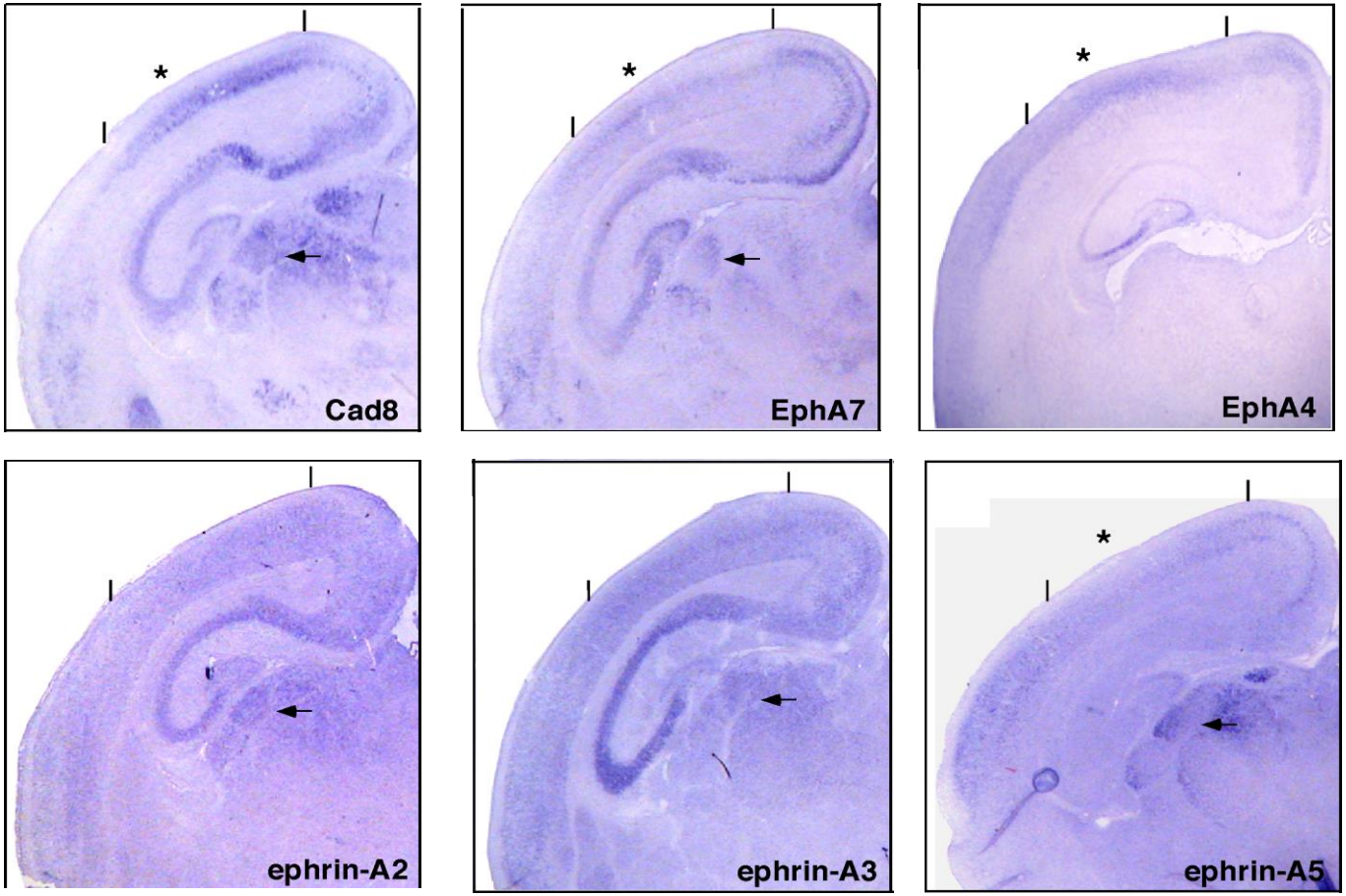
We sought to determine how topographic maps in the visual system were created.

- Does neural activity play a role?
- What are the molecular cues?

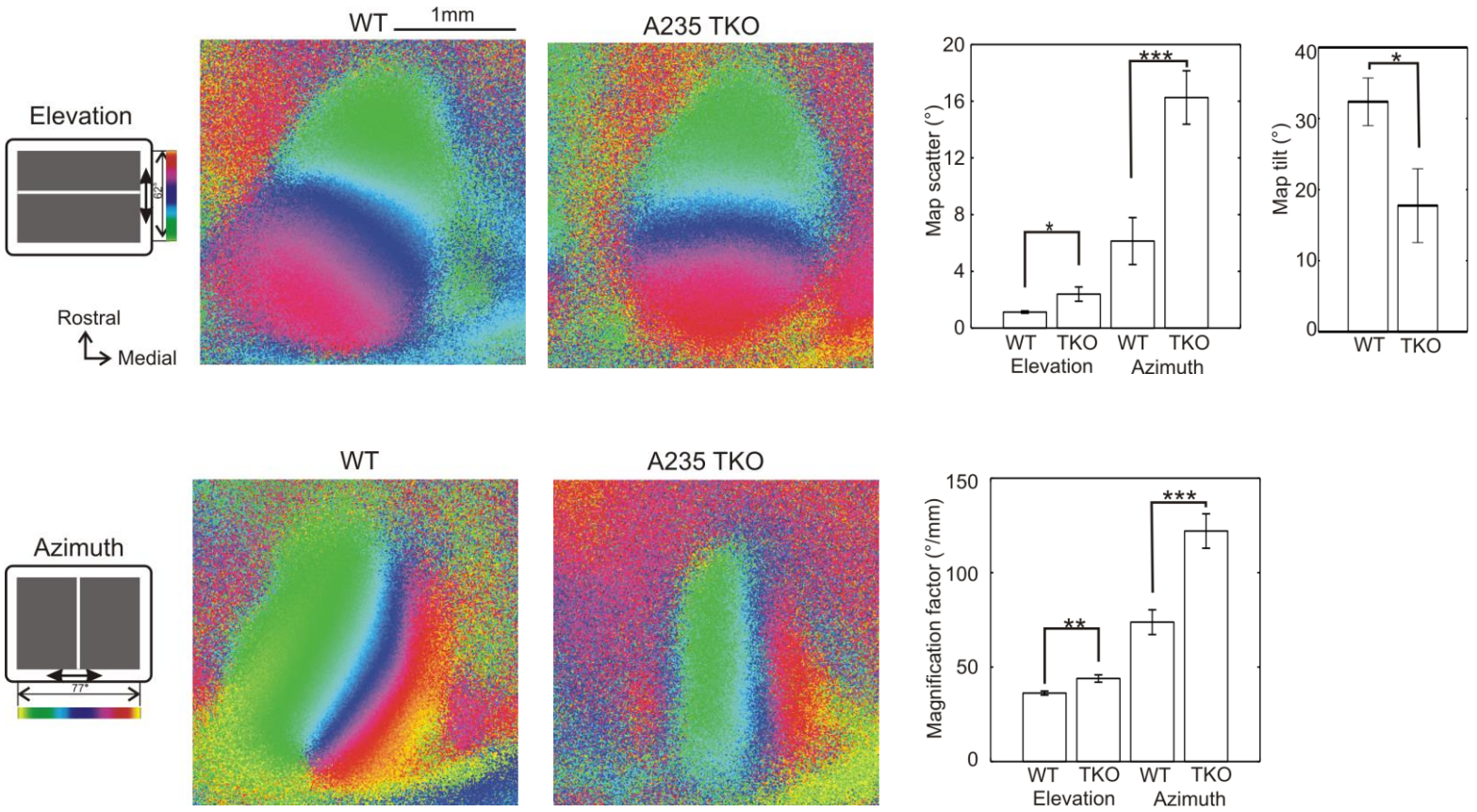
The Eph family of receptor tyrosine kinases and their cell-surface bound ligands, ephrins, are likely candidates as **effectors** for the neuronal interactions that make cortical maps.



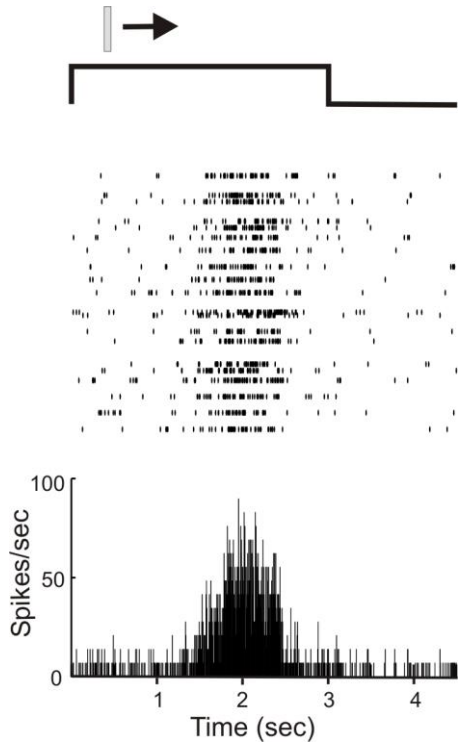
Expression of EphA Receptors and ephrin-A Ligands in the Developing Visual Cortex

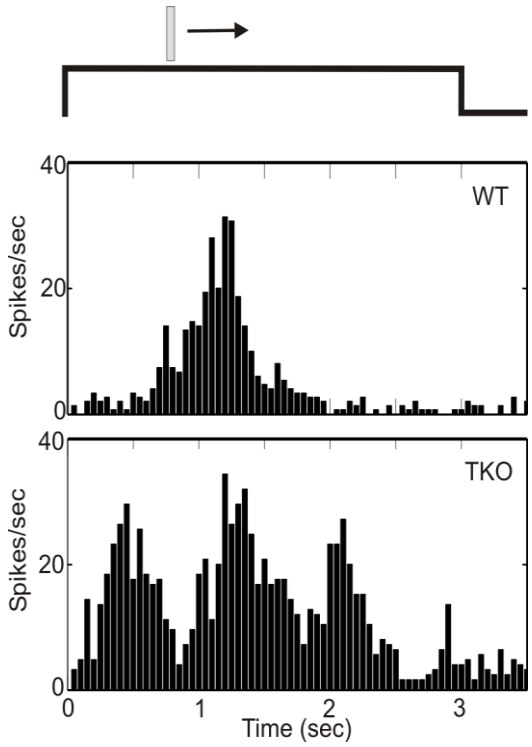


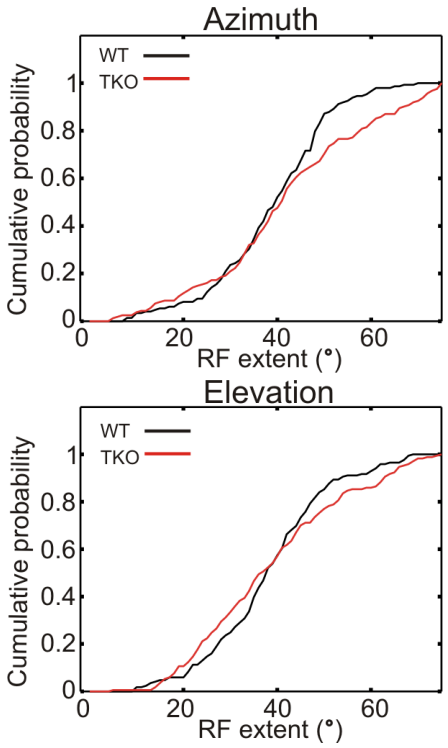
Retinotopic Maps in the Cortex of Ephrin-A TKOs



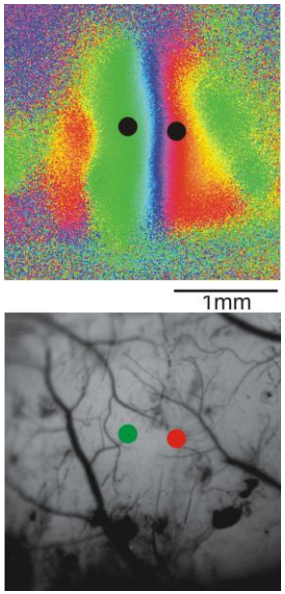
Cortical Response Patterns in Ephrin-A TKOs

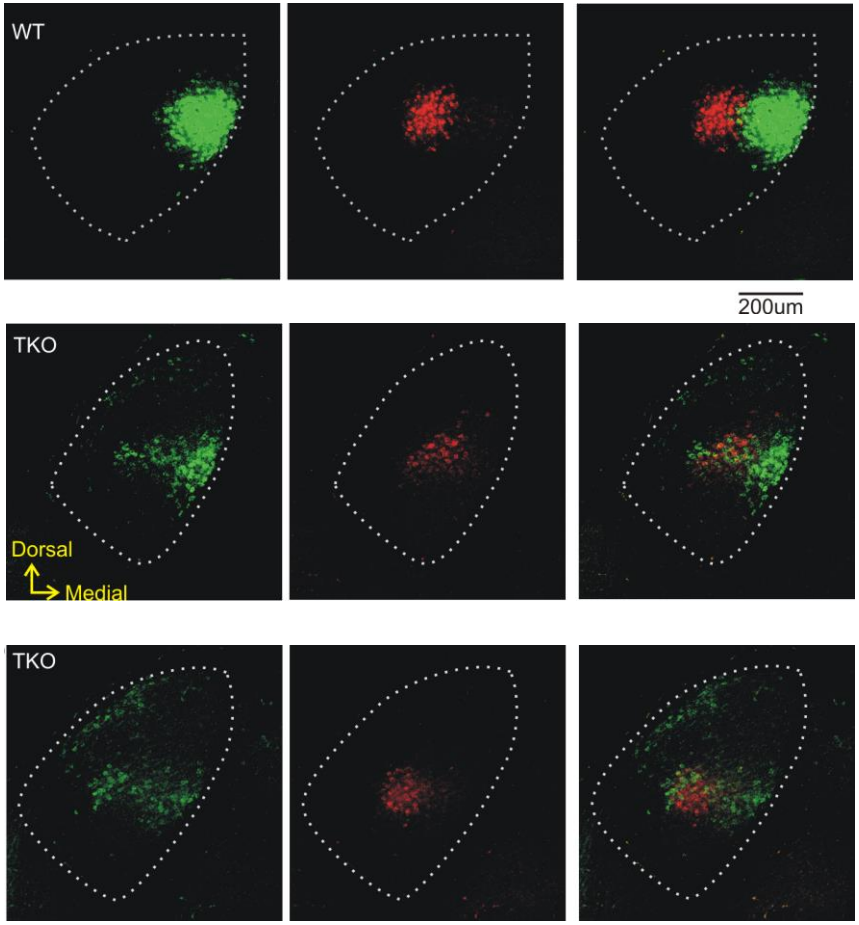




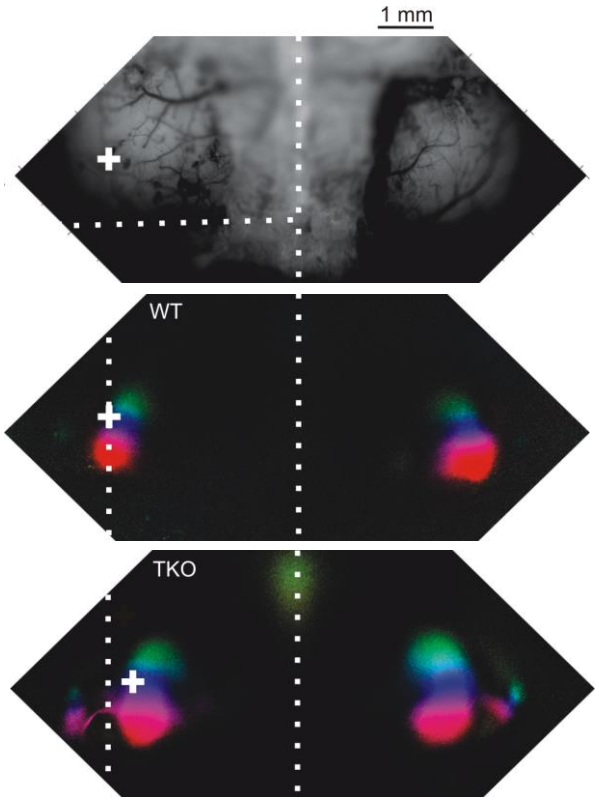


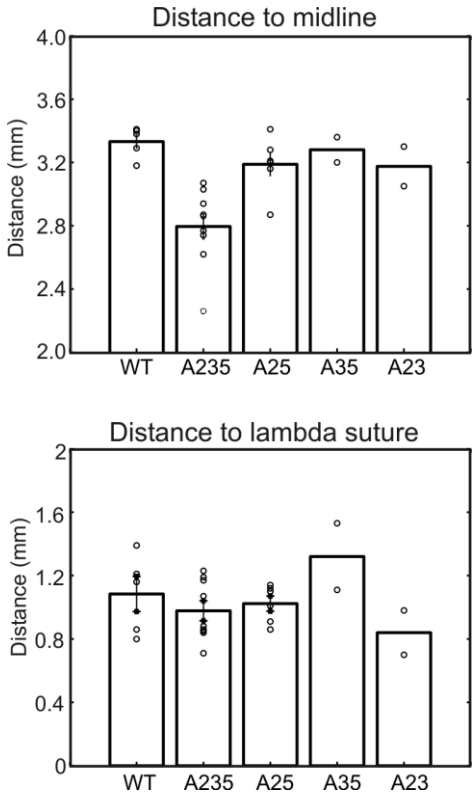
Geniculocortical Projection Patterns in Ephrin-A TKOs



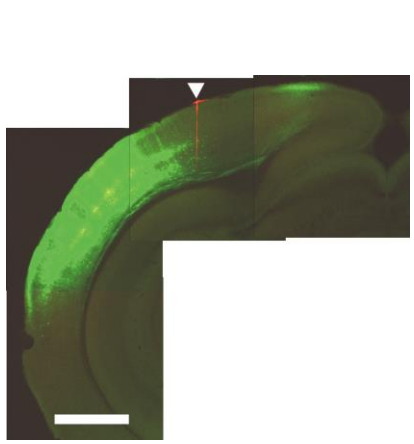


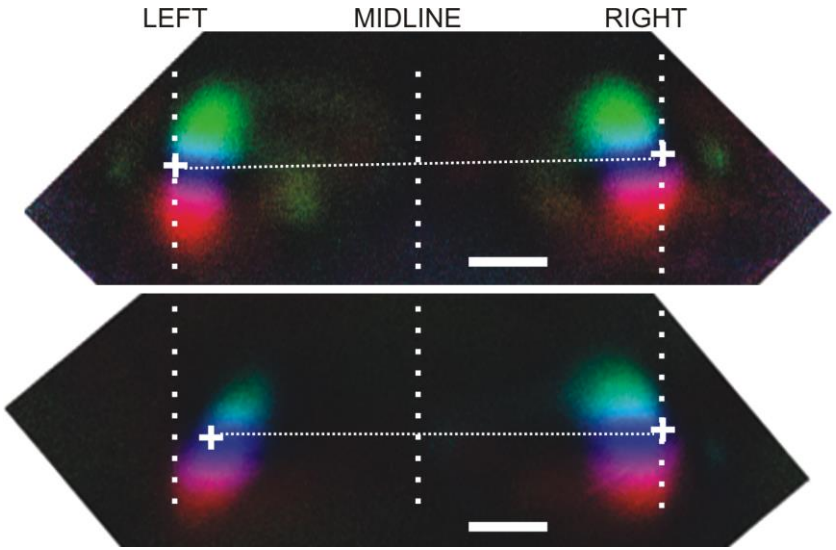
Ephrin-A TKOs Have Mediolateral Positioning Defects in Primary Visual Cortex

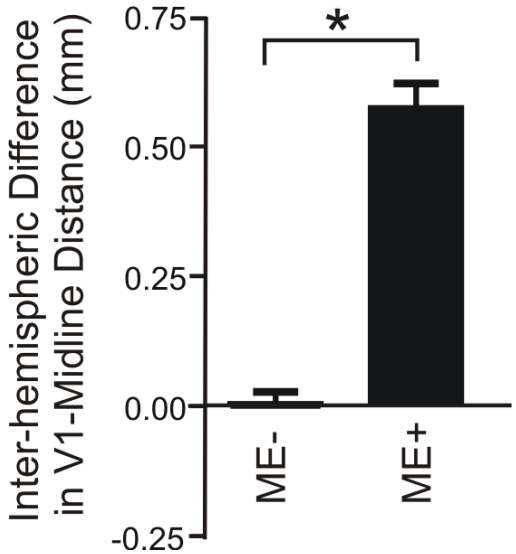




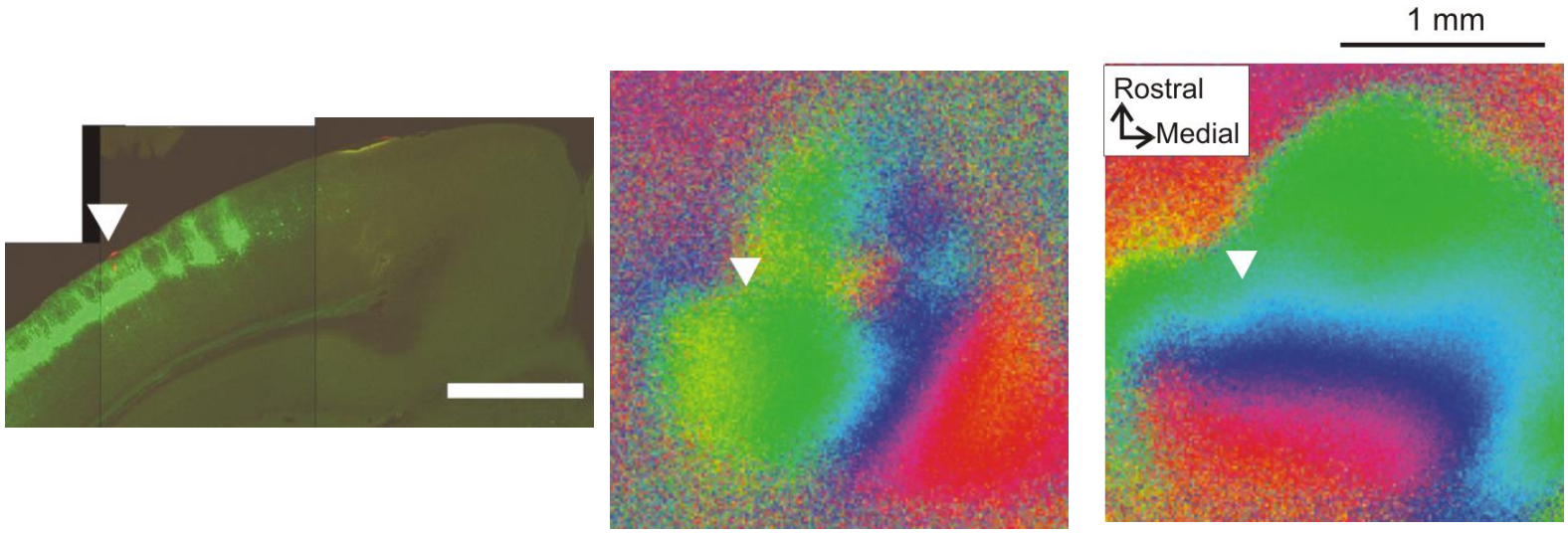
Lateral Misexpression of Ephrin-A5 Shifts V1 Medially in WT mice



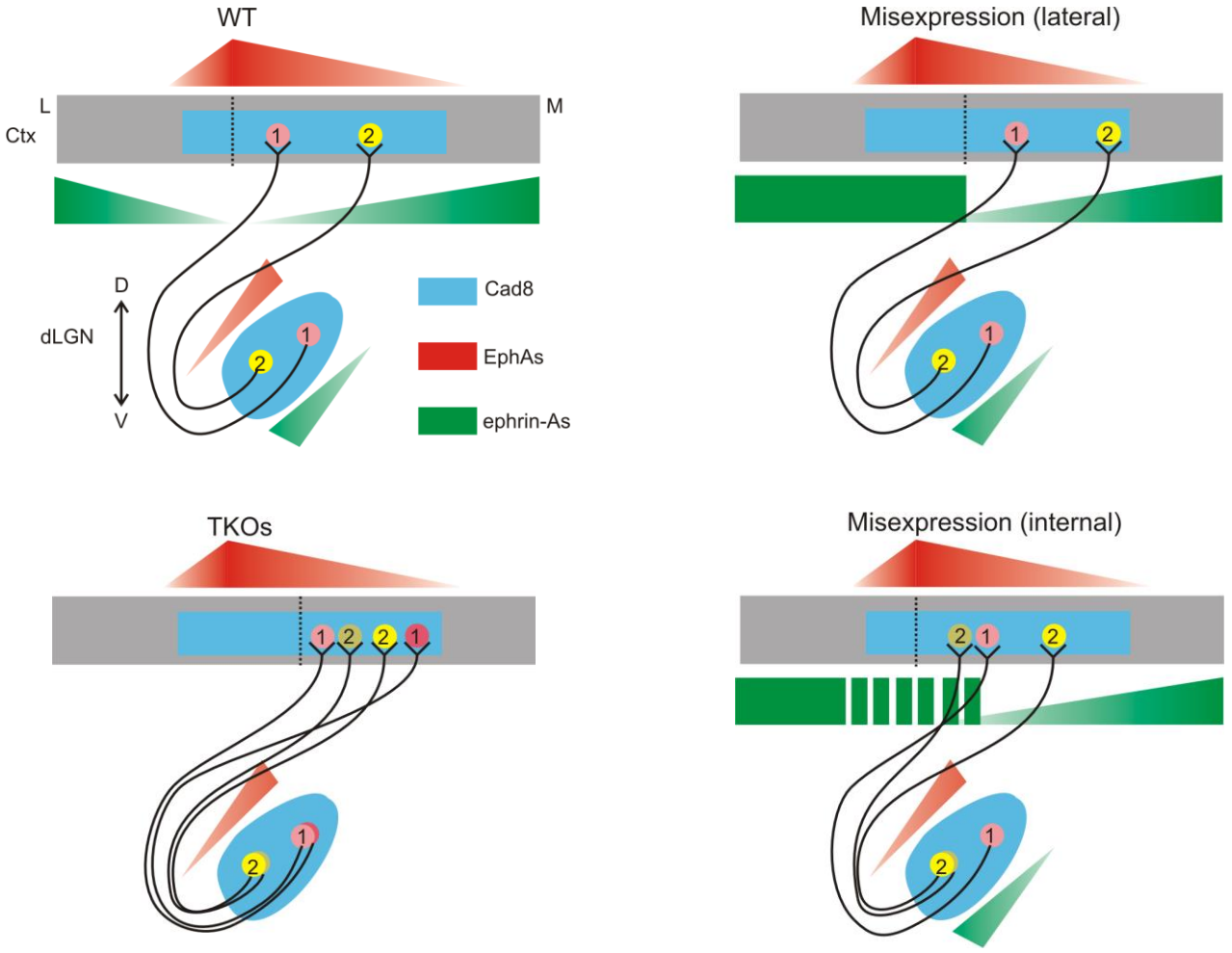




Misexpression of Ephrin-A5 within V1 Disrupts Functional Retinotopy



EphA/ephrin-A interactions in the primary visual cortex are required for proper position and internal order of the map



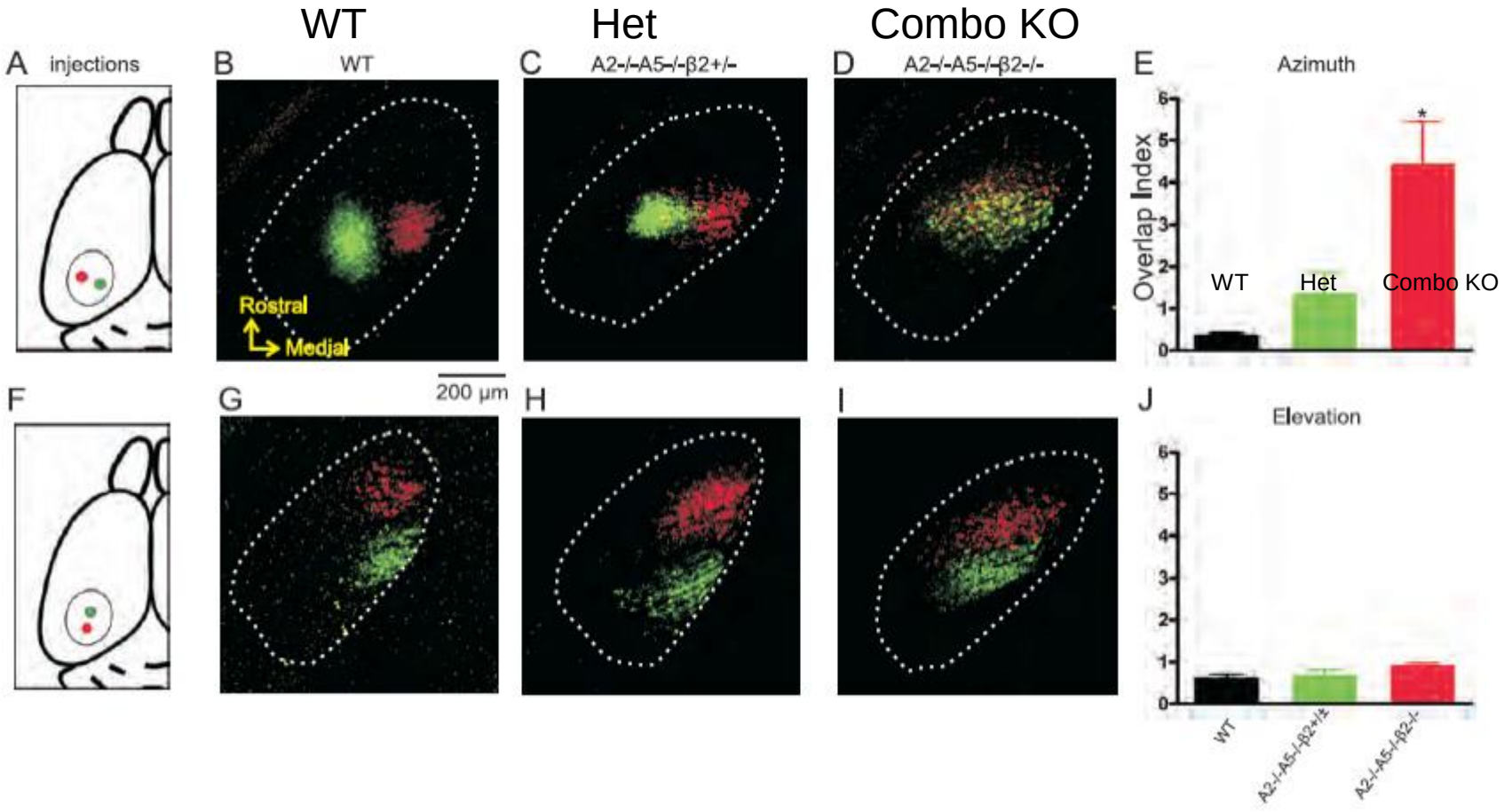
Either activity disruption or ephrin-A knockout or misexpression disrupt the cortical map. But with either manipulation, a clear but messy map remains.

Do ephrin-A signaling and structured activity account *entirely* for the map of azimuth?

We answered this question by making Combined Knockout mutant mice with defects in both ephrin-A signaling and structured neuronal activity.

Cang, Niell et at, Neuron, 2008

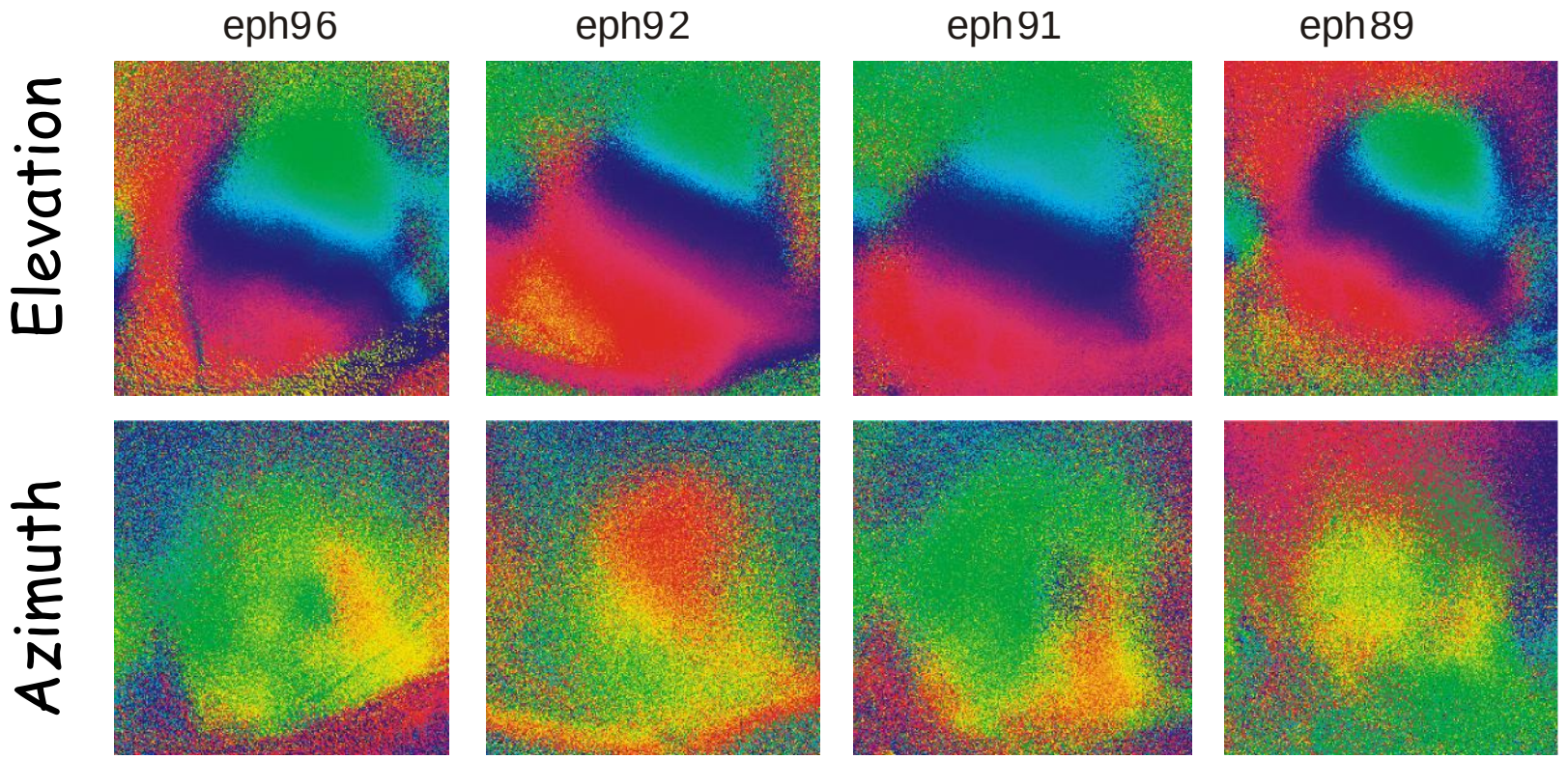
Combining the ephrin-A knockout with the nicotinic β 2 knockout (A2 $^{-/-}$A5 $^{-/-}$ β 2 $^{-/-}$) completely destroys the azimuth maps seen with anatomical tracing. Connections seem completely scrambled in azimuth, but elevation is OK.



Cang, Niell et at, Neuron, 2008

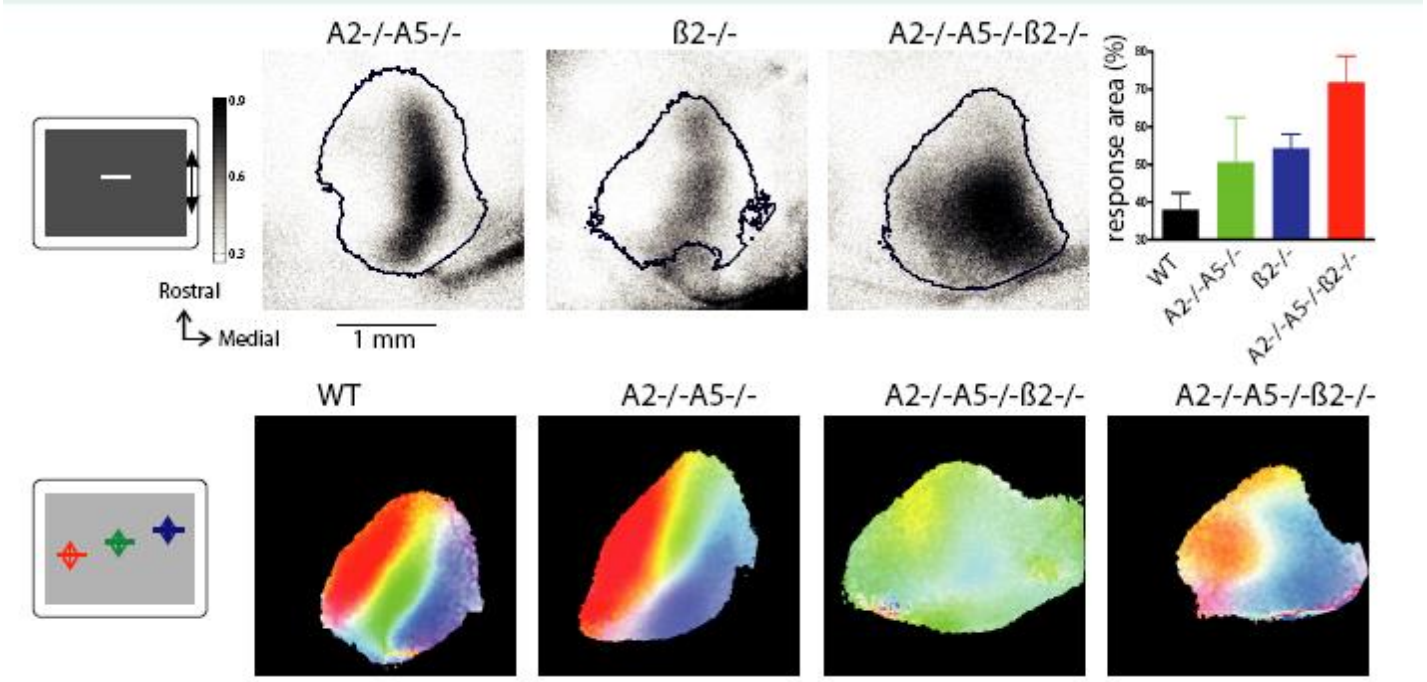
Intrinsic signal maps of azimuth in combination knockout (A2 $^{-/-}$A5 $^{-/-}$ β 2 $^{-/-}$) are nearly absent.

Elevation maps are nearly normal



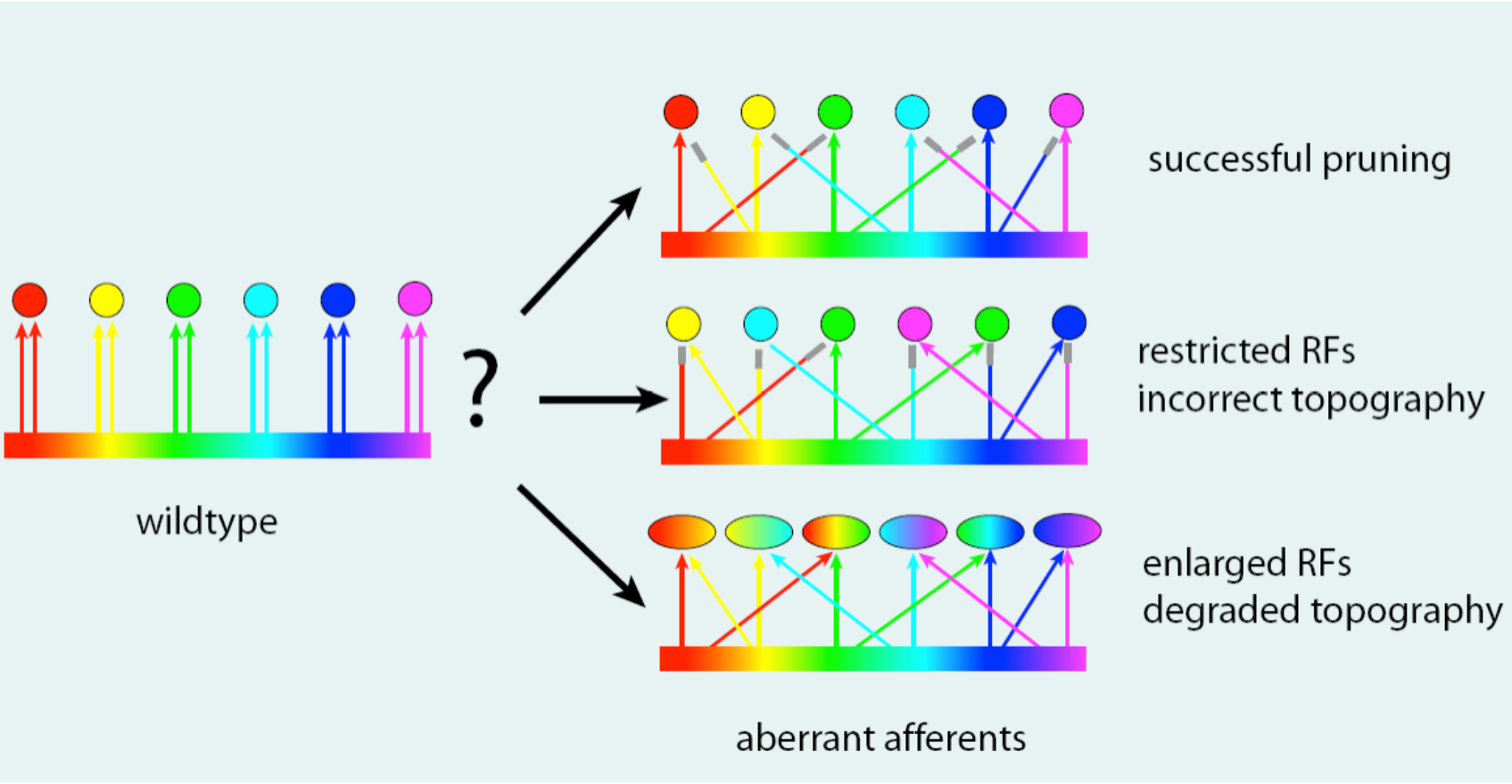
Cang, Niell et at, Neuron, 2008

Mapping with short bars reveals widespread response to smallest stimulus, with slight remnant topography in Combo Knockout ($A2^{-/-}A5^{-/-}\beta\ 2^{-/-}$)



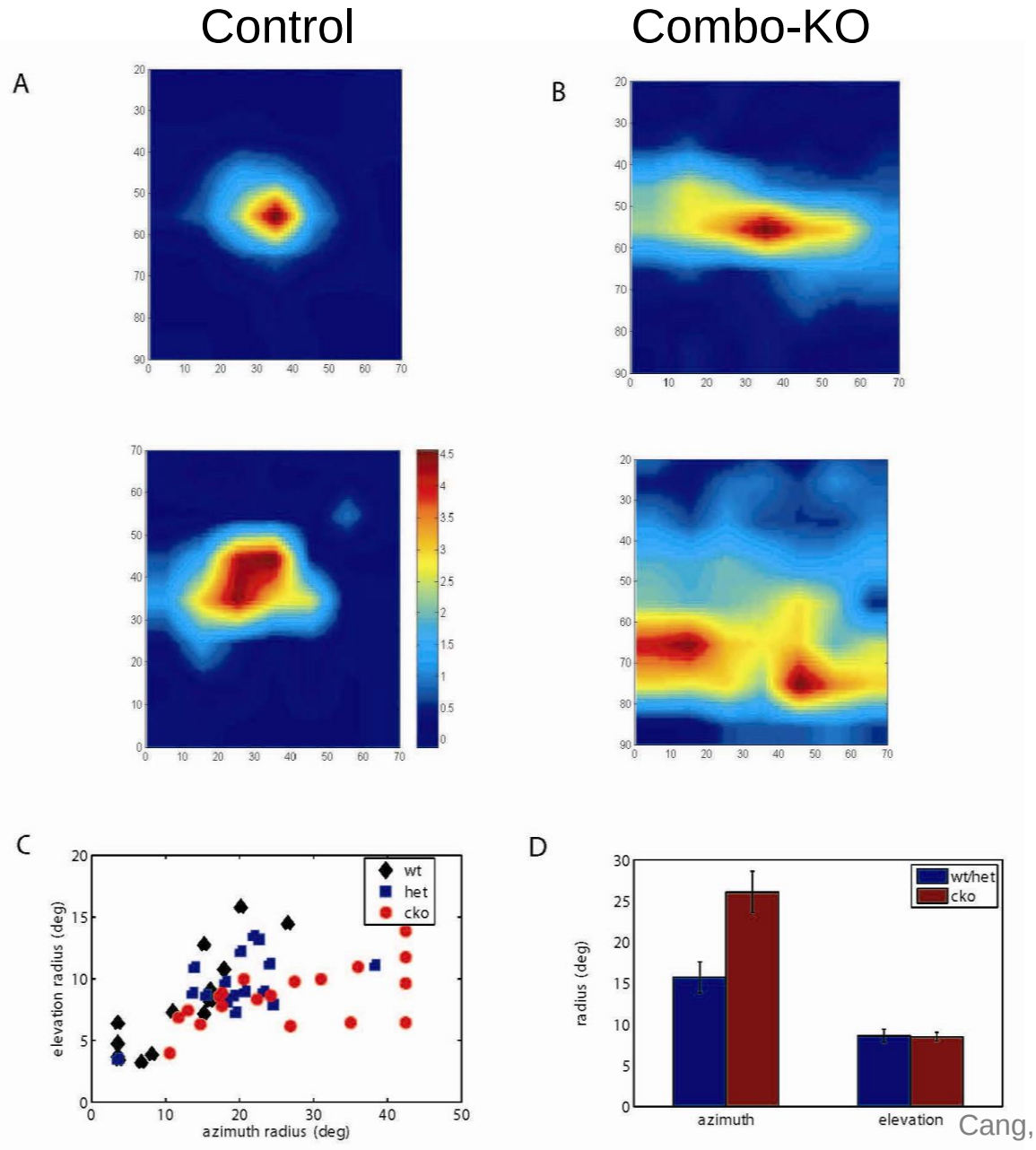
Cang, Niell et at, Neuron, 2008

How do the cortical cells cope with the miswiring of their inputs?



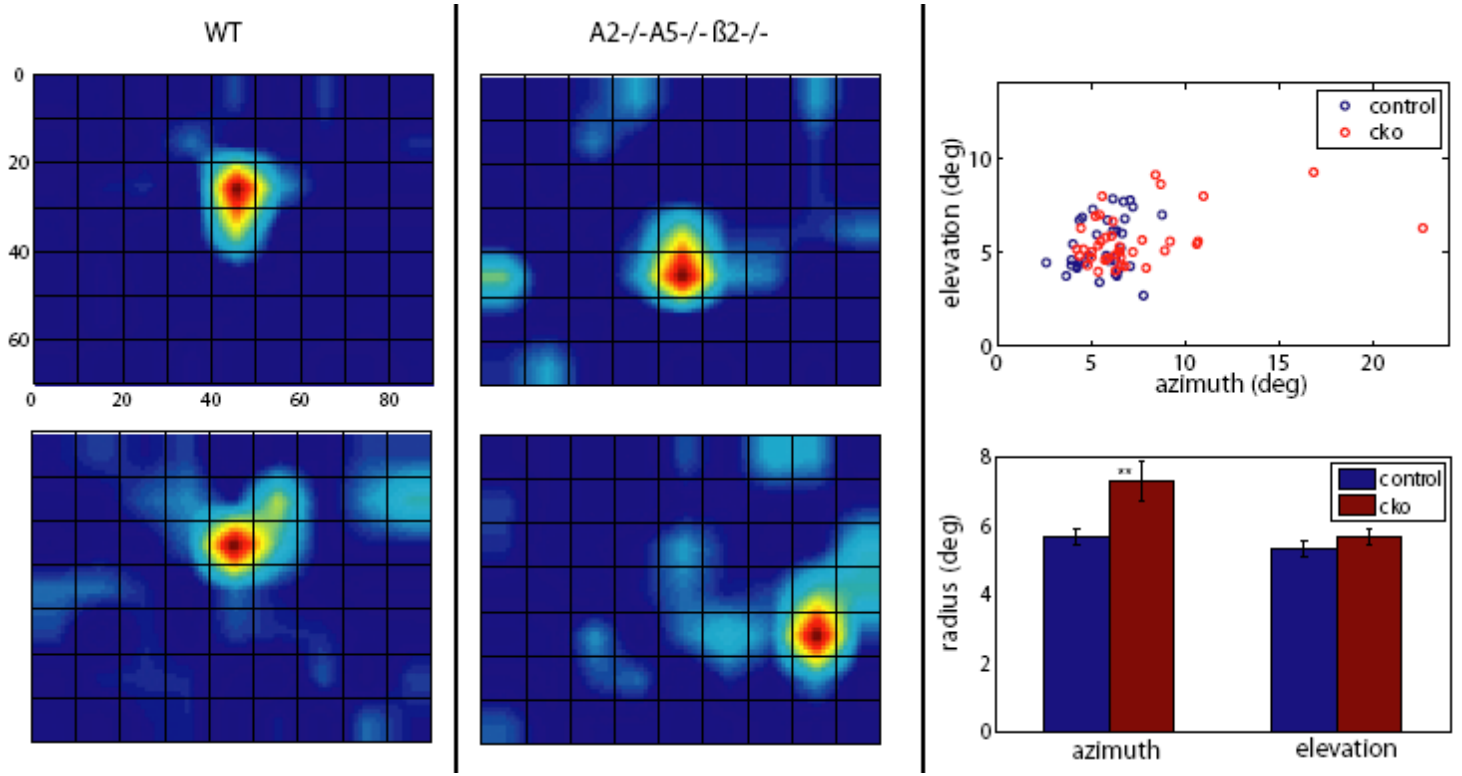
Cang, Niell et at, Neuron, 2008

Extended receptive fields of single units in visual cortex



Cang, Niell et at, Neuron, 2008

Receptive fields of single neurons in Lateral Geniculate Nucleus are nearly normal in size



The scatter in LGN RF position can account for not more than 1/3 of the effect of these mutations on cortical RFs.

Cang, Niell et at, Neuron, 2008

Conclusions on the Formation of Azimuth Maps in V1

The formation of the topographic map in visual cortex, complete by 1 week of age, and not binocular plasticity, is the analogy to whisker barrel plasticity.

Molecular cues and structured neural activity can partially compensate for one another, but when both are gone, the azimuth maps in cortex are nearly absent.

Neural activity and ephrin-A signaling are not epistatic. They are distinct mechanisms that cooperate to organize maps in early development.

Amazingly, elevation maps are nearly normal. Therefore, the axes of the cortical map are actually Cartesian and are organized independently.

Cang, Niell et at, Neuron, 2008

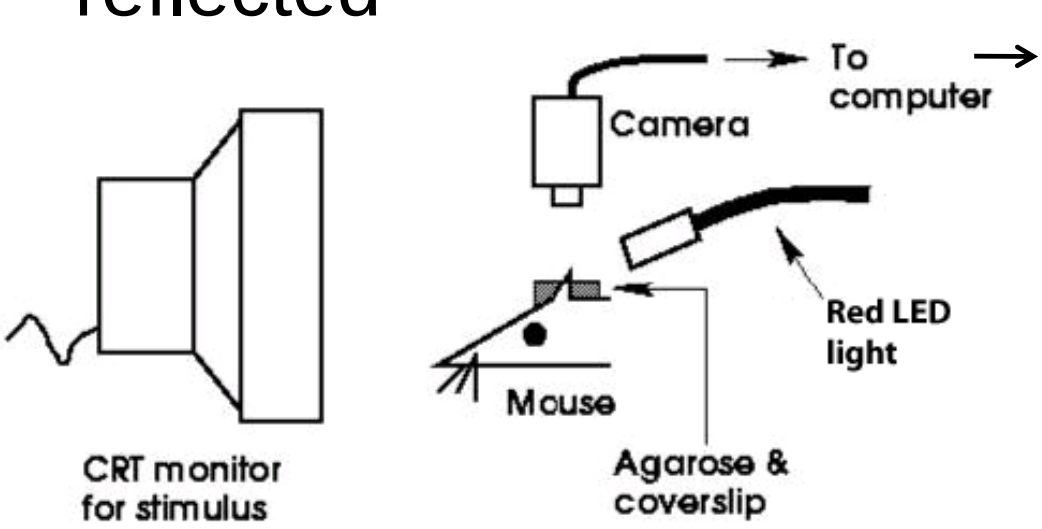
Map Formation in the Superior Colliculus

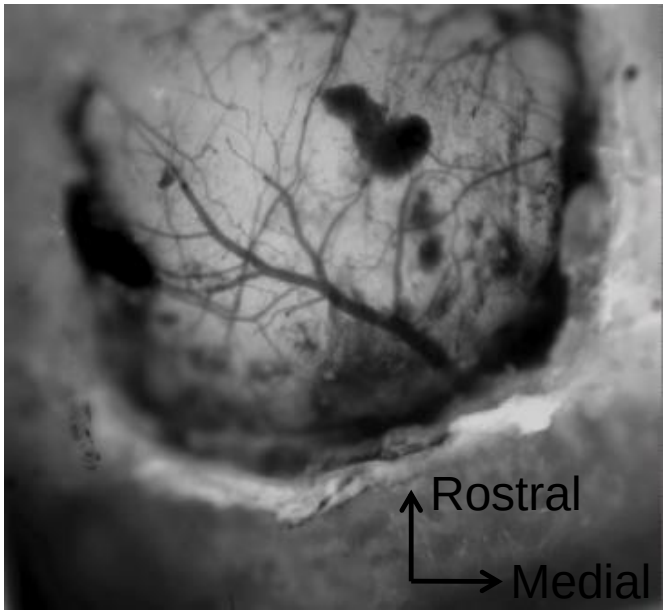
The superior colliculus also receives input from the retina and has a topographic map, the azimuth axis of which is organized by eprin-A signaling and structured neural activity.

We have mapped the SC in the same mutants and modeled the findings.

Optical imaging of the intrinsic signal

- Brain activity causes a local change in blood oxygenation, changing the amount of red light reflected

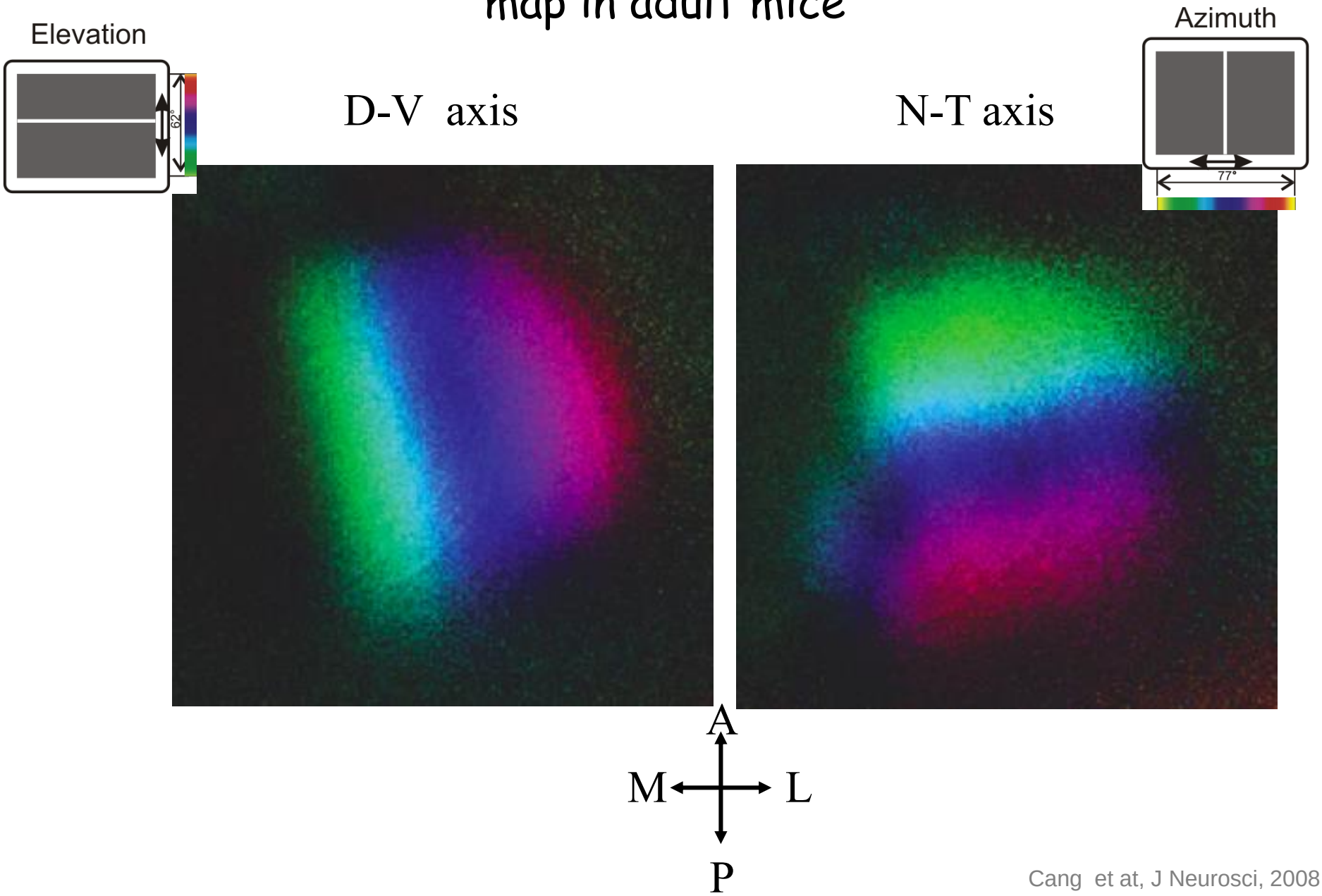




mod. from Lee *et al*. 2006

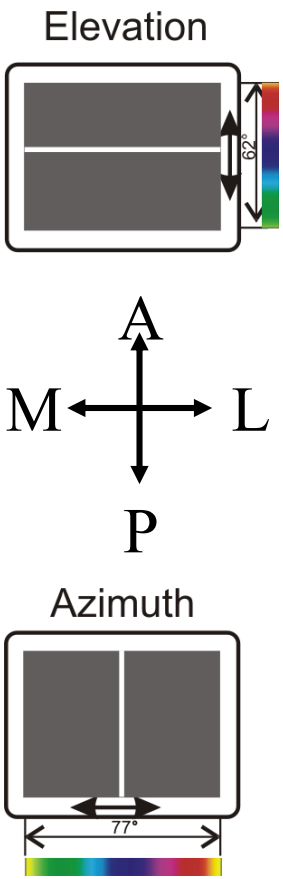
- For Superior Colliculus (SC) imaging, the overlying cortex is aspirated away

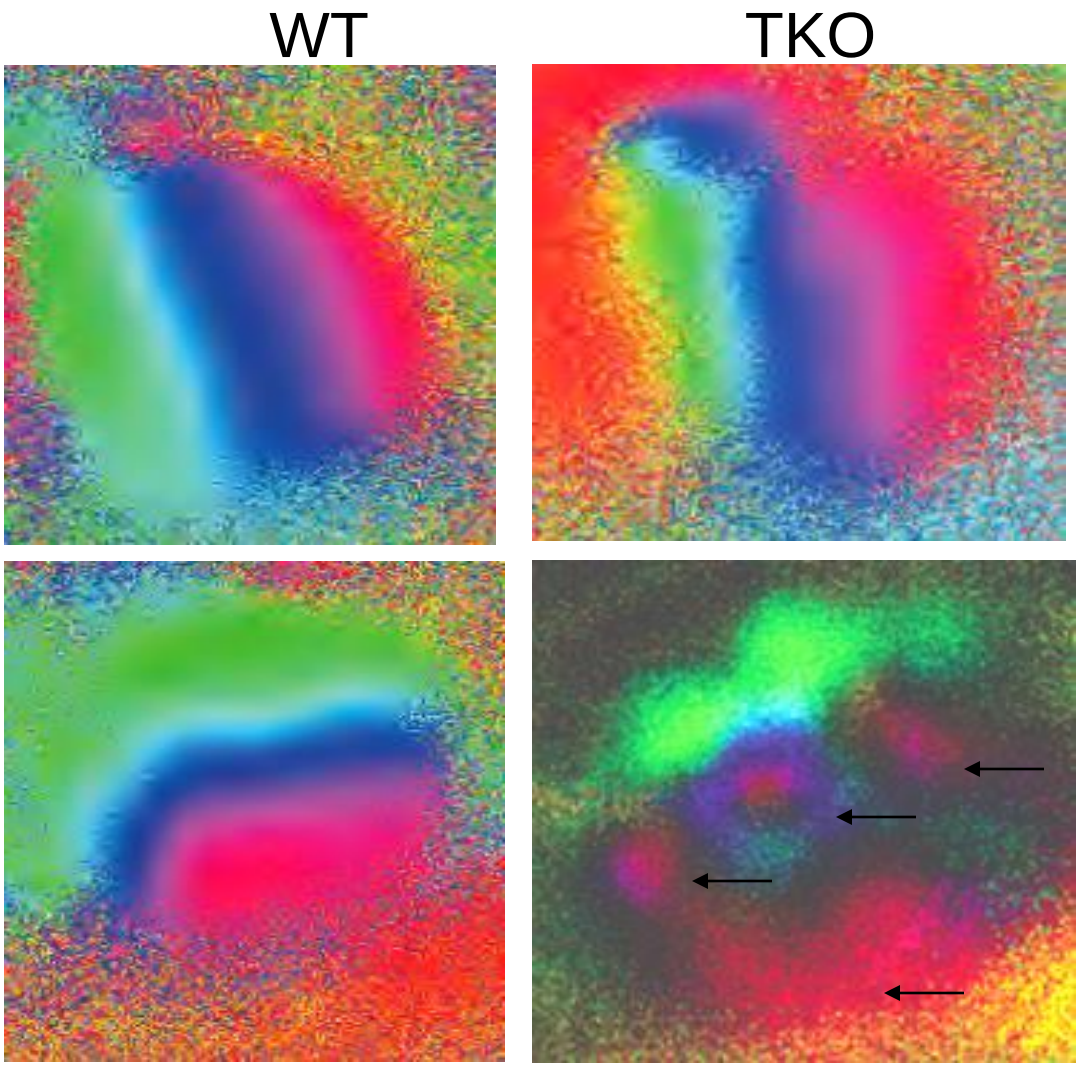
Intrinsic signal optical imaging of the Superior Colliculus map in adult mice



Cang et at, J Neurosci, 2008

"Patchy" functional maps of azimuth in SC of ephrin-A2/A3/A5 TKO mice with normal retinal activity





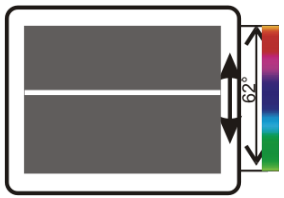
Cang et at, J Neurosci, 2008

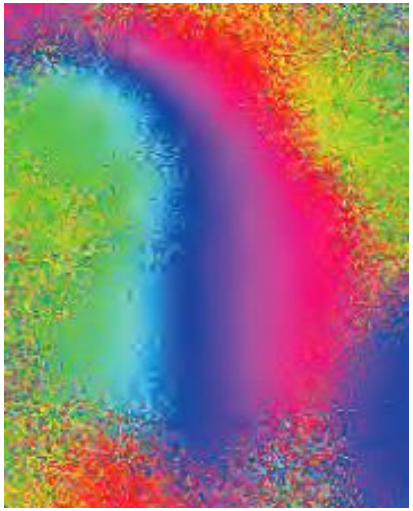
Ephrin-A2/A5; β 2 combination mutant mice (in which both ephrin signaling and structured activity are deficient) lack functional topography along the nasal-temporal mapping axis of the Superior Colliculus

wild type

ephrin-A2/A5/β2 tko

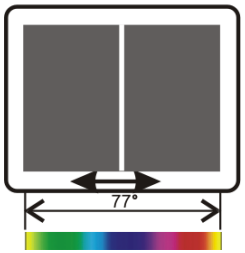
Elevation

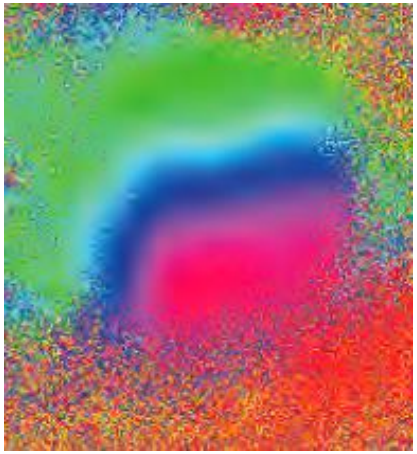


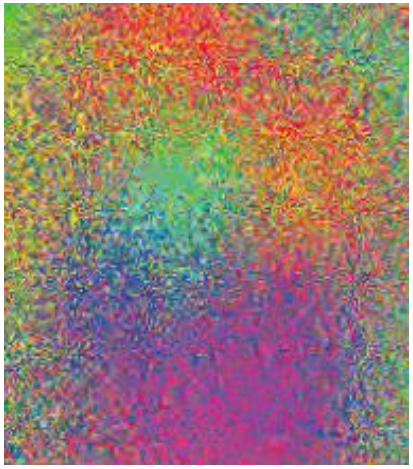


Elevation

Azimuth







Azimuth

Cang et at, J Neurosci, 2008

Conclusions on Interaction of Neural Activity and Molecular Cues in Map and RF Formation:

The same problem, map formation, using the same signals, is solved by 3 different structures in 3 different ways:

Map polarity and global order in the SC appears entirely determined by ephrin-A signaling. Without it, but with structured activity, maps are locally tightly organized but globally nonsensical. Without both, almost no azimuth map.

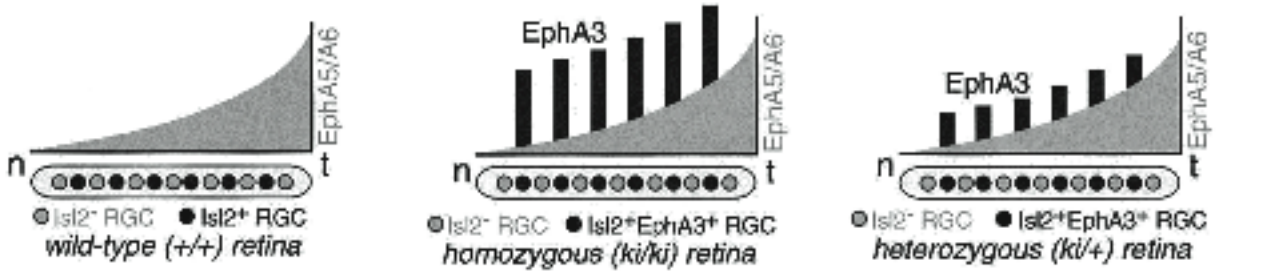
In V1, map polarity and global order are preserved but messy with either structured activity or ephrin-A signaling. With defects in both, the azimuth map is nearly absent. In the absence of the azimuth map, individual cells have extremely wide RFs.

In LGN, both map polarity and global order are preserved well with defects in either structured activity or ephrin-A signaling. With defects in both, individual cells have normal-size RFs, but these are abnormally scattered.

Misexpression of EphA receptors in a population of retinal ganglion cells to alter retinotectal (SC) maps

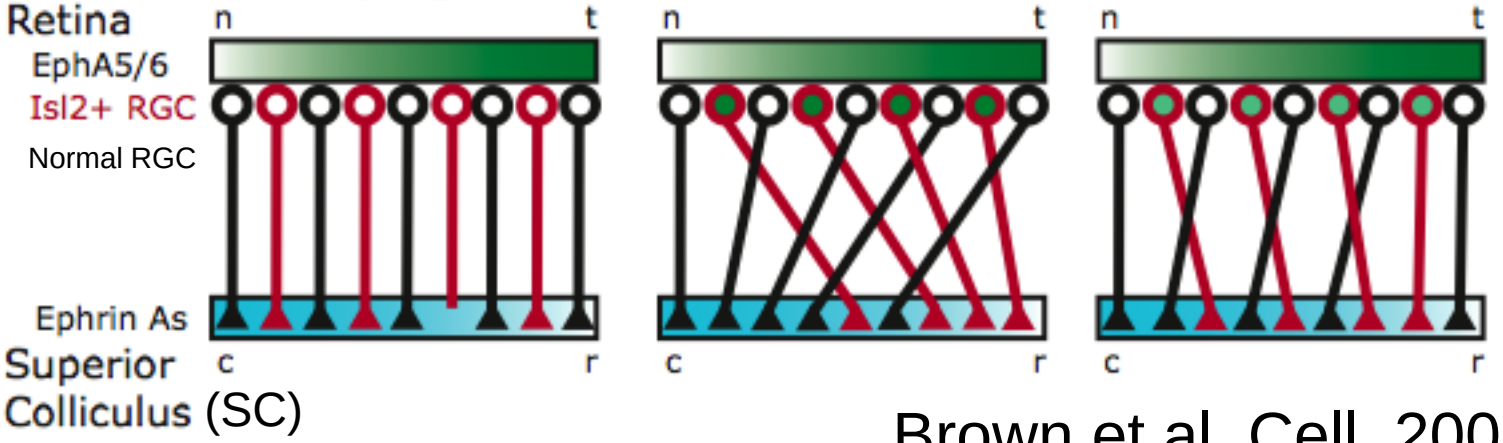
Wildtype (wt/wt) | **Homozygote (ki/ki)** | **Heterozygote (ki/wt)**

Levels of EphA in the Retina:



from Brown *et al.* 2000

Retinotectal Mapping:

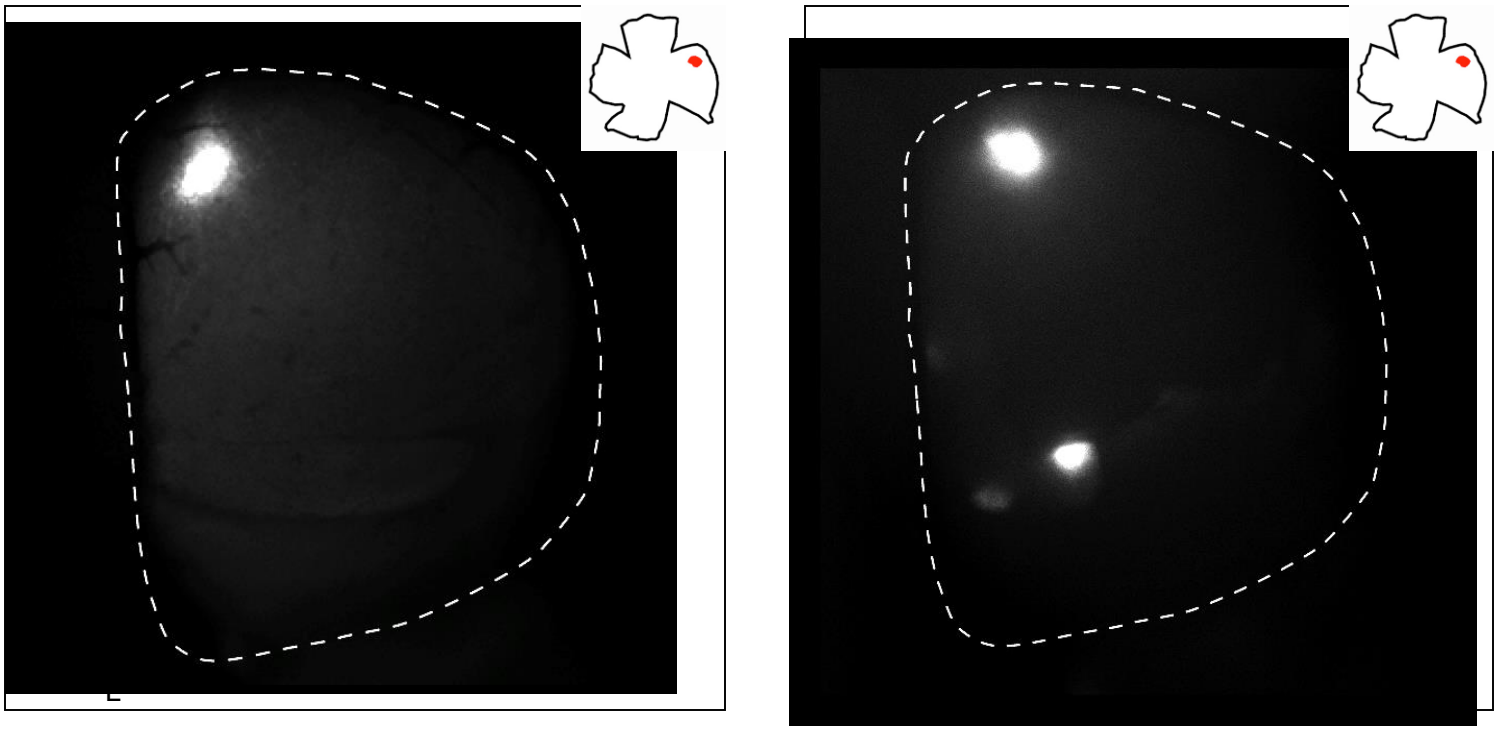


Brown et al, Cell, 2000

Each region of the retina projects to two places in the Superior Colliculus (SC) of EphA3$^{ki/ki}$ mice, suggesting that the azimuth map is duplicated

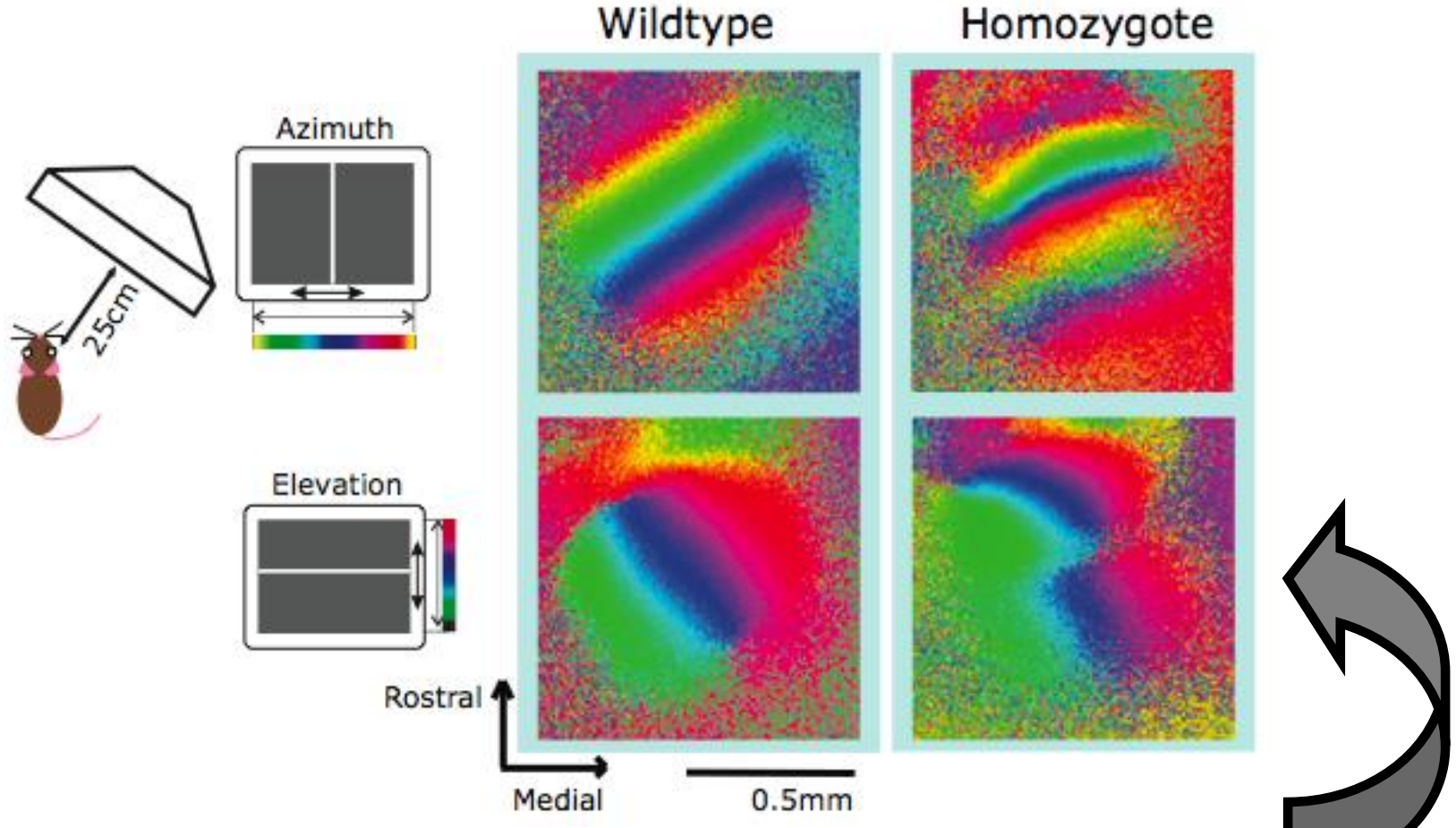
WT

EphA3$^{ki/ki}$



Brown et al., *Cell* 2000

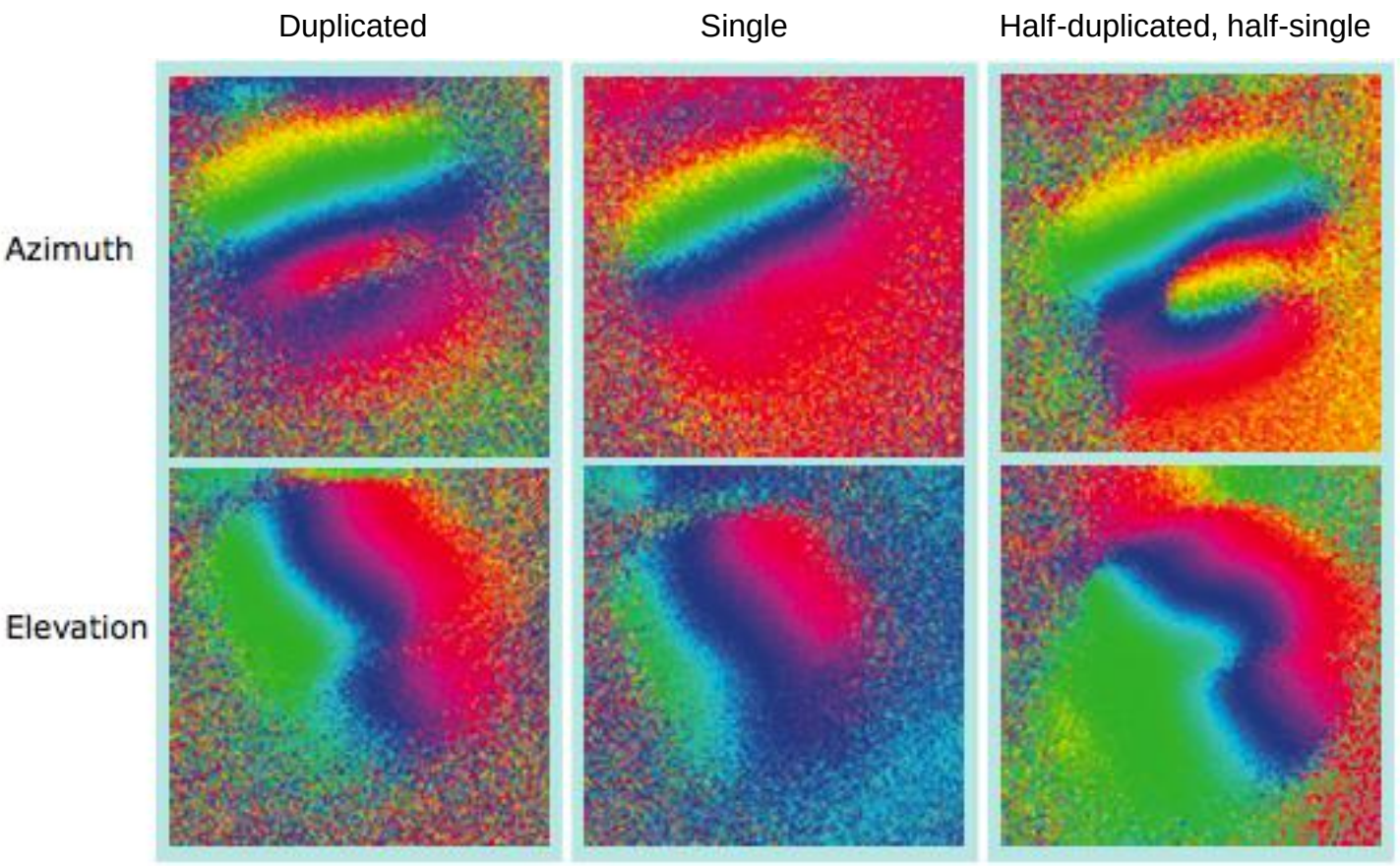
Intrinsic signal imaging confirmed the duplication of the azimuth map in SC of EphA3$^{ki/ki}$ mice. It also showed changes in the representation of elevation that made each of the duplicated maps isotropic and fully coherent.



Note discontinuity in elevation map of EphA3$^{ki/ki}$ mice

Owens et at, 2009

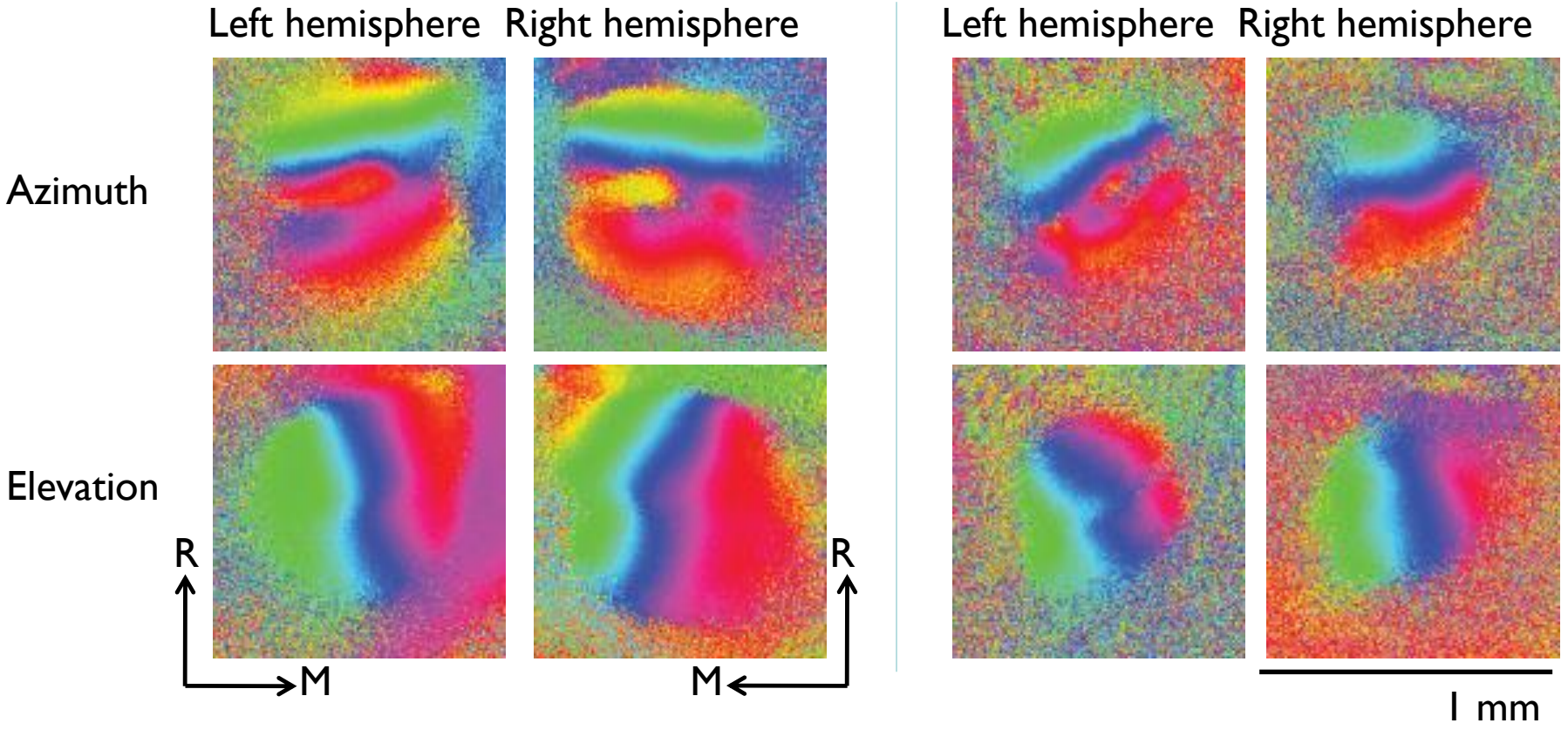
Heterozygous $EphA3^{ki/+}$ mice showed both normal and duplicated azimuth maps in SC, sometimes in the same individual. Elevation was remapped in each duplicated case to make the maps isotropic and coherent.



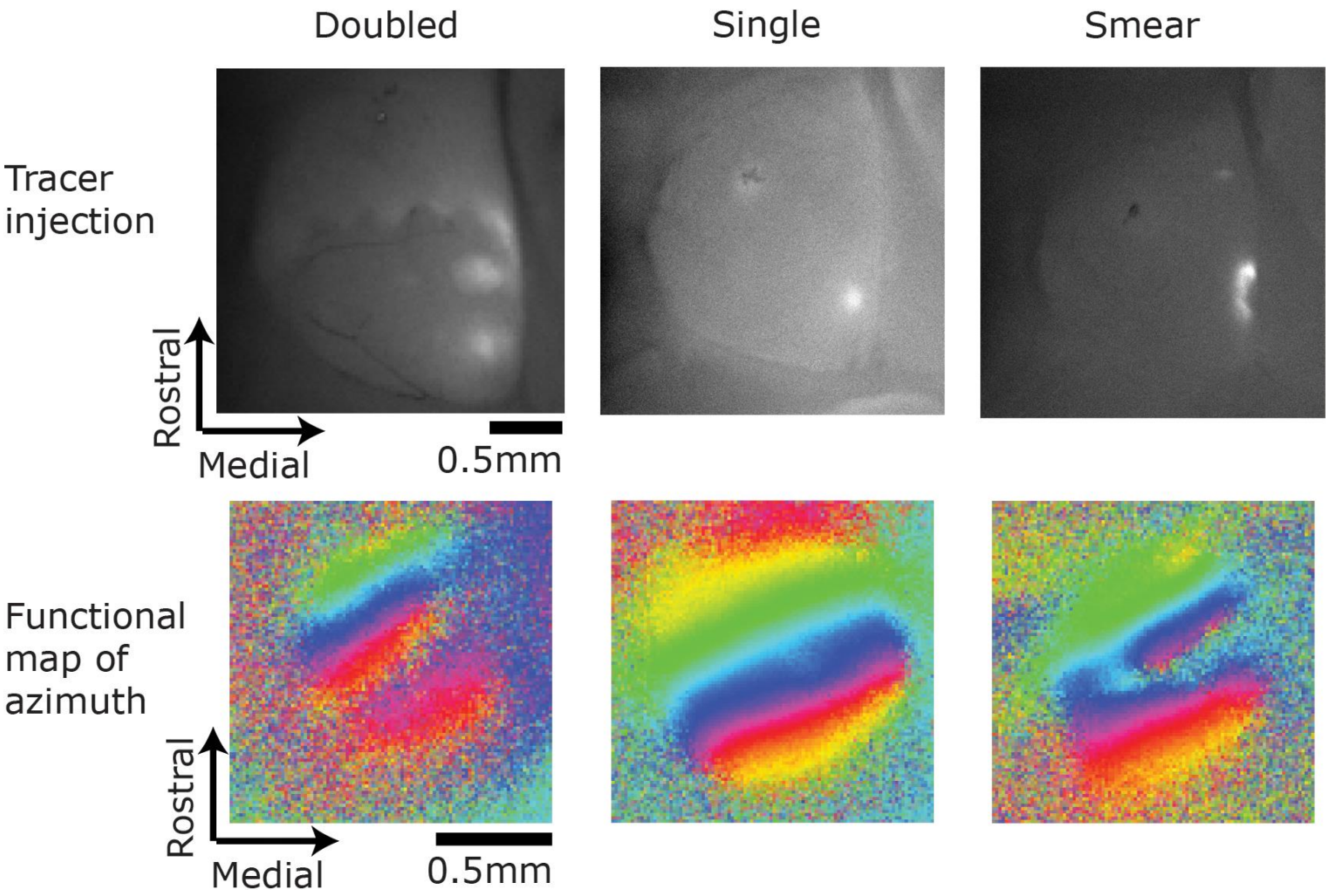
Owens et at, 2009

Variability in maps of heterozygous mice is not the result of genetic differences between individuals

- We imaged both left and right SCs in single animals



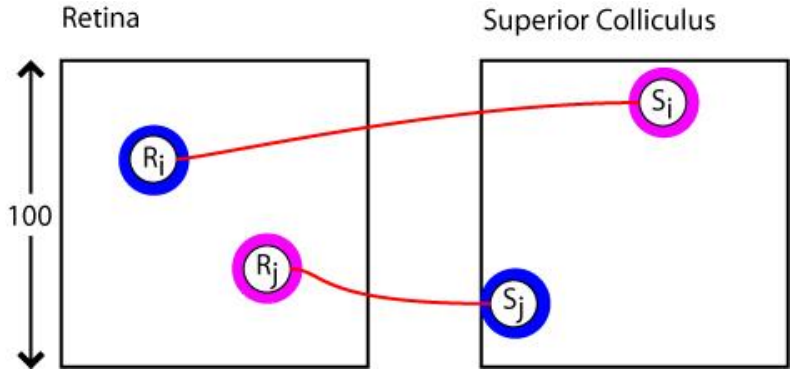
In heterozygous mice, individual anatomy is consistent with functional maps



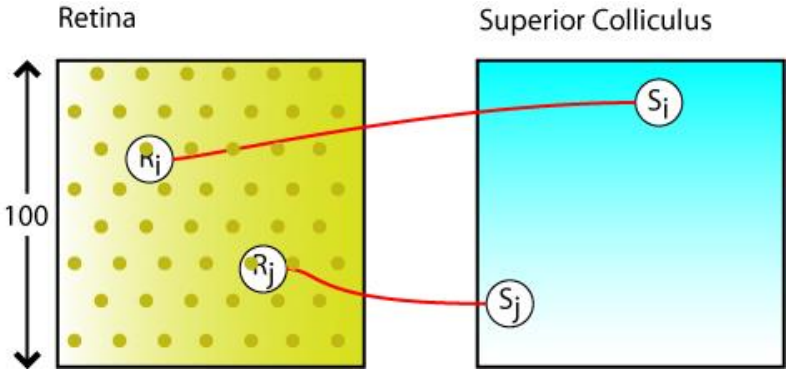
Work done with Jason Triplett, UCSC

The Koulakov model for the *isl2-EphA3* knockin mouse

A: Patterned neural activity



B: EphA/ephrin-A gradients



- $Isl2^+$ RGCs

Probability of inputs switching places is based on two factors:

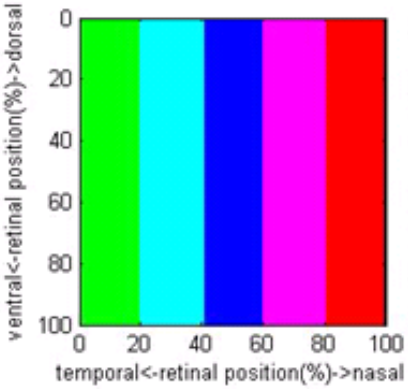
- A. Patterned neural activity: P↑ if switching puts S_i and S_j near SC cells that receive input from RGCs near R_j and R_i
- B. EphAs: Each cell in R is given an EphA value; each cell in S is given an ephrin-A value. P ↑ if switching would make the EphA values of R_i and R_j not correspond to the correct ephrin-A values of S_i and S_j

  To model *Isl2-EphA3* knock-in, a value ΔR is added to the EphA value of alternating cells in the retina.

Koulakov model recapitulates map heterogeneity in heterozygous mice

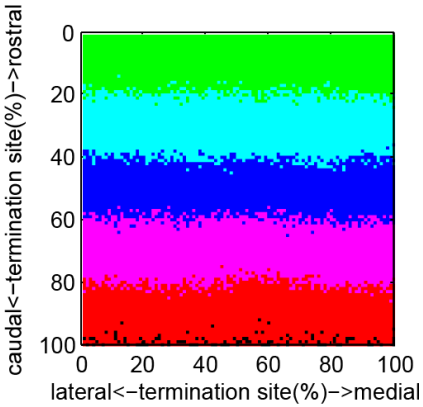
Retina

Azimuth

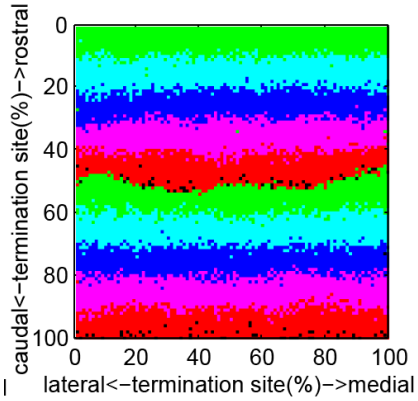


Superior Colliculus

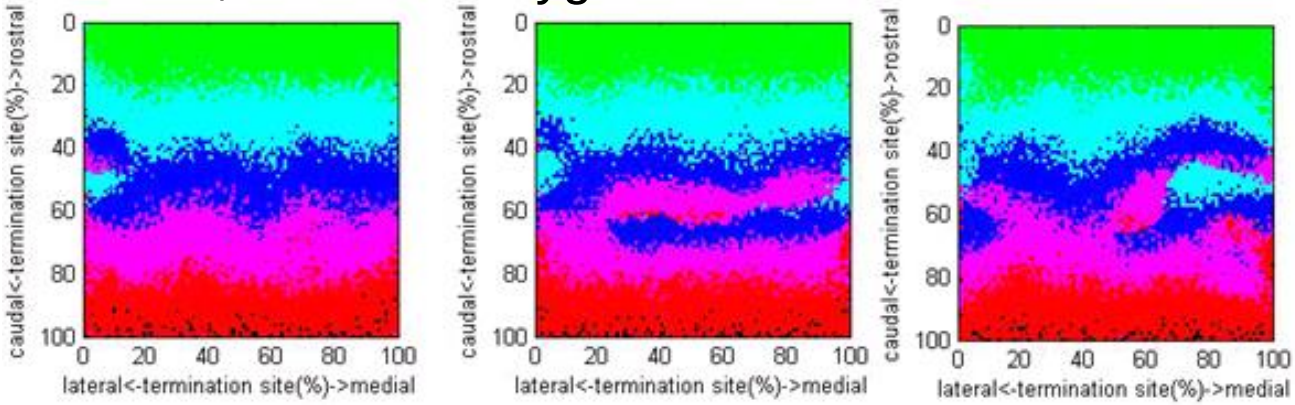
ΔR=0; WT-like



ΔR=0.70; Like homozygous mouse

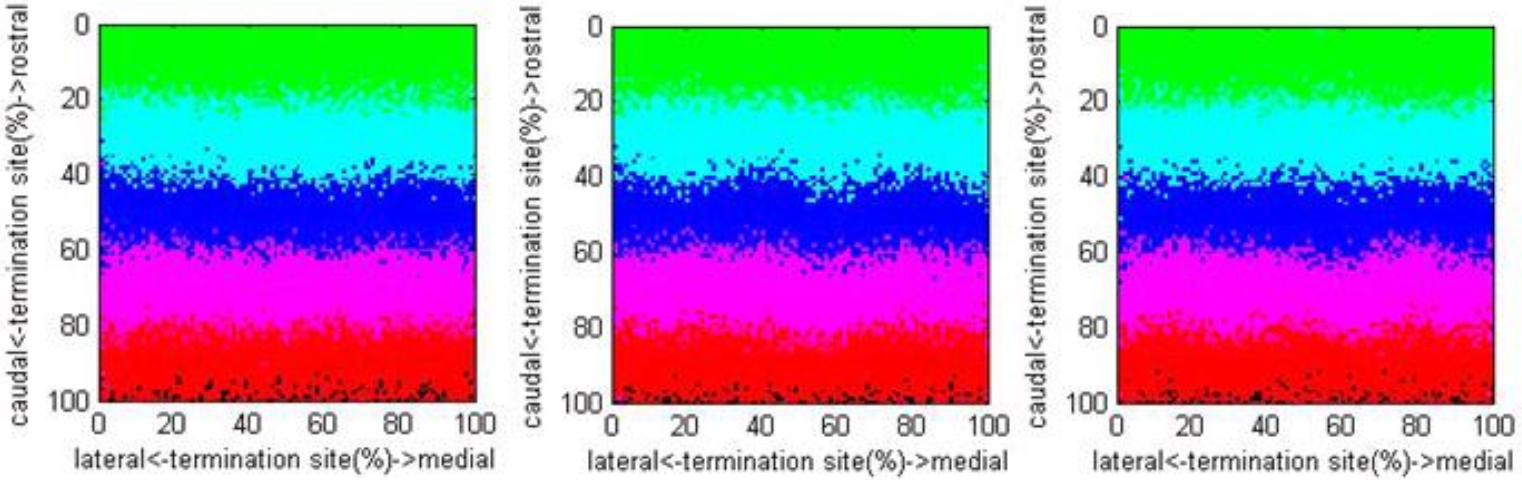


ΔR=0.35; Like heterozygous mouse

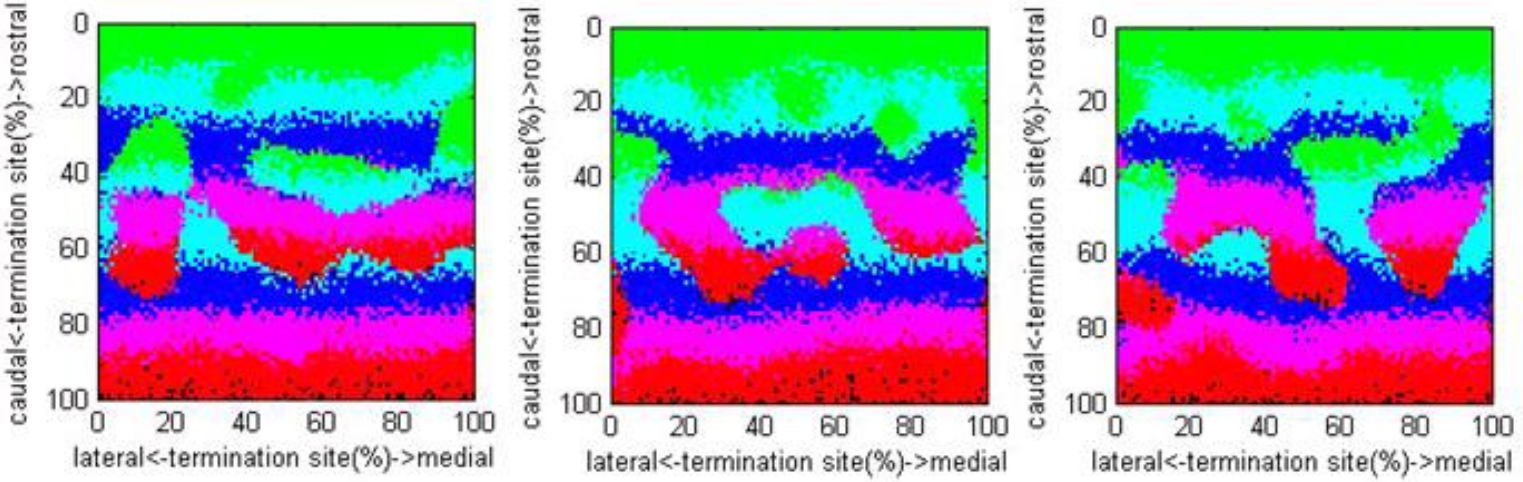


Heterozygous SC is at a critical point of instability

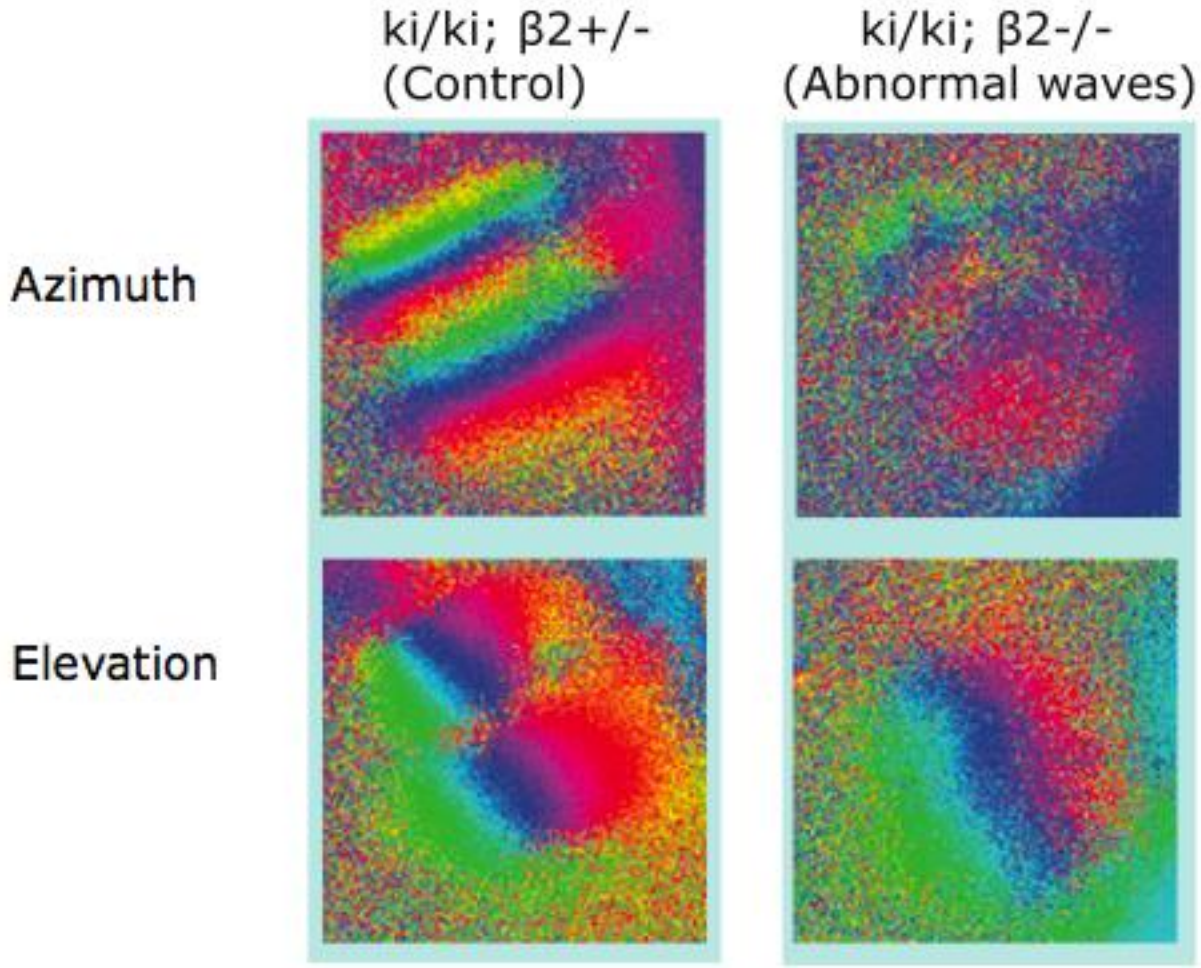
ΔR=0.30;
14% decrease
in EphA3



ΔR=0.40;
14% increase
in EphA3



Coherence of duplicated SC maps requires structured activity and fails in combination EphA3$^{ki/ki}$; $\beta2^{-/-}$ mice



Owens et at, 2009

Findings

- Maps in the heterozygous *Isl2-EphA3* knock-in mouse are heterogeneous.
- Heterogeneity is not dependent on individual genetic differences.
- It is not a result of functional suppression: an individual's functional map is consistent with its anatomy.
- It is a consequence of the exact amount of EphA3 over-expression.
- Depends on retinal waves.

Conclusions so far from EphA3$^{ki/ki}$ mice

Doubling the expression of an EphA, even one that is not normally in RGCs, alters the molecular gradient sensed in the SC. The Isl2+ and Isl2- RGCs make separate maps in the SC.

Neural activity organized these maps and makes them fully isotropic and coherent. This requires changing the normal representation of elevation, creating a discontinuity in elevation, just as there is a discontinuity in azimuth, between the two separate, coherent maps in anterior and posterior SC.

In the heterozygous EphA3$^{ki/+}$ mice, the influence of the molecular gradient on map formation is smaller and is almost perfectly matched in strength to the influence of activity-dependent coherence and isotropy. The result is variable--sometimes a duplicated map, sometimes a single map, and sometimes both in the same animal. All such arrangements are continuous and coherent.

How do brain areas connect with one another?

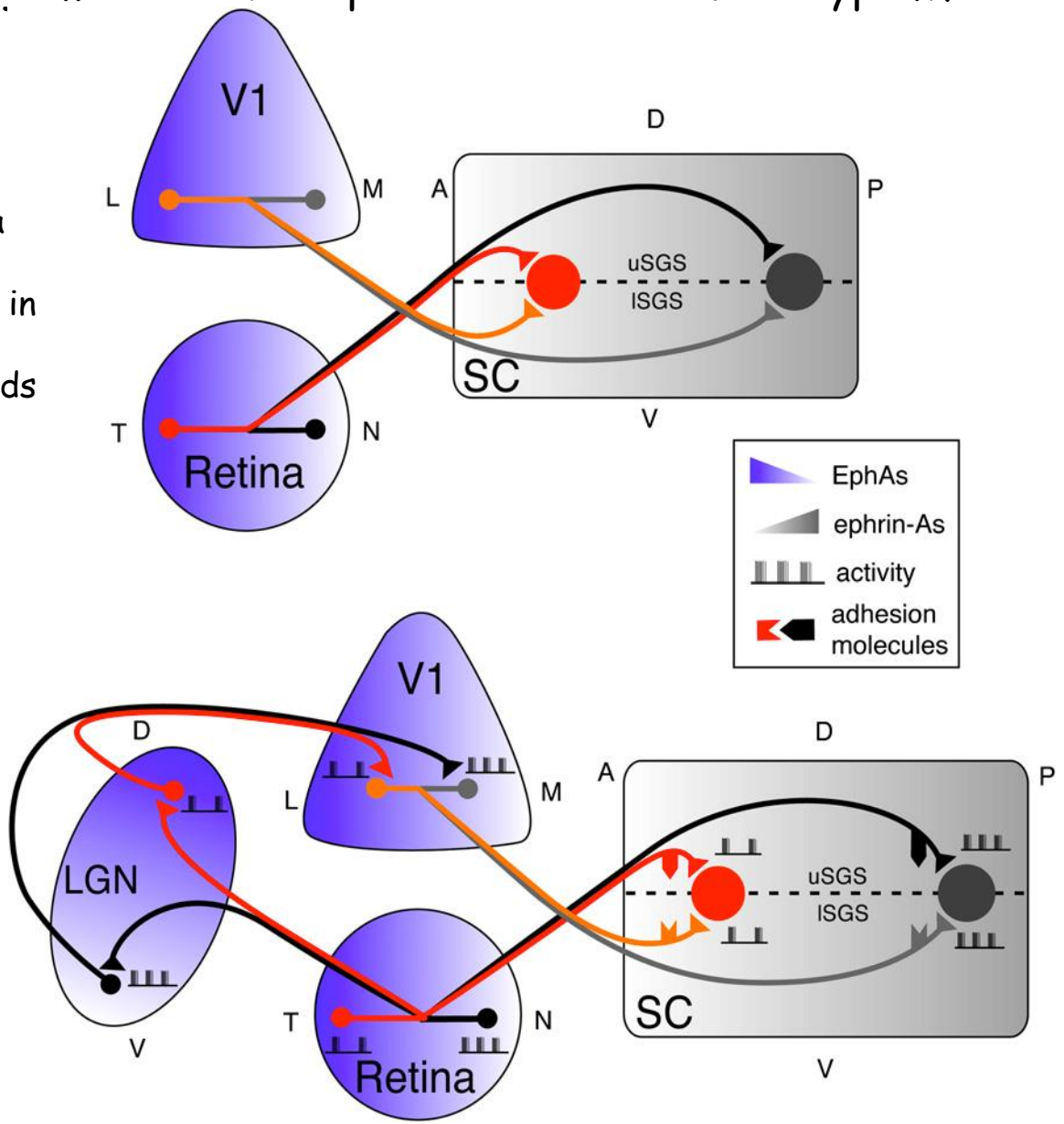
Models of Visual Map Alignment in the Superior Colliculus Wild-Type Mouse

Gradient-Matching Model:

Graded expression of EphA receptors (blue) in both the retina and primary visual cortex (V1) are used to guide topographic mapping in the superior colliculus (SC), which expresses repulsive ephrin-A ligands (gray) in a gradient in both retinal and cortical recipient layers.

Retinal-Matching Model:

Retinocollicular mapping is established first through the use of graded EphAs and ephrin-As. Then, V1 projection neurons terminate in areas with similar activity patterns or with RGCs expressing complementary cell surface molecules.

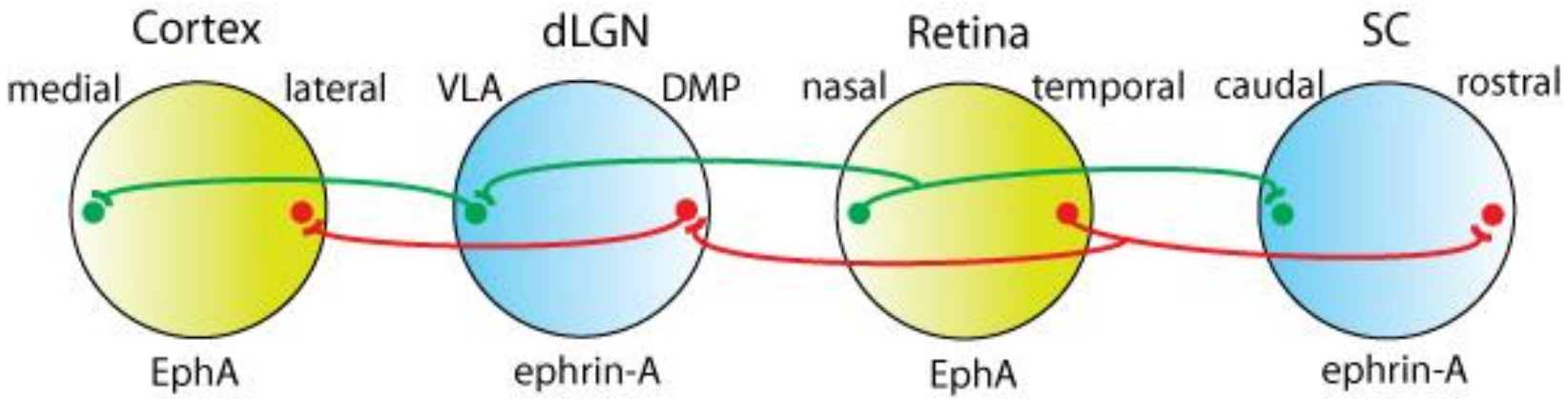


Triplett et at, 2009

These models are hard to distinguish from one another because EphA gradient in Visual Cortex is like that in Retina

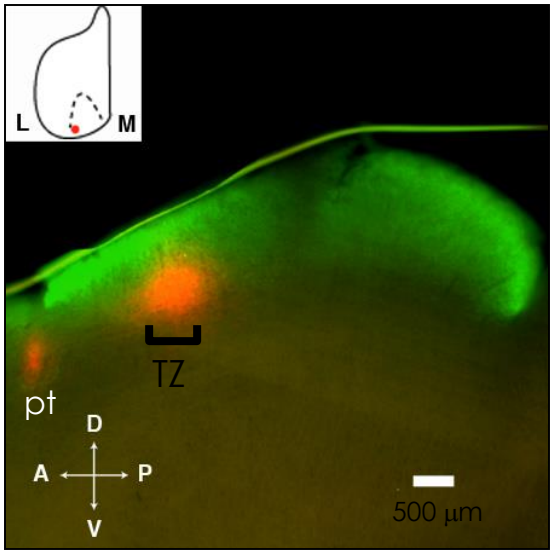
Axon guidance molecules: Eph/Ephrin-As

- Are membrane molecules that bind to each other and signal bidirectionally and repulsively
- All EphAs bind all ephrin-As and vice-versa
- Matching gradients of Eph/ephrin-As in visual areas

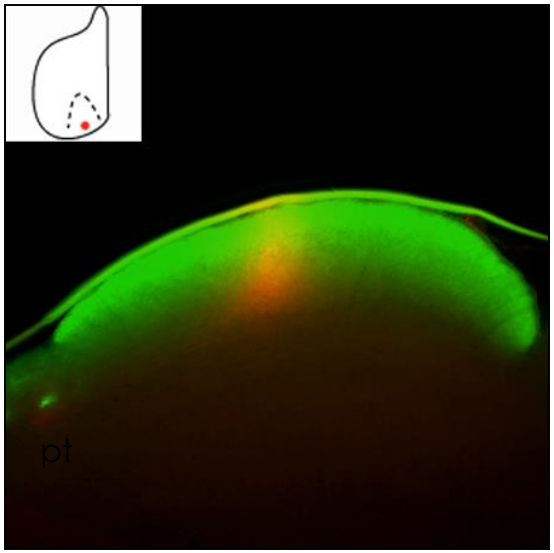


Anatomical tracing shows that V1→SC Corticotectal projection is topographic in WT mice

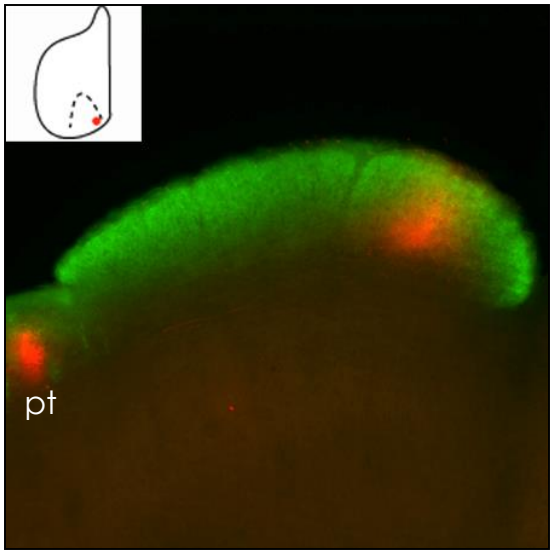
Lateral V1



Central V1



Medial V1



Green = CTB-488 labels entire retinal projection from Contralateral Eye

Red = DiI after focal injection into visual cortex Termination Zone in SC

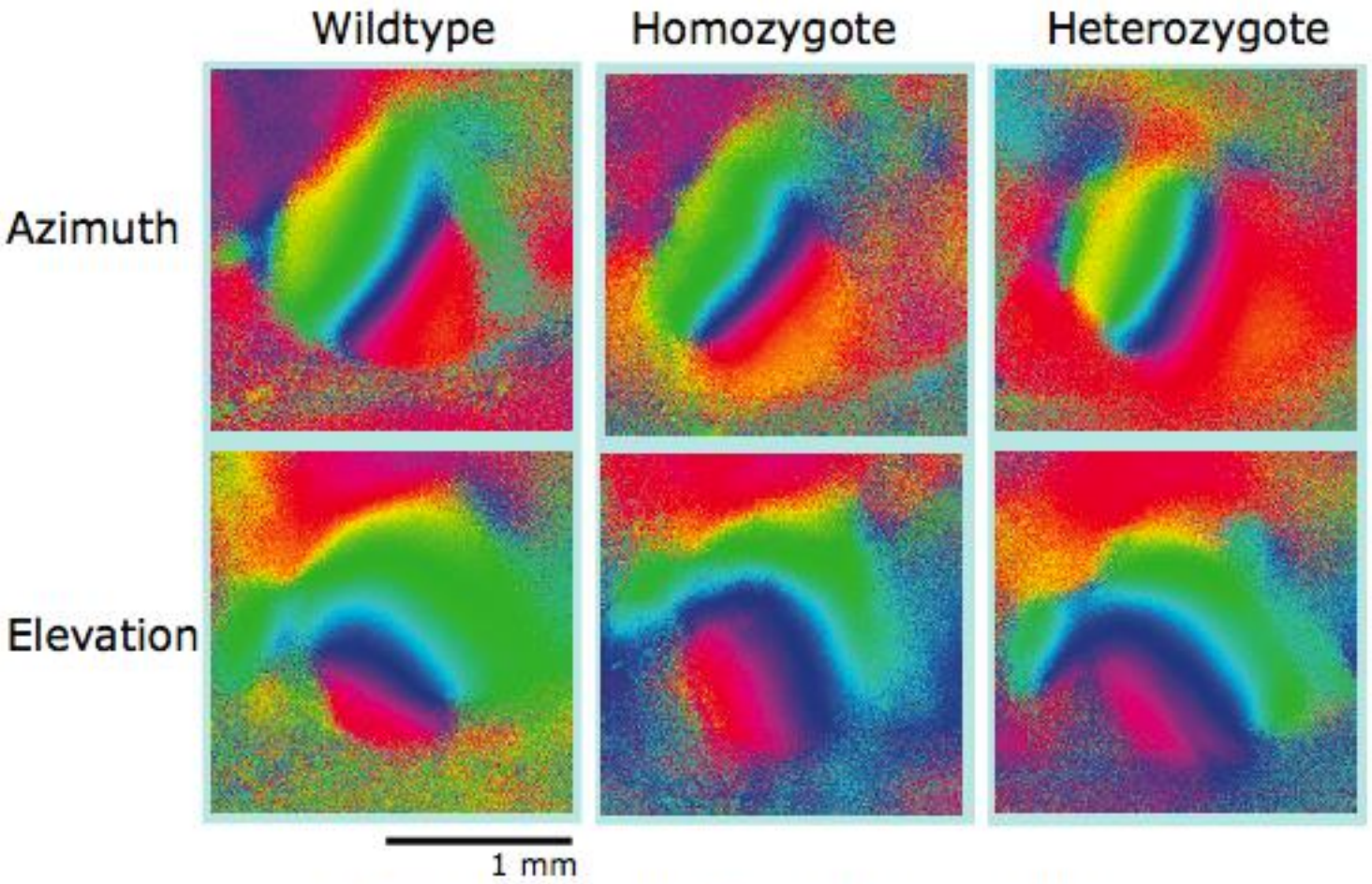
TZ = terminal zone in SC of cortical input

Pt= pretectum (also receives cortical input)

Triplett et at, 2009

EphA3$^{ki/ki}$ mice have a single map in the visual cortex, despite their duplicated map in SC

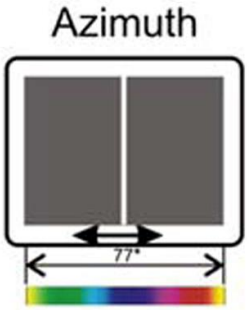
Maps of Visual Cortex

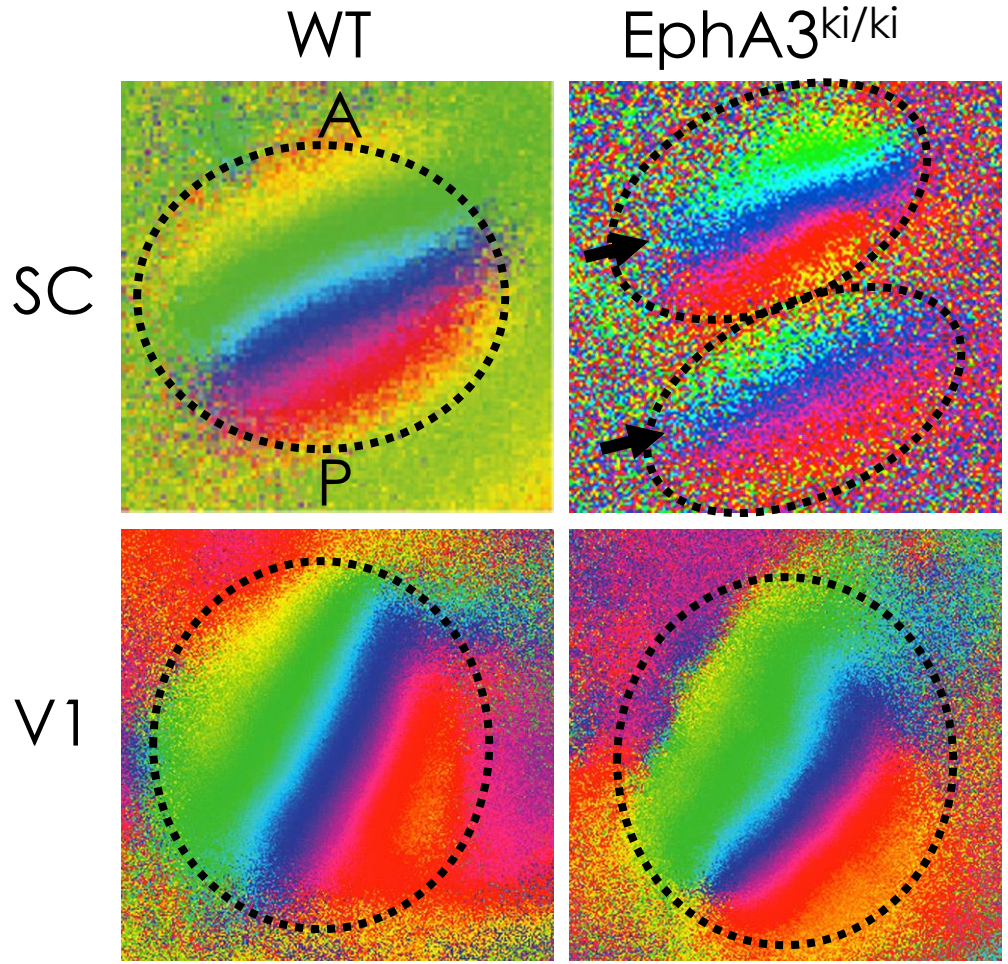


(n=22 of 22 for ki/ki, 14 of 14 for wt/ki)

Owens et at, 2009

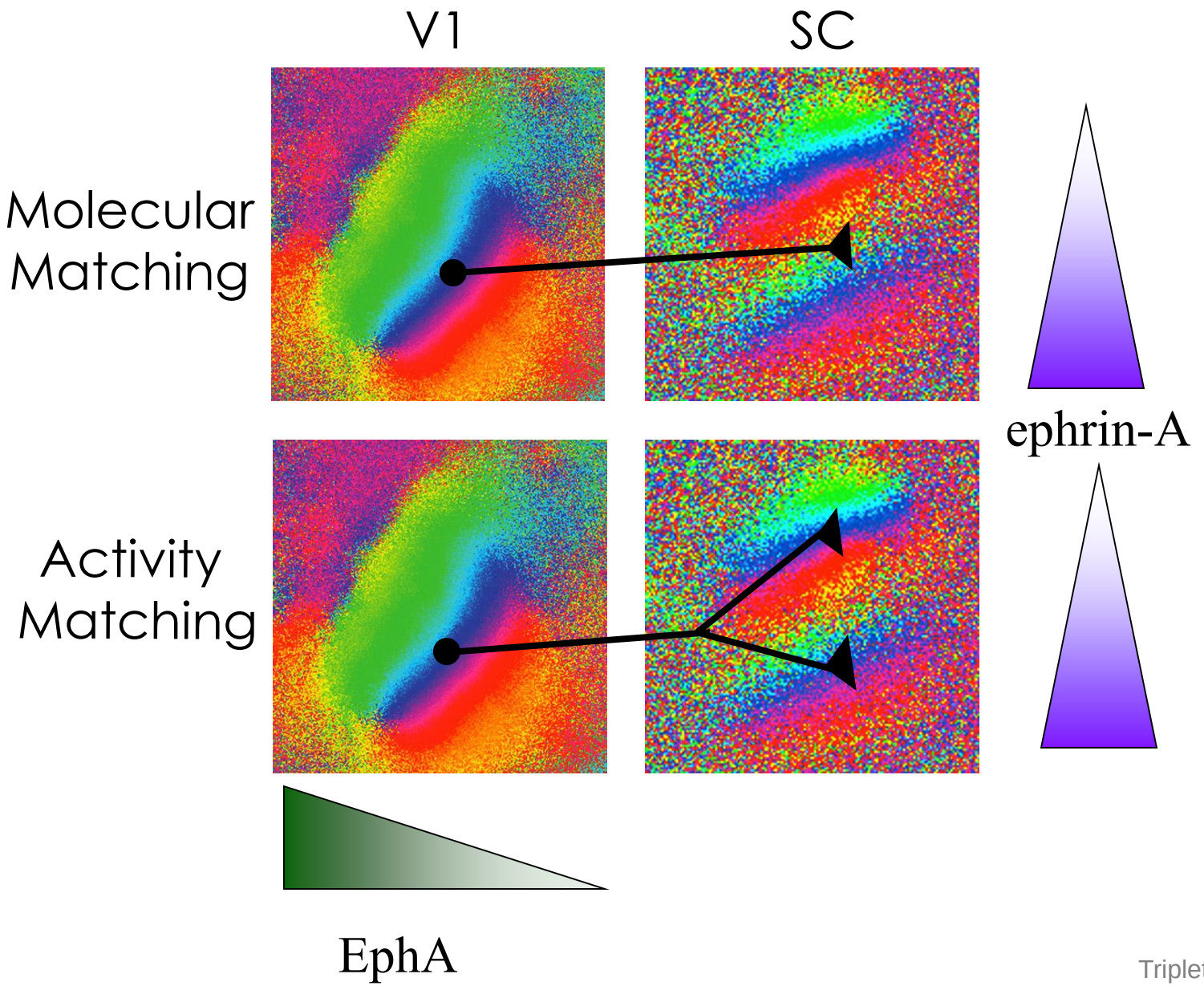
EphA3$^{ki/ki}$ have a duplicated map in SC, and a single map in V1. How do they connect? Experiment to test the rules for connections.





Jianhua Cang, Melinda Owens

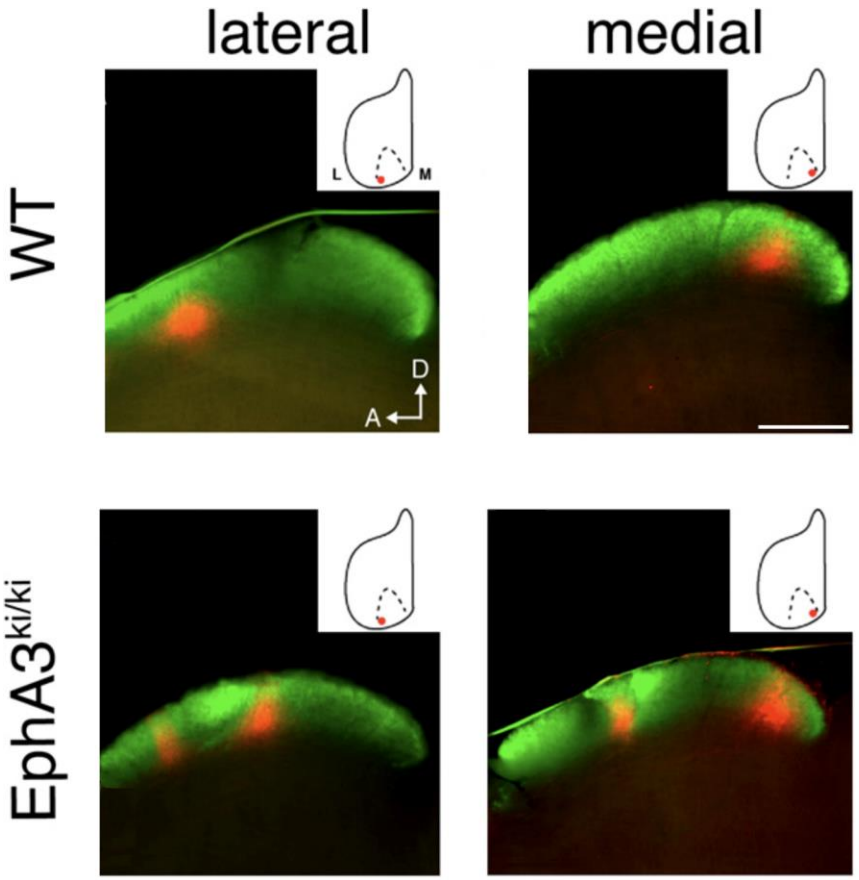
Possible outcomes in EphA3$^{ki/ki}$

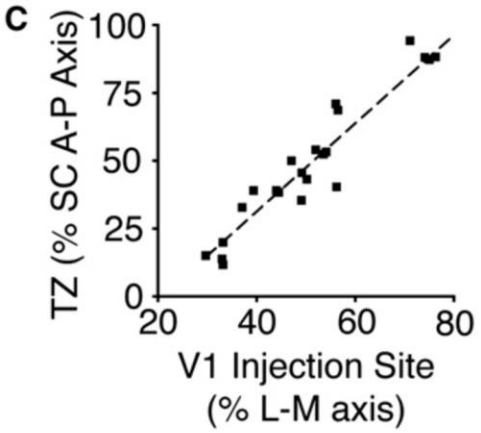


Triplett et at, 2009

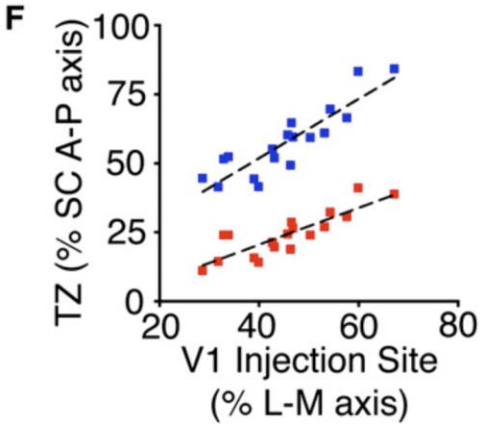
V1→SC Projections Form Two Termination Zones in EphA3$^{ki/ki}$ Mice

Tracer injection site in V1





One map from V1 to SC in WT mice.



Two maps from V1 to SC in EphA3$^{ki/ki}$ Mice, one to anterior SC and the other to posterior SC

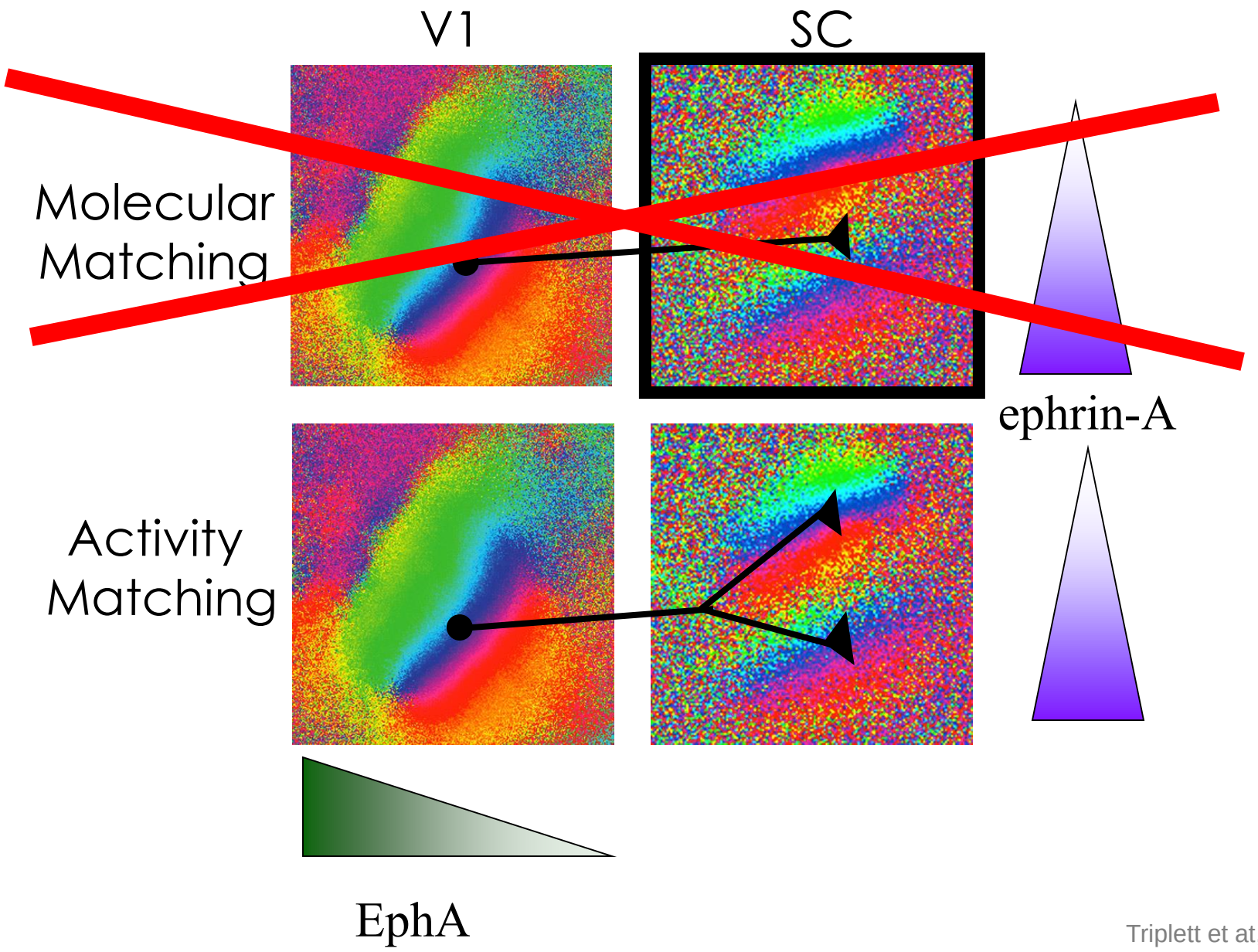
Above: Below:

Red = DiI from cortical tracer injection

Green = CTB-488 from contralateral retina

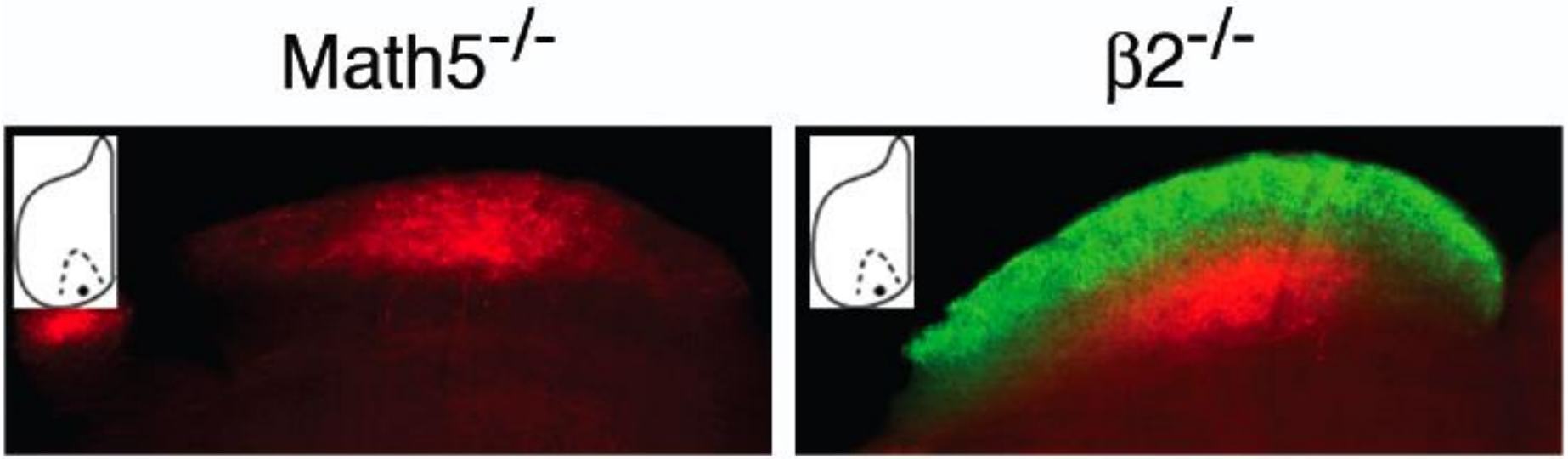
Triplett et at, 2009

Possible outcomes in EphA3$^{ki/ki}$



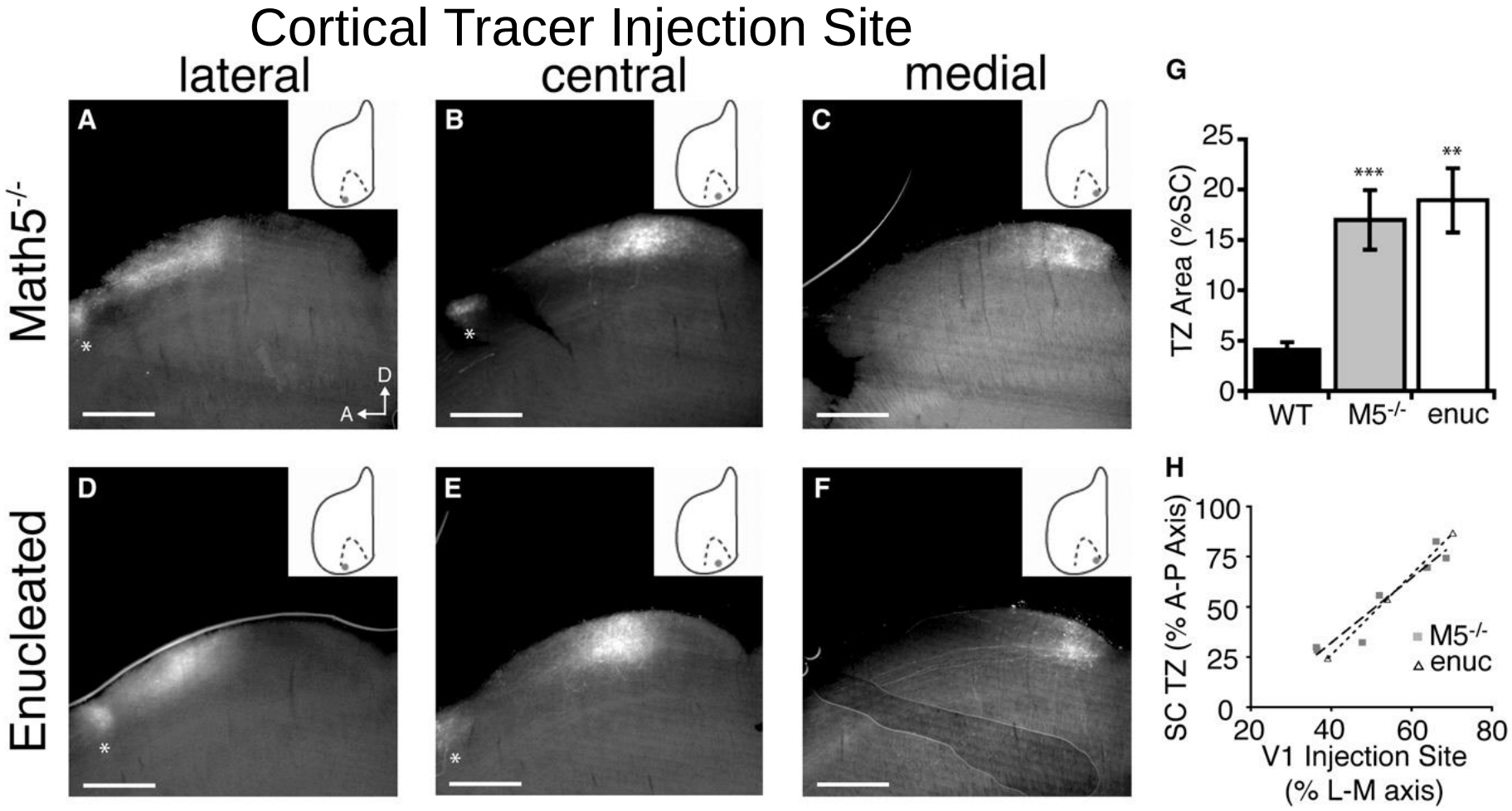
Triplett et at, 2009

Precise topography of the V1-SC projection requires retinal input and structured retinal activity.



Triplett et at, 2009

The Corticocollicular Projection has Normal Order but is Much Less Precise When Retinal Input is Degraded or Absent

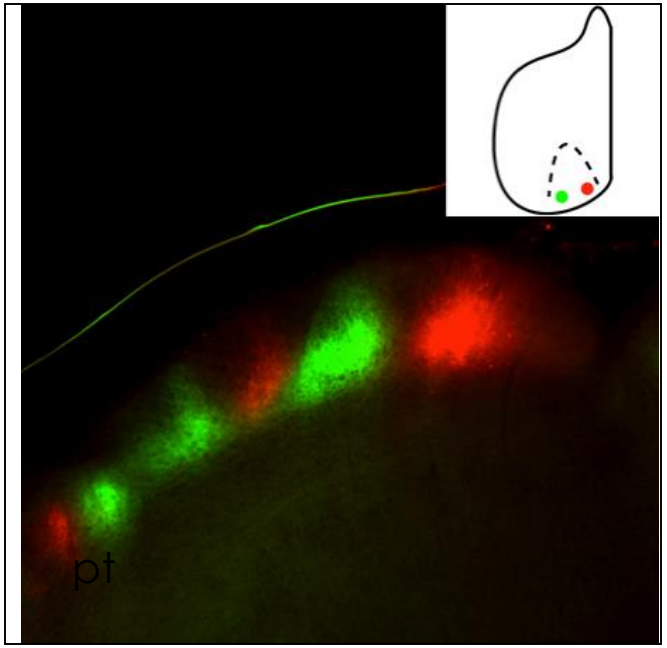


Cortical termination zones in SC are bigger but generally topographic when retinal input is severely reduced by removing most RGCs in Math5 knockout or eliminated by enucleation.
(*=pretectum, not SC)

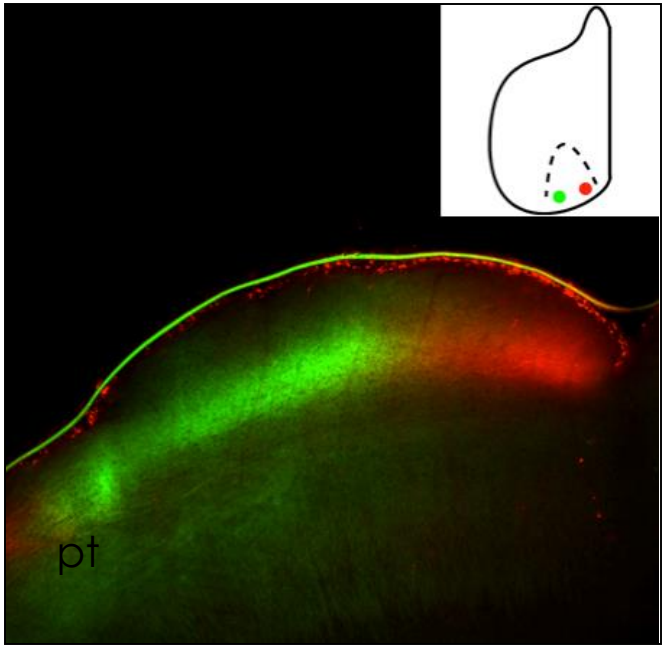
Triplett et at, 2009

Disruption of retinal waves in EphA3$^{ki/ki}$ prevents duplication of corticotectal projection

EphA3$^{ki/ki}$

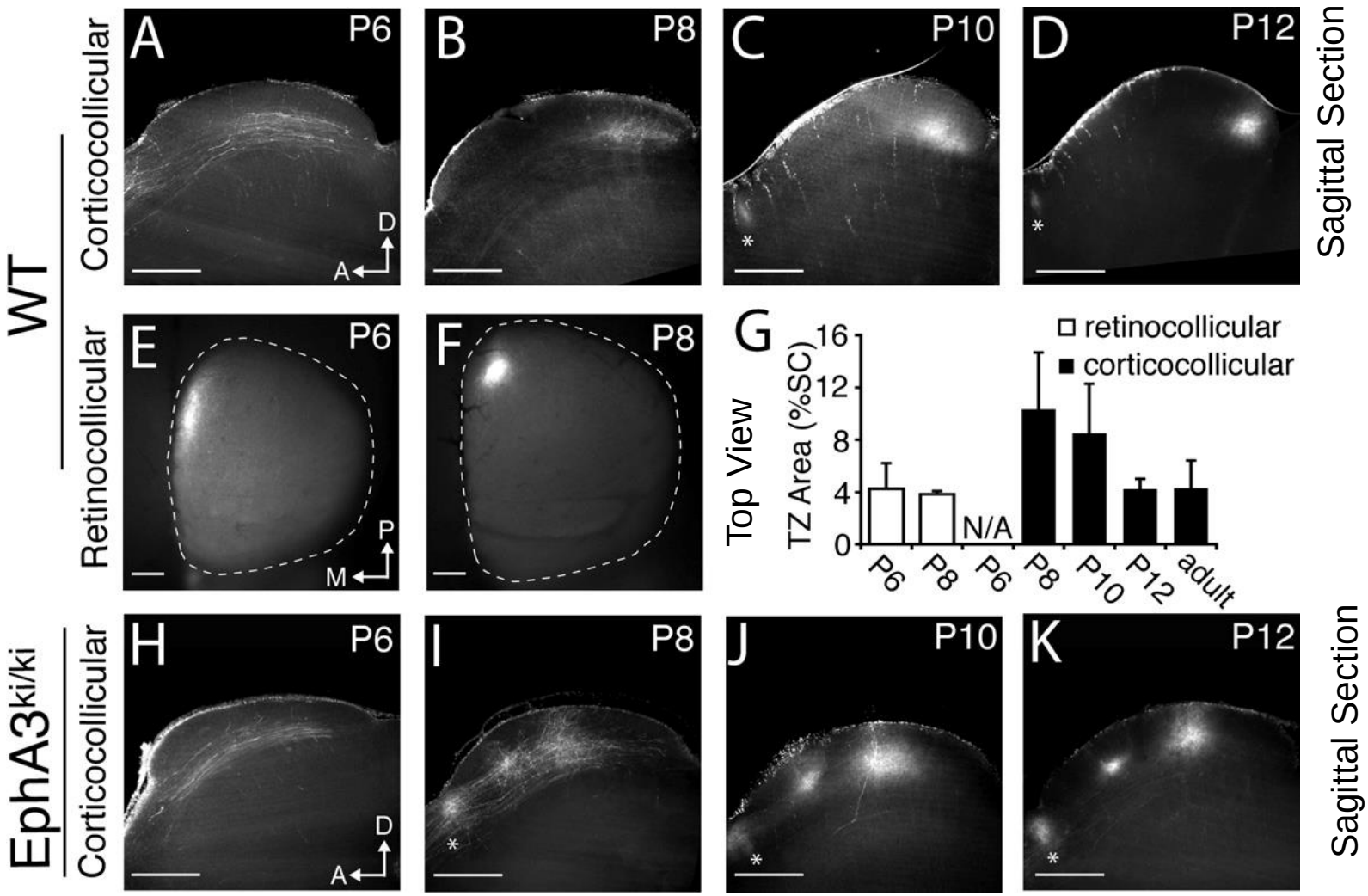


EphA3$^{ki/ki}$/β2$^{-/-}$



Triplett et at, 2009

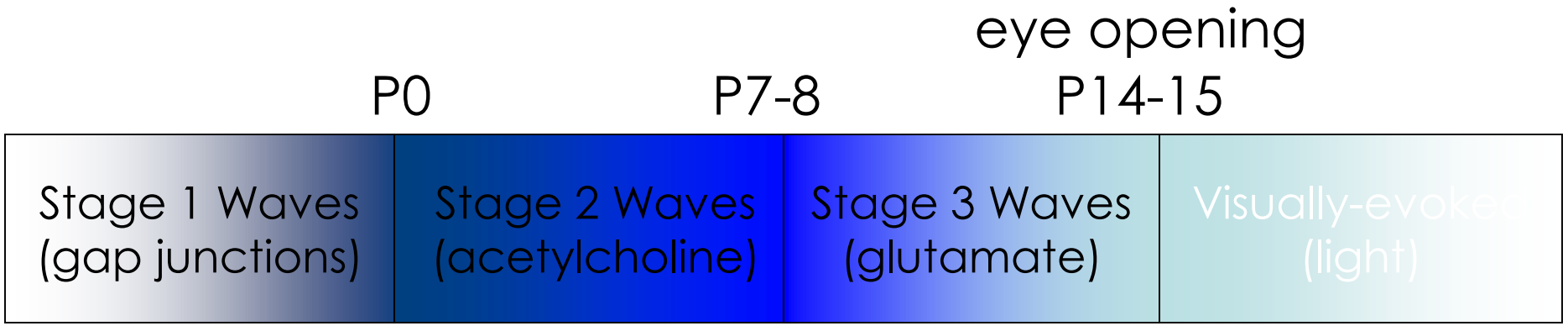
Retinotectal map forms first, and is perfectly precise by P8.
Corticotectal map refines only after retinal map is established.



Time Course of V1-SC Mapping Similar in WT and EphA3$^{ki/ki}$ Mice

Triplett et at, 2009

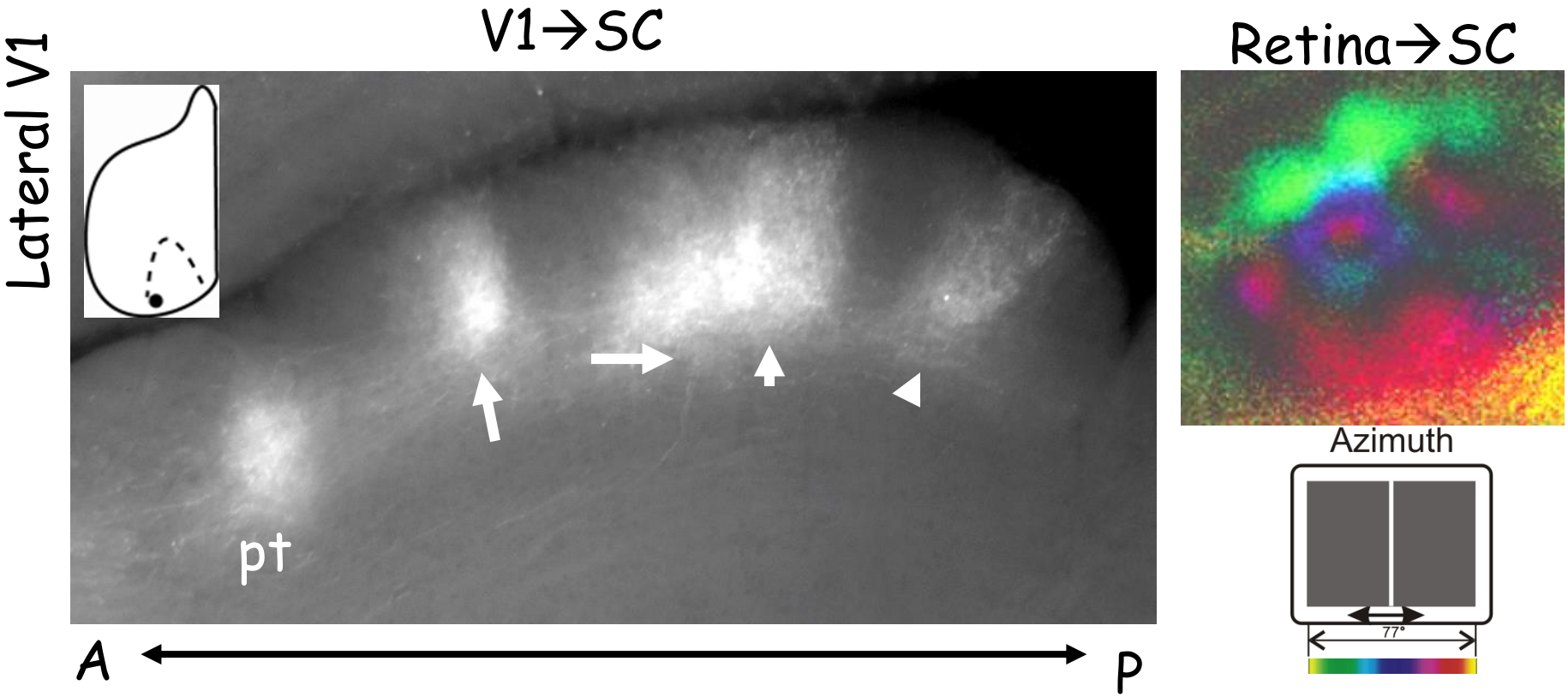
Corticotectal refinement in WT mice occurs after retinotectal refinement but during spontaneous retinal waves



Huberman, *Ann Rev Neurosci*, 2008

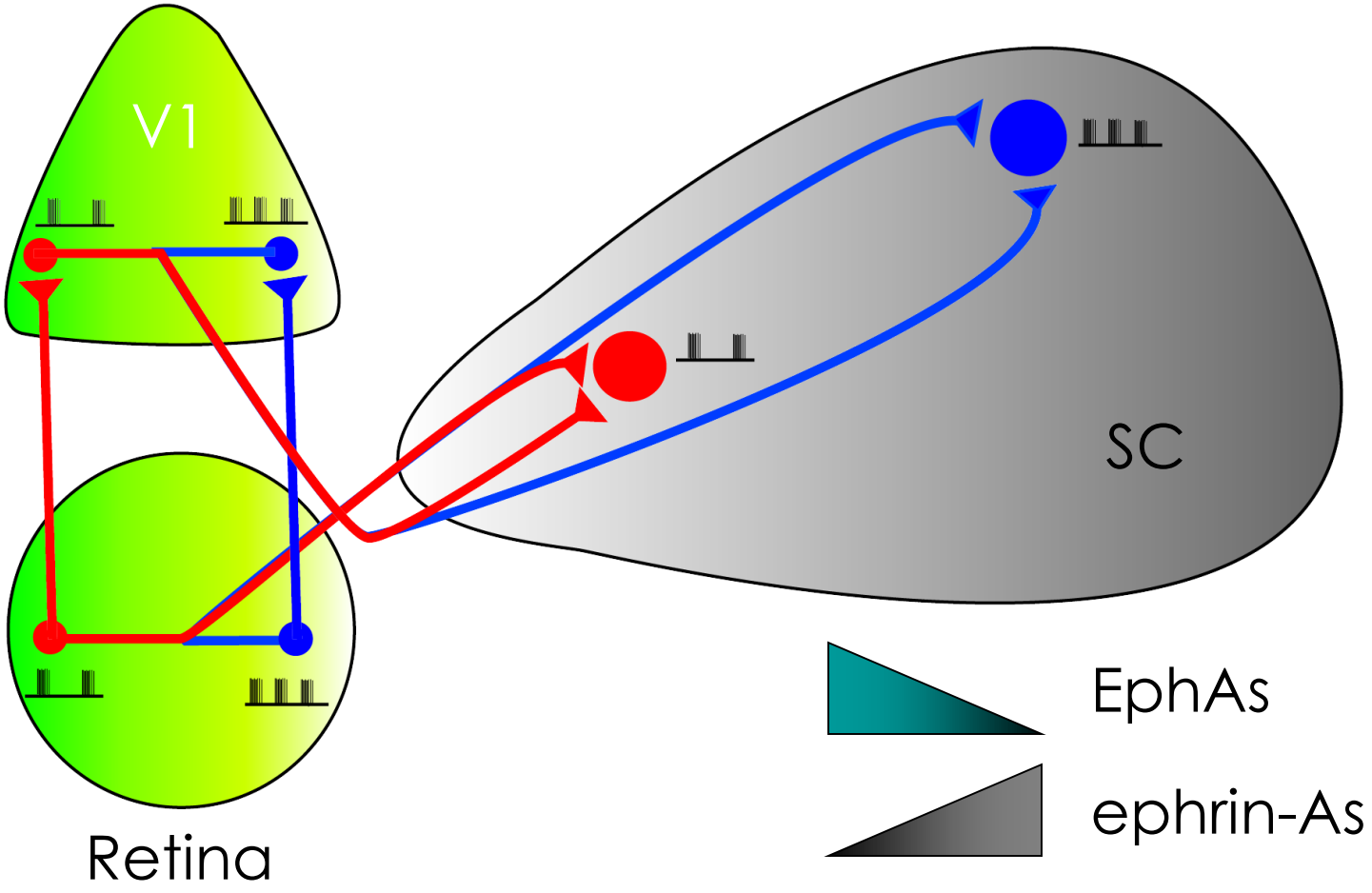
Triplett et at, 2009

V1->SC projection in TKO mice also matches expected etina->SC topography with "patchy" reiterated maps, confirming retinal matching hypothesis.



Cang et al, 2008; Triplett et al, 2009; Owens et al 2009;

Final Model: The retinotectal map serves as a template and waves provide signal for alignment



Triplett et at, 2009

Conclusion about connections between maps:

The corticotectal map aligns with the map or maps made by the retinotectal projection when there is structured neuronal activity that correlates the activity of neighboring retinal ganglion cells. This alignment may override the corticotectal EphA/ephrin-A gradients.

With structured neuronal activity, the retinotectal map can bifurcate (or worse) in the presence of altered ephrin-As, but it remains isotropic and coherent. Without structured activity, the maps fail to refine.

These results, together with Knudsen laboratory findings on alignment of auditory and visual maps in owl SC, provide strong evidence that correlated activity aligns and refines connections between different brain areas.

General Conclusions

For many years, neuroscience believed that map formation in the brain relied on molecular gradients and was refined by neural activity.

Now we know the identity of the molecular gradients responsible for the azimuth map. We also know the timing and character of the necessary neural activity.

We see that the interaction of these molecular signals with neural activity can have different outcomes depending on the relative timing and strength of these interactions, as well as on intrinsic factors regulating the numbers of connections that cells will keep (as in the LGN).

We also see that matching of neural activity is a general strategy that can account for connections among many maps in the brain.

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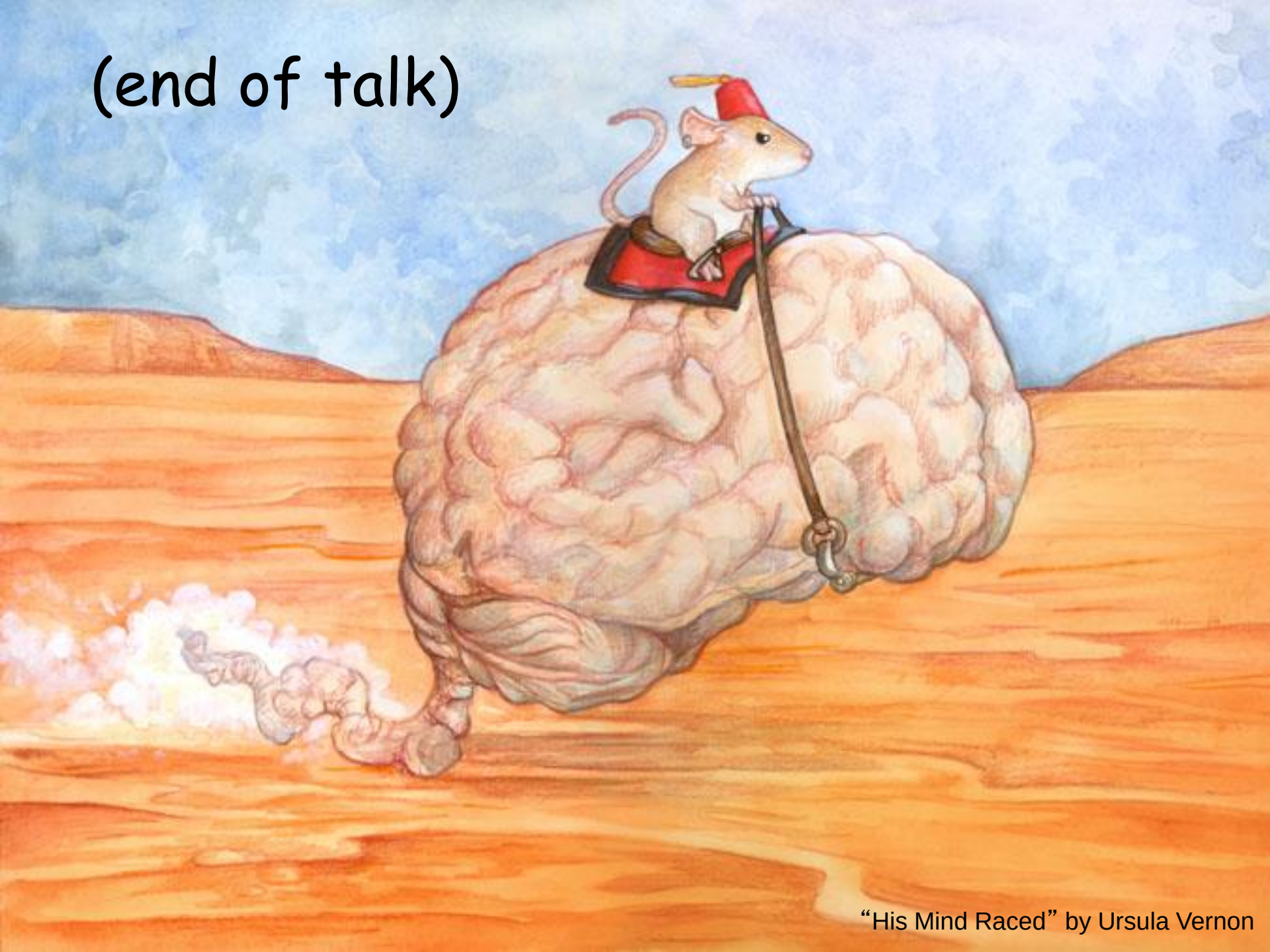
(end of talk)



“His Mind Raced” by Ursula Vernon

Bibliography for Part 2

Reviews: Cang J and Feldheim DA (2013) . Developmental mechanisms of topographic map formation and alignment.Annu Rev Neurosci. 36:51-77, and Feldheim DA and O'Leary DD. (2010) Visual map development: bidirectional signaling, bifunctional guidance molecules, and competition. Cold Spring Harb Perspect Biol. 2: a001768 PMC2964178

Owens, M.T., Feldheim, D.A., Stryker, M.P. and Triplett, J.W. (2015) Stochastic interaction between neural activity and molecular cues in the formation of topographic maps. Neuron 87: 1261–1273. PMC4583656

Triplett, J.W., Owens, M.T., Yamada, J., Lemke, G., Cang, J., Stryker, M.P. and Feldheim, D.A. (2009) Retinal input instructs alignment of visual topographic maps. Cell 139: 175-185. PMC2814139

Cang, J., Wang, L., Stryker, M.P. and Feldheim, D.A. (2008) Roles of ephrin-As and structured activity in the development of functional maps in the superior colliculus. J. Neurosci. 28: 11015-11023. PMC2588436

Cang*, J.C., Niell*, C.M., Liu, X., Pfeiffenberger, C., Feldheim, D.A. and Stryker, M.P., (2008) Selective Disruption of One Cartesian Axis of Cortical Maps and Receptive Fields by Deficiency in Ephrin-As and Structured Activity. (* co-first authors). Neuron 57: 511-523. PMC2413327

Cang, J.C., Renteria, R.C., Kaneko, M.., Liu, X., Copenhagen, D.R. and Stryker, M.P. (2005) Development of precise maps in visual cortex requires patterned spontaneous activity in the retina. Neuron 48: 797-809 PMC2562716

Cang*, J.C., Kaneko*, M., Yamada, J., Woods, G., Stryker, M.P., and Feldheim, D.A. (2005) Ephrin-As Guide the Formation of Functional Maps in the Visual Cortex (* co-first authors). Neuron 48: 577-589. PMC2424263