Development of topographically organized maps and circuits:

Roles of molecular targeting, spontaneous activity, and post-natal experience

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Objectives

Genetics
Spontaneous (peri-natal) activity,
Experience-dependent circuit formation.

- The optic tectum/ superior colliculus
- Frog eye rotation
- Growth cones and axon guidance
- Chemo-affinity hypothesis of Sperry

- Ephrins/Ephs gradients; attraction, repulsion, terminal zone formation
- Role of pre-natal spontaneous activity on map refinement
- How does one fool development? KO, Ki, conditional KO, pharmacology and what are the limitations?
- Different maps (areas) different rules?
- Cell-type specific? Target area selection? Laminar specific segregation? Axonal segregation?
- What is left for post-natal experience?
The primary function of the optic tectum is to localize the stimulus in space and to cause the animal to orient to the stimulus by moving its body and eyes.
Retina-to-tectum projection re-wired

Fig. 21. When the eye is rotated 180°, the frog's prey catching behavior is inverted. (after Sperry, 1956).
Figure 23.4 **Signals in embryo affect growth cones and growing axons (Part 1)**
Chemo-affinity hypothesis – by Roger Sperry-1963

Chemical markers on growing axons are matched with complementary chemical markers on their targets through attraction or repulsion to establish precise connections.

Membrane stripe assay

RGC from nasal retina show no preference for anterior or posterior colliculus
RGC from temporal retina show preference for anterior colliculus

1990s
Discovery of ELF-1 (Eph ligand family 1) and RAGS (repulsive axon guidance signal)

Expressed in low to high gradient in the OT and are ligands of the receptor tyrosine kinase, EphA3 (that is expressed in a high to low temporal=nasal gradient by RGCs).

Ephrin-A2 and Ephrin-A5 are axon repellants and serve as axonal guidance molecules.
Mechanisms of topographic mapping in the vertebrate visual system
Visual areas in the brain are mapped topographically. In the mammalian visual system, photoreceptors in the retina detect photons and transfers this information to multiple visual areas in the brain, including the superior colliculus (SC) in the midbrain and the dorsal lateral geniculate nucleus (dLGN) in the thalamus. The dLGN then transfers this information to the primary visual cortex (V1). Each of the projections is mapped topographically such that neighboring inputs in the visual field (shown as different colors) maintain their relationships as information is transferred, creating a visuotopic map in each processing area.
Stage III waves begin at approximately P10–P12 in mice and ferrets and are mediated by glutamate released from retinal bipolar cells (Bansal et al. 2000, Wong et al. 2000, Zhou & Zhao 2000) (Figure 1). Stage III waves persist until around the time of eye-opening (Bansal et al. 2000, Demas et al. 2003, Syed et al. 2004, Wong et al. 1993).

Fiber optic recordings of calcium-sensitive dyes revealed the presence of propagating calcium waves in the cortex of newborn mice (Adelsberger et al. 2005).

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**Arborization phase:** P0-P3-4 in mice

Molecular guidance cues such as EphA/ephrinA signaling

**Dynamic phase:** P3-4 to P8-9

Remodeling of retino-collricular map by activity dependent arbor plasticity and competition for collricular resources
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Schematic diagrams of the types of molecular cues that direct wiring specificity in developing visual circuits.

(a) **Graded expression of guidance cues** in axons and in their targets can guide specific patterns of visual connections according to matching of ligand and receptor levels.

(b) **Homophilic adhesion cues** expressed in the axons and dendrites of pre- and postsynaptic neurons can lead to highly specific patterns of connectivity (red cells connect to red cells, green cells to green cells, etc.). Similarly, the expression of adhesion molecules in neurons within one structure (*shown in blue*; schematic of the mouse LGN) and within the specific layer of their targets can induce highly specific patterns of connectivity.

(c) **Adhesive cues expressed among axons arising from a common cell type can segregate these axons into distinct fiber tracts** which can then lead to segregation of their axons within the final target.

(d) **Different adhesion cues expressed at different sites along the dendritic arbor** of an individual postsynaptic neuron can **segregate synaptic inputs arising from different cells/sources at the subcellular level** and thereby impact the receptive field properties of the postsynaptic neuron.
Developmental stages in mapping temporal-nasal retinal axis on anterior-posterior axis of colliculus/tectum in mice and chicks

Initially RGC axons enter the SC at the anterior end and project to the posterior SC **overshooting** their topographically appropriate termination zone (dashed circles).

Then branching occurs from the axon at the topographically specific location followed by **pruning of the posterior axon**.

At later stages the branches are **stabilized**, and **refined to form strong synapses**.

In mice this process starts late in embryogenesis and lasts through the **first week of life** (E, embryonic day post conception; P, postnatal).
Regulation of topographic-specific interstitial branching along anterior–posterior axis of tectum/colliculus: the critical determinant of retinotopic mapping

All models have as recurring theme: graded repellent activity that preferentially affects temporal RGC axons is achieved by **EphA forward signaling**.

EphA is expressed in a high to low temporal (T) to nasal (N) gradient by RGCs, and ephrin-As in a low to high anterior (A) to posterior (P) gradient in the optic tectum (OT)/superior colliculus (SC).

**This receptor-legend pairing** generates through EphA **forward signaling** a **high to low AP gradient of repellent activity** that **preferential affects temporal RGC axons**

This, in principle is sufficient to topographically guide RGC axonal growth cones along the AP axis to their appropriate terminal zone (TZ). This mapping mechanism is **adequate in lower vertebrates**.

However, a single repulsive gradient **cannot generate the topographic interstitial branching** of RGC axons along the AP axis observed in chicks and rodents, because branching would occur at a greater frequency more **anterior** to the TZ.

This is not observed in vivo—branching is focused on the AP position of the TZ and is **lower both anterior and posterior it**.
In principal, topographic-specific interstitial branching could be achieved by a model with a gradient of molecules with branch promoting activities that cooperate with the EphA forward signaling inhibition of branching.

Branching is prevented posterior to the correct TZ by ephrin-A repellent activity mediated by EphA forward signaling, and does not occur anterior to the correct TZ because the branch promoting activity is subthreshold.

Branching occurs at the AP position in which the branch promoter is suprathreshold and the branch inhibitor is subthreshold.

TrkB, in a similar distribution to EphAs in the retina and BDNF in the OT/SC have the appropriate activities to act as the graded branch promoter, and cooperate with the graded branch inhibitor generated by EphA/ephrin-A forward signaling.

(D) topographic-specific interstitial branching in the chick OT and mouse SC. Topographic-specific branching is generated by opposing gradients of branch inhibiting molecules combined with a branch promoting activity.

Branching occurs preferentially at a trough in the opposing repellent gradients that is subthreshold for branch inhibition and is located at the correct AP position of the TZ.

At the position of this trough, the activity of a branch promoter (such as BDNF signaling through TrkB) is suprathreshold for branch inhibition and generates branching at this topographically correct location for the TZ.
Forward and reverse signaling through ephrin-As and Bs

**Forward:** in Eph containing cells—binding to EphA receptors results in activation of GTPase—leading to **growth cone collapse** (also regulation of Tsc2).

**Reverse:** in the ephrin-bearing cells—mediated by co-receptors TrkB and p75NTR—resulting in **axonal branching and axon repulsion**.

**Forward** signaling through EphB—**growth cone attraction or repulsion**.

**Reverse** signaling through eprinBs is important for **synapse maturation**.
**Paradox:** In ephrinA/EphA repulsion model, Cell-cell contact is required for signaling to occur, but contact must be terminated for repulsion to occur. 

**Solutions:** cleavage of the extracellular domain of either protein or Endocytosis of receptor-ligand complex (via GTPase RAC)
Signalling mechanisms: intracellular signalling pathways

Forward

Eph forward signalling results in phosphorylosine-mediated recruitment and tyrosine phosphorylation of intracellular effector proteins, which regulate the activity of small Rho family GTPases, thus modulating cytoskeletal dynamics.

Reverse

Ephrin A reverse signalling involves interaction with co-receptors that can mediate either attraction or repulsion via as-yet-unknown mechanisms. Ephrin B reverse signalling occurs via phosphotyrosine- and PDZ domain-dependent signalling pathways. In cases where generic Eph/ephrin names are given, multiple family members operate by this mechanism.
Functional imaging of adult SC in wild type and mutant mice. Top panels show that the D-V retinal projection onto the medial-lateral axis of the SC is largely normal in the mice indicated. Bottom panels show that the N-T retinal projections map onto the anterior–posterior axis of the SC. Note that ephrin-A KOs have a patchy map (arrows highlight red regions [nasal axon mapping regions] that are noncontiguous), whereas ephrin-A2/A5/b2 tKOs have no map, specifically along the N-T mapping axis. Amazingly, the EphA3-ki mouse maps are duplicated within the SC (arrows mark regions of duplication). (Adapted from Cang et al. 2005b, 2008b; Triplett et al. 2009.)
Expression patterns of Ephs and ephrins in the retinocollicular system

A) The temporal-nasal (T-N) axis of the retina maps along the anterior-posterior (A-P) axis of the SC/OT. Graded expression of EphA and ephrin-A expression patterns in each structure in the mouse are indicated by blue and red bars, respectively. EphAs are expressed high temporally and anteriorly, while ephrin-As are expressed high nasally and posteriorly in complementary gradients.

B) The dorsal-ventral (D-V) axis of the retina maps along the lateral-medial (L-M) axis of the SC/OT. Graded EphB and ephrin-B expression patterns in each structure are indicated by blue and red bars, respectively. In the retina, EphBs are expressed in a V > D gradient, while ephrin-Bs are expressed in a complementary D > V gradient. In the SC, EphBs are expressed uniformly across the L-M axis, and ephrin-B1 expression is high at the midline, but is steeply reduced laterally.
Structure of the retino-collicular map in EphA/ephrin-A knockout and transgenic mice  (A–B)
In wild type mice, the retina is mapped topographically onto the SC. Temporal RGCs (blue dots) map to anterior SC, while nasal RGCs (red dots) project to posterior SC. Intrinsic optical imaging reveals topography by measuring the visual responses in the SC to a drifting bar along the N-T axis of the retina.

(C–D) Genetic deletion of ephrin-As or EphAs results in aberrant topographic map formation. **Anatomical tracing reveals multiple termination zones along the A-P axis of the SC for both temporal and nasal RGCs**. Functionally, this results in areas of the SC with **topographically inappropriate responses**, with the general polarity of the map intact.

(E–F) Relative levels of EphA expressed are used to map along the A-P axis. In **Islet2-EphA3 knock-in mice**, **half of retinal ganglion cells express exogenous EphA3**, resulting in neighboring cells with drastically different EphA expression levels. This results in a **duplicated** retino-collicular map as assessed by anatomical tracing (E) and functional imaging studies (F).
Retinal Waves: Development of orderly connections depends on activity before visual input!

The development of orderly connections in the mammalian visual system depends on action potentials in the optic nerve fibers, even before the retina receives visual input. In particular, it has been suggested that correlated firing of retinal ganglion cells in the same eye directs the segregation of their synaptic terminals into eye-specific layers within the lateral geniculate nucleus. Such correlations in electrical activity were found by simultaneous recording of the extracellular action potentials of up to 100 ganglion cells in the isolated retina of the newborn ferret and the fetal cat. These neurons fired spikes in nearly synchronous bursts lasting a few seconds and separated by 1 to 2 minutes of silence. Individual bursts consisted of a wave of excitation, several hundred micrometers wide, sweeping across the retina at about 100 micrometers per second. These concerted firing patterns have the appropriate spatial and temporal properties to guide the refinement of connections between the retina and the lateral geniculate nucleus.

**Fig. 5.** Time course of spike activity over the electrode array during a burst covering the time interval from 889 s to 893 s of Fig. 4. Successive frames show the averaged firing rate over successive 0.5-s intervals. Each of 82 neurons is represented with a small dot at its approximate spatial location over the electrode array. The dot area for an electrically active cell is increased proportionally to the averaged firing rate of the neuron during the respective 0.5-s interval.
Disruption in temporal and spatial pattern of retinal waves with nAchR KO
Figure 6. A Hebbian Model of Visual Map Development Recapitulates the Anatomical Phenotype Observed in b2(TG) Mice (A) Schematic of the computational model. RGCs and SC neurons are represented by a one dimensional array of spatially arranged computational units, and retinocollicular synaptic weights develop according to a standard Hebbian rule. (B) Each row in the diagrams displays the afferent connectivity to one SC neuron at the end of a simulation. The size of the boxes indicates the strength of the corresponding synaptic connections, while their color indicates ocularity (red ipsilateral and green contralateral; see scales at bottom).

Large retinal waves result in both eye specific segregation (red or green, not yellow) and refinement of axonal arbors (narrow diagonal bands). Small waves, in contrast, generate robust retinotopic refinement in the monocular zone but result in dramatically impaired eye segregation as well as poor retinotopic refinement in the binocular zone (yellow and broad connectivity patterns).

(C–E) Quantification of simulation results for eye specific segregation in the binocular SC and retinotopic refinement in the monocular and binocular SC. (C) Eye segregation is dramatically degraded by small waves in these simulations. (D) Retinotopic refinement is comparable for small and large waves in the monocular SC. (E) Retinotopic refinement is worse for small waves than large waves in the binocular SC. Eye segregation and retinotopic-refinement indices were averaged over SC neurons.
Interactions between molecular cues and structured retinal activity
In the development of cortical topographic maps


1. Disruption of azimuthal maps in Ephrin-A2/A5/beta2 KO
2. Receptive fields of cortical neurons—enlarged in the azimuthal direction
3. LGN RFs.
4. LGN topography
Figure 1. Cortical Azimuth Maps Are Severely Disrupted in Ephrin-A2A5-β2 Combination KOs

A–C) Cortical azimuth maps of an A2−/−A5−/−β2+/- (A), an A2+/-A5+/-β2−/- (B), and an A2−/-A5−/-β2−/- combination KO (C). The color code used to represent positions of different azimuthal lines on the stimulus monitor is shown to the left of panel (A). **Note that the lack of retinotopic organization in the map of A2−/-A5−/-β2−/- combination KO.**

(D) Quantification of map scatters for the azimuth maps of these genotypes. (E–H) Elevation maps of the same three mice and quantification of their map scatter. The color code is shown to the left. Error bars represent SEM.
Substantial size differences in midbrain targets and distinct mechanisms of map development exhibited by model systems.

(B) In mouse and chick RGC axons enter the OT/SC over a broad LM extent and significantly **overshoot** their future termination zone (TZ, circle).

**Interstitial branches form** de novo from the axon shaft around the correct anterior–posterior (A–P) position of their future TZ.

**Branches** are directed along the lateral–medial (L–M) axis of the OT/SC **toward the position of the future TZ**, in which they arborize in a domain encompassing the forming TZ.

The broad, loose array of arbors is refined to a dense TZ in the topographically appropriate location.

(C) In frogs and fish the RGC **growth cone extends** to the TZ in which it forms a terminal arbor through a process of terminal branching of the growth cone and **backbranching immediately behind the growth cone**. The tectum expands as terminal arborizations elaborate and refine into a mature TZ.
Schematic of (a) the mature retinotopic map in the SC, (b) the immature unrefined retinotopic map in the SC (c) Disruptions in retinotopic mapping in ephrin-A2/5−/− or EphA5−/− mice. Multiple dense termination zones are observed along the N-T axis of the target.

(d) Disruptions in retinotopic mapping in EphB2/3−/− mice or in response to disrupting Wnt/ryk signaling; RGC terminals shift more medially (ryk disruption; or shift more laterally (EphB2/3 knockout;)

(e) The overall retinotopic map forms when stage II retinal waves are eliminated (because ephrin-A2/5 signaling is still intact), but RGC axons fail to form dense terminal arbors in their correct topographic locations and are abnormally broad.

(f) When stage II waves are prevented in ephrin-A2/5−/− mice, N-T mapping of RGC projections is abolished.
Fig. 4. Schematic representations of the eye-specific projection patterns to the LGN of the ferret (panels a–j) and the mouse (panels k–t) during normal development (b, l) The early pre-refined pattern of RGC inputs to the LGN in the newborn ferret (b) and mouse (l). Red areas of the LGN correspond to territory occupied by RGC axons arising from the right (red) eye, and green areas correspond the territory occupied by RGC axons from the left (green) eye. Yellow corresponds to the LGN territory where red and green axons from the two eyes overlap.

The asterisk above *NP1/2 and *P25 in panel t refers to the fact that the lack of eye-specific segregation observed in the P10 NP1/2−/− mouse changes to a pattern similar to panel p by P25.

By contrast, C1q−/− mice and MHCI−/− mice exhibit defects in eye-specific segregation until at least P25.
Figure 3. Disruption of Geniculo-cortical Map in Ephrin-A2A5-β2 Combination KO (A–D) Retrogradely labeled dLGN neurons of a WT (B), an A2−/−A5−/−β2+/− (C), and an A2−/−A5−/−β2−/− (D). Neurons were labeled by injections of CTB-Alexa 488 (green) and CTB-Alexa 568 (red) at 500 μm apart in V1 along lateromedial axis (A). In all the panels, dotted lines mark the border of dLGN. **Note the overlap between the green and red cells in (D).** (E) Quantification of overlap between the two groups of labeled cells in the dLGN along the azimuth axis. (F–J) Retrograde labeling and quantification for dLGN neurons when the tracers were injected along elevation axis. Error bars represent SEM.
RF structure in V1 from 2 WT vs. 2 triple KO V1 neurons

Figure 4. Single-Unit Recording in Visual Cortex Demonstrates that the Receptive Fields of Cortical Neurons in Combination KOs Are Selectively Enlarged in the Azimuthal Direction (A and B)
Representative receptive fields measured with moving short bars of two WT neurons (A1, 2) and two ephrin-A2A5-β2 combination KO neurons (B1, 2). Axes in degrees of visual space, color represents magnitude of response. (C) Receptive field radii in degrees, by Gaussian fit to sweeping short bar data, for all single units recorded. (D) Average receptive field size in azimuth and elevation from (C). (n = 31 units in control, 23 units combination KOs, from 5 animals each.) Error bars represent SEM.
Figure 1. Models of visual map alignment in the superior colliculus of the wild type mouse
(A) 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 recipient layers. N, nasal; T, temporal; A, anterior; P, posterior; D, dorsal; V, ventral; L, lateral; M, medial; uSGS, upper stratum griseum superficiale; lSGS, lower stratum griseum superficiale.

(B) 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.
Figure 2. EphA3ki/ki Mice Have Duplicated Functional Maps in the SC and a Single functional Map in V1

(A–D) Intrinsic optical imaging signal obtained from V1 (A and B) and SC (C and D) of WT adult mice presented a drifting bar stimulus along the azimuth (A and C) or elevation (B and D) axis. bar, 500 μm.

(E–H) Intrinsic optical imaging signal obtained from V1 (E and F) and SC (G and H) of EphA3ki/ki animals presented a drifting bar stimulus along the azimuth (E and G) or elevation (F and H) axis. bar, 500 μm.
FIGURE 1|Spontaneous retinal waves mediate eye-specific segregation and retinotopic refinement of retinofugal projections. The axons of retinal ganglion cells (RGCs) target the dorsal lateral geniculate nucleus (dLGN) of the thalamus and the superior colliculus (SC). RGC projections from opposite eyes are segregated into ipsilateral and contralateral regions (black/gray oppositions). RGC projections from each retina are retinotopically organized (colored regions). Note that for simplicity we have depicted retinotopic maps only for one eye. Retinotopic maps and eye-specific segregations form for both eyes.
Under Normal conditions, RGCs exhibit high local correlations while activity of the two retinas is minimally correlated. This pattern of activity supports both eye-specific Segregation and retinotopic maps in both the dLGN and SC.

High correlations between retinal waves of opposite retinas induced by optogenetic stimulation is detrimental to eye-specific segregation while retinotopic maps are unaffected (Zhang et al., 2011).

Disruption of local correlations either by an increase in Uncorrelated firing or by abnormally elevated correlations between distant RGCs (global correlations), such as that observed in b2KO and Retb2-cKO mice, is detrimental to retinotopic map formation. Local correlations are sufficient for normal retinotopic maps in Retb2-cKO, Rxb2-cKO and b2(TG) (Xu et al., 2011, 2015; Burbridge et al., 2014).

High global correlations paired with high inter-retina correlations such as that observed in the b2KO mouse, is detrimental to both eye-specific segregation and retinotopic maps (Xu et al., 2011; Burbridge et al., 2014).
These data demonstrate that normal levels of spontaneous neuronal activity and “small” retinal waves are not sufficient to mediate the segregation of retinal afferents with respect to eye of origin in the dLGN and SC but are sufficient to mediate normal retinotopy (in the absence of binocular competition) throughout the dLGN and SC.

Daily binocular intravitreal injection of CPT-cAMP, a nonhydrolyzable membrane permeable analog of cAMP, beginning at P2 in b2(TG) mice significantly improves eye-specific segregation in both the dLGN and SC.

During the simulation, retinal activity gradually modifies the pattern of retinocollicular connectivity through Hebbian synaptic plasticity rules so that after each retinal wave some of the synapses are potentiated and others are weakened, depending on the size, position and eye of origin of the wave.

In simulations with large retinal waves (WT mice), inputs from the two eyes segregate so that neurons in the binocular SC become responsive to input from only one eye (Figure 6B). Large waves also induce retinotopic refinement of retinocollicular projections, both in the monocular and binocular SC, by strengthening retinotopically correct projections and weakening spatially inappropriate ones.

Simulations with small retinal waves reproduce both the monocular and binocular mapping phenotype of b2(TG) mice. In the monocular SC (or throughout the SC in one-eye enucleated animals), small-wave simulations result in retinotopic refinement, but in the binocular SC, both eye segregation and retinotopic refinement are impaired.

Afferents from the two eyes still compete in the “small-wave” scenario, but competition in this case does a poor job distinguishing between afferents from the two eyes, resulting in degraded eye-specific segregation.
Figure 6. A Hebbian Model of Visual Map Development Recapitulates the Anatomical Phenotype Observed in b2(TG) Mice (A) Schematic of the computational model. RGCs and SC neurons are represented by a one dimensional array of spatially arranged computational units, and retinocollicular synaptic weights develop according to a standard Hebbian rule. (B) Each row in the diagrams displays the afferent connectivity to one SC neuron at the end of a simulation. The size of the boxes indicates the strength of the corresponding synaptic connections, while their color indicates ocularity (red ipsilateral and green contralateral; see scales at bottom).

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Neuronal activity may be permissive for brain development, acting in a passive way to promote gene expression, cell survival and neurite outgrowth, or instructive for brain development, by actively guiding circuit formation through specific spatiotemporal patterns of neural activity.

Whole animal b2KO mice lack expression of any b2-nAChRs, and RGC activity levels and retinal waves are severely perturbed in vivo. b2(TG) mice, which have b2-nAChR expression confined to the GCL but missing elsewhere in the retina and brain, have normal levels of RGC activity but truncated or “small” retinal waves. Rx-b2cKO have greatly reduced b2-nAChR expression throughout the retina, and correspondingly reduced RGC activity and truncated retinal waves both in vitro and in vivo.

Eye segregation … globally but not strictly locally correlated activity across the retina appears necessary for eye-specific segregation, along with a minimum overall level of activity that is close to that normally found during development. In Rx-b2cKO mice, retinal activity is locally but not globally correlated, like in b2(TG) mice, and eye specific segregation is completely disrupted. A minimum level of activity not too far below that found normally in the retina, along with correlated activity among RGCs across large swathes of the retina, but not between retinas, are necessary for the emergence of eye-specific segregation.

Topographic refinement: waves with a distinct wave front that propagates slowly across the retina and produces local correlations in activity between RGCs that are much stronger than global (long distance) correlations, appears necessary for normal retinotopic refinement. Even activity levels dramatically reduced below the norm, as in Rx-b2cKO, can produce normal retinotopic refinement as long as the activity is strongly correlated locally between nearby RGCs.

Retinotopic refinement in ventral-temporal (“binocular zone”) RGCs in Rx-b2cKO is normal only when competition between the two eyes is removed by monocular enucleation. Despite the fact that the development of refined retinotopy and eye-specific segregation are interdependent, each map is sensitive to distinct features of spontaneous retinal activity, including overall activity levels and RGC correlations.
Previously, using b2(TG) mice (Xu et al., 2011), we provided evidence to show that reducing the spatial extent of waves (their “size”) disrupted eye-specific segregation without interfering with retinotopy (so long as competition between the eyes was removed by monocular enucleation). Overall levels of retinal activity in b2(TG) mice are not altered in comparison to WT mice. This data demonstrated that merely the presence of normal levels of spontaneous activity is not sufficient to promote eye-specific segregation. Rather, eye-specific segregation is sensitively dependent on the pattern of spontaneous activity, and “large” waves with correlations amongst RGCs across long distances are necessary for normal segregation.

Here, we used an additional manipulation of b2-nAChR expression (the Rx-b2cKO mice) to examine the relationship between retinal activity and visual map development. In Rx-b2cKO mice, retinal activity levels are very substantially reduced in comparison to control and b2(TG) mice. Retinal waves are also altered, with some “small” and normally propagating waves, like in b2(TG) mice (Xu et al., 2011), along with some “flashy” waves, similar to those observed in b2KO mice (Burbridge et al., 2014).

Overall retinal activity levels in Rx-b2cKO mice were reduced to a level comparable to that seen in b2KO mice in vivo. Despite the dramatic reduction in retinal activity levels, the overall anatomical phenotype in Rx-b2cKO mice was indistinguishable from that observed in b2(TG) mice, with normal retinotopy (in the absence of between eye competition), but disrupted eye segregation. In summary, given the anatomical and physiological (in vivo and in vitro) phenotype of the Rx-b2cKO mice described here, we infer that even low levels of local (but not long-distance or “global”) correlated activity are sufficient to promote retinotopic refinement.

However, the absence of long-range correlations in RGC activity in Rx-b2cKO and b2(TG) mice prevent the emergence of normal eye-specific segregation.

It remains an open question and important future area of investigation to elucidate precisely what cellular and molecular mechanisms are responsible for translating distinct features of spontaneous retinal activity into refined and accurate maps of the visual world.
References

15. Arroyo, D.A. and Feller, M.B. 2016 Spatiotemporal features of retinal waves instruct the wiring of the visual circuitry. Front. Neural Cir. 10:
Xu et al Crair Neuron 2011: Figure 1. b2(TG) Mice Express b2-nAChRs Only in the Ganglion Cell Layer of the Retina, Have Normal RGC Firing Properties When Considered in Isolation, but Have Small Retinal Waves

(A) Expression of b2-nAChRs in the b2(TG) retina is controlled by a Tet-Off system, formed through the expression of both NSE-tTA and TetOp-b2 transgenes. (B) b2-nAChRs are broadly expressed in WT mice, with no [125I]A85380 binding in b2(KO) mice. In b2(TG) mice, binding is detected only in the optic tract, dLGN and SC. Enucleating both eyes completely eliminates binding in b2(TG) mice, demonstrating that b2-nAChRs in b2(TG) mice are expressed on RGC axon terminals.

(C) In situ hybridization for b2-nAChR mRNA in P4 WT and b2(TG) mice. In WT mice, b2-nAChR mRNA expression is broad, but highest in the ganglion cell layer (GCL) and inner nuclear layer (INL, arrow in top panel). In b2(TG) mice, b2-nAChR mRNA expression is concentrated in the GCL and much weaker in other retinal layers (arrow in bottom panel).

(D) Spontaneous RGC activity in P4 retina recorded in Ringer’s solution at 37°C. RGC activity is synchronous across the entire multielectrode recording array (shown in gray at bottom) in WT mice, while there are only local patches of synchronous activity in b2(TG) mice. (E) Retinal ganglion cell firing rates in WT and b2(TG) mice are similar (p = 0.51, two-tailed Student’s t test) and sensitive to the b2-nAChR antagonist, DHbE.

(F) A wide range of RGC firing parameters were compared between WT and b2(TG) mice under a range of conditions (see also Table 1 and Table S2). Illustrated here are four of these parameters, including burst frequency, spike frequency in a burst, and percent of time firing greater than 10 Hz. Only parameters related to the spatiotemporal pattern of the waves, not spiking properties (independent of waves), differed between WT and b2(TG) mice. By far the largest difference between WT and b2(TG) mice is wave size (p < 0.002, two-tailed Student’s t test). (G) Correlation index (cross correlation) of RGC activity is broad in WT mice, but falls off more steeply with separation in b2(TG) mice. dLGN, lateral geniculate nucleus; SC, superior colliculus; ONL, outer nuclear layer; RPE, retinal pigment epithelium. Triasterisk, p < 0.001, two-tailed Student’s t test. Error bars are SEM.
Figure 3. V1-SC Projections Form Two Termination Zones in EphA3ki/ki mice (A and B) Parasagittal sections of the SC after focal injection of Dil (red) in V1 and whole eye fill with CTB-488 (green) in the contralateral eye, which labels all RGCs. In WT mice lateral V1 injections result in TZs in the anterior SC, while medial V1 injections give rise to TZs in the posterior SC. (C) Corticocollicular TZ location expressed as a percent of SC anterior-posterior axis plotted against the V1 injection site, expressed as percent of the lateral-medial axis of the cortical hemisphere. Line represents best-fit regression, $R^2 = 0.9135$. $n = 23$

(D and E) In EphA3ki/ki mice lateral V1 injections result in two termination zones in the anterior and central SC, while medial injections result in two termination zones in the central and posterior SC. (F) Corticocollicular TZ location expressed as a percent of SC A-P axis plotted against the V1 injection site expressed as percent of the lateral-medial axis of the cortical hemisphere. Line represents best-fit regression, $R^2 = 0.7828$ and $0.7131$ for posterior (blue) and anterior (red) TZs, (G) Quantification of corticocollicular TZ overlap with the retinal recipient layer in WT and EphA3ki/ki mice. (H) Corticocollicular TZ area expressed as a percent of SC area in WT and EphA3ki/ki mice. Data are represented as mean +/- SEM, $n = 18$. **, $p < 0.01$ by ANOVA and Tukey's HSD post-hoc analysis.
Figure 6. Spontaneous cholinergic waves are required for map alignment in EphA3ki/ki mice

(A) Whole mount SC after focal injection of DiI (white) in nasal retina. In EphA3ki/ki mice, two distinct retinocollicular TZs were observed (arrowheads) in the appropriate topographic positions. bar, 500 μm; M, medial; P, posterior (B) Parasagittal section of the SC in (A) revealing two distinct retinocollicular TZs (arrowheads). bar, 500 μm; A, anterior, D, Dorsal. (C) Parasagittal SC section after focal injection of Dil (red) and DiA (green) in V1. In EphA3ki/ki mice, each single injection results in two corticocollicular TZs, which are interdigitated. (D) Representative intensity profile plots from two EphA3ki/ki mice after focal injection of Dil (red) and DiA (green) in V1. (E) Whole mount SC after focal injection of Dil (white) in nasal retina. In EphA3ki/ki/β2−/− mice, showing two broad TZs (arrowheads). (F) Parasagittal section of the SC in (D) showing two broad TZs (arrowheads). Images in (D) and (E) are from the same SC. (G) Parasagittal SC section after focal injection of Dil (red) and DiA (green) in V1.

(B) In EphA3ki/ki/β2−/− mice, each single injection results in a single, broad TZ, which are not interdigitated. (H) Representative intensity profile plots from two EphA3ki/ki/β2−/− mice after focal injection of Dil (red) and DiA (green) in V1.
Figure 2. Retinotopic Map Refinement, but Not Eye-Specific Segregation, Is Rescued in the SC of b2(TG) Mice

(A and B) Focal Dil injections into dorsal retina result in a spot of label in the SC (whole-mount, dorsal view). The target zone spot in b2(KO) mice and b2(TG) mice treated with doxycycline is much larger than in WT and b2(TG) mice. (C and D) Whole-eye (vitreal) injections of Alexa-conjugated cholera toxin dye bulk label most RGC axon projections in the SC. Contralateral axons are green, ipsilateral red. Contralateral axons (green) project to the most superficial (SGS) layer of the SC (sagittal sections), ipsilateral eye axons (red) project to the SO layer just inferior to the contralateral axons. A large fraction of axons from the ipsilateral eye extend into the SGS layer in both b2(KO) and b2(TG) mice (D, top) and overlap with projections from the contralateral eye (D, bottom), indicating poor eye segregation. M, medial; C, caudal; R, rostral; SGS, stratum griseum superficial; SO, stratum opticum. Scale bars ,500 mm for all figures. Biasterisk, p < 0.01, and triasterisk, p < 0.001, two-tailed Student’s t test. Error bars are SEM. See also Figures S1 and S8.
Figure 3. Binocular Competition Interferes with Retinotopic Map Refinement
(A and B) Focal Dil injections around the periphery of the retina results in focal target spots in the SC of WT mice, but much larger target zones in b2(KO) mice (see also Figure S2). In b2(TG) mice, target zones are completely restored in regions of the SC that receive monocular input but remain enlarged in the regions that receive input from both eyes (shown in gray). (C and D) Focal Dil injections into ventral-temporal retina, which projects bilaterally, labels a spot in the rostromedial portion of the contralateral SC in WT mice. A similar injection in b2(KO) and b2(TG) mice results in a much larger target zone. (E and F) Enucleation of one eye at birth restores retinotopic refinement of ventral-temporal RGCs in b2(TG) mice, but not in b2(KO) mice. M, medial; C, caudal; T, temporal; D, dorsal. Biasterisk, p < 0.01, two-tailed Student’s t test. Error bars are SEM. See also Table S1.
Figure 4. Retinotopic Map Refinement, but Not Eye-Specific Segregation, Is Rescued in the dLGN of b2(TG) Mice

(A and B) Focal Dil injections into dorsal retina result in a large spot of label in the dLGN (coronal sections) of b2(KO) mice, but small spots in WT and b2(TG) mice. (C and D) Focal Dil injections into ventral-temporal retina labels a focal target spot in the contralateral dLGN of WT mice, but produces a much larger target zone in both b2(KO) and b2(TG) mice. (E and F) Enucleation of one eye improves retinotopic refinement of ventral temporal RGC axons in the dLGN of b2(TG) mice, but not b2(KO) mice. (G–I) In the dLGN (coronal sections) of WT mice, RGC projections from the contralateral eye (green) are strictly excluded from the ipsilateral RGC axon terminal region (red). In b2(KO) and b2(TG) mice, ipsilateral eye projections have an expanded termination zone and intermingle with projections from the contralateral eye. (J and K) Two measures of eye-specific segregation in the dLGN show that eye segregation is much better in WT mice (0.33 ± 0.07, mean ± SD for Fraction ipsi only; 3.42 ± 0.51, mean ± SD, for Segregation index) than b2(KO) mice (0.24 ± 0.08, mean ± SD, for Fraction ipsi only; 2.11 ± 0.25, mean ± SD for Segregation index) or b2(TG) mice (0.20 ± 0.08, mean ± SD, for Fraction ipsi only; 2.27 ± 0.78, mean ± SD, for Segregation index). Biasterisk, p < 0.01, and triasterisk, p < 0.001, two-tailed Student’s t test. Error bars are SEM. Scale bars, 500 mm for all figures. See also Figure S4.
Figure 5. Daily Binocular Injections of CPTcAMP Rescue Eye-Specific Segregation in b2(TG) Mice

(A) Example coronal sections show that binocular CPT-cAMP injections correct eye-specific segregation defects in the dLGN of b2(TG) mice compared to saline injection controls. Contralateral axons are labeled green, and ipsilateral axons are labeled red with whole eye (vitreal) injections of Alexa-conjugated cholera toxin. (B) The fraction of dLGN with segregated ipsi projections is larger in CPT-cAMP-treated b2(TG) mice (0.31 ± 0.19, mean ± SD) than saline treated b2(TG) mice (0.16 ± 0.12, mean ± SD, 10% threshold shown, difference was consistent across a range of thresholds). (C) Eye-specific segregation in the dLGN measured with a segregation index was significantly improved in CPT cAMP-treated b2(TG) mice (2.46 ± 0.31, mean ± SD) in comparison to that of saline-treated b2(TG) mice (1.70 ± 0.36, mean ± SD). (D) Eye-specific segregation in the SC improves significantly in b2(TG) mice when treated with daily binocular injections of CPT-cAMP. (E) Summary quantification of eye segregation measured as the fraction of the contralateral (SGS) layer with ipsi label (10% threshold shown, the difference was consistent across a range of thresholds). Fewer ipsilateral axons project to the contralateral (SGS) layer in CPT-cAMP-treated b2(TG) mice (22.43% ± 5.29%, mean ± SD) than in saline-treated b2(TG) mice (37.03% ± 2.32%, mean ± SD). (F) Summary quantification of binocular overlap of ipsi (red) projections with contralateral (green) projections in the SGS layer. In CPT-cAMP-treated b2(TG) mice, the overlap was 22.15% ± 5.16% (mean ± SD). In saline-treated b2(TG) mice, the overlap was 35.95% ± 2.01% (mean ± SD). Triasterisk, p < 0.001, two-tailed Student’s t test.
Neuronal activity may be permissive for brain development, acting in a passive way to promote gene expression, cell survival and neurite outgrowth, or instructive for brain development, by actively guiding circuit formation through specific spatiotemporal patterns of neural activity.

Whole animal b2KO mice lack expression of any b2-nAChRs, and RGC activity levels and retinal waves are severely perturbed in vivo b2(TG) mice, which have b2-nAChR expression confined to the GCL but missing elsewhere in the retina and brain, have normal levels of RGC activity but truncated or “small” retinal waves. Rx-b2cKO have greatly reduced b2-nAChR expression throughout the retina, and correspondingly reduced RGC activity and truncated retinal waves both in vitro and in vivo.

Eye segregation …globally but not strictly locally correlated activity across the retina appears necessary for eye-specific segregation, along with a minimum overall level of activity that is close to that normally found during development. In Rx-b2cKO mice, retinal activity is locally but not globally correlated, like in b2(TG) mice, and eye specific segregation is completely disrupted. A minimum level of activity not too far below that found normally in the retina, along with correlated activity among RGCs across large swathes of the retina, but not between retinas, are necessary for the emergence of eye-specific segregation.

Topographic refinement: waves with a distinct wave front that propagates slowly across the retina and produces local correlations in activity between RGCs that are much stronger than global (long distance) correlations, appears necessary for normal retinotopic refinement. Even activity levels dramatically reduced below the norm, as in Rx-b2cKO, can produce normal retinotopic refinement as long as the activity is strongly correlated locally between nearby RGCs.

Retinotopic refinement in ventral-temporal (“binocular zone”) RGCs in Rx-b2cKO is normal only when competition between the two eyes is removed by monocular enucleation. Despite the fact that the development of refined retinotopy and eye-specific segregation are interdependent, each map is sensitive to distinct features of spontaneous retinal activity, including overall activity levels and RGC correlations.
Previously, using b2(TG) mice (Xu et al., 2011), provided evidence to show that reducing the spatial extent of waves (their “size”) disrupted eye-specific segregation without interfering with retinotopy (so long as competition between the eyes was removed by monocular enucleation). Overall levels of retinal activity in b2(TG) mice are not altered in comparison to WT mice. This data demonstrated that merely the presence of normal levels of spontaneous activity is not sufficient to promote eye-specific segregation. Rather, eye-specific segregation is sensitively dependent on the pattern of spontaneous activity, and “large” waves with correlations amongst RGCs across long distances are necessary for normal segregation.

Overall retinal activity levels in Rx-b2cKO mice were reduced to a level comparable to that seen in b2KO mice in vivo. Despite the dramatic reduction in retinal activity levels, the overall anatomical phenotype in Rx-b2cKO mice was indistinguishable from that observed in b2(TG) mice, with normal retinotopy (in the absence of between eye competition), but disrupted eye segregation. In summary, given the anatomical and physiological (in vivo and in vitro) phenotype of the Rx-b2cKO mice described here, we infer that even low levels of local (but not long-distance or “global”) correlated activity are sufficient to promote retinotopic refinement.

Here, we used an additional manipulation of b2-nAChR expression (the Rx-b2cKO mice) to examine the relationship between retinal activity and visual map development. In Rx-b2cKO mice, retinal activity levels are very substantially reduced in comparison to control and b2(TG) mice. Retinal waves are also altered, with some “small” and normally propagating waves, like in b2(TG) mice (Xu et al., 2011), along with some “flashy” waves, similar to those observed in b2KO mice (Burbridge et al., 2014).

However, the absence of long-range correlations in RGC activity in Rx-b2cKO and b2(TG) mice prevent the emergence of normal eye-specific segregation.

It remains an open question and important future area of investigation to elucidate precisely what cellular and molecular mechanisms are responsible for translating distinct features of spontaneous retinal activity into refined and accurate maps of the visual world.
Figure 3 Different RGC types show different levels of dependence on ephrin-As in topographic mapping. A. Whole mount brain with unilateral Dil injection into SC indicated by arrow. B. Flat-mounted retina (ganglion cell layer up) from an ephrin-A5 mutant mouse following injection of Dil into the contralateral SC. Scale bar, 500 mm. Bottom inset: topographically correct, A5-/- insensitive cluster of Dil1 cells. Top inset: topographically incorrect, A5-/- insensitive Dil1 cells. Scale bars, 100 mm. C, E. Maximal projections of A5-/- insensitive (C), and A5-/- sensitive (E), Dil1 RGCs, in an ephrin-A5 mutant mouse. D, F. Expression of CART or SMI-32 in a subset of the Dil-labeled cells shown in C and E, respectively. Arrowheads label single RGCs as follows: purple, CART1/Dil1; yellow, SMI321/Dil1. Scale bars, 25 mm.
The topographic maps of different RGC types are misaligned in ephrin-A mutant mice. A1–C1. Three examples of whole mount images of SC showing Dil-labeled RGCs from focal injections in the nasal retina in ephrin-A2/A5 double knockouts. Each shows a TZ at the correct topographic location (white arrows) and multiple ectopic TZs at incorrect topographic locations (yellow arrowheads). White dotted lines show the dimensions of the SC; posterior is at the top, medial is to the left. Small black arrows show the approximate locations of the parasagittal sections shown in panels A2–A4, B2–B4, and C2–C4. A: anterior; D: dorsal; M: medial; P: posterior. Scale bar: 250 mm.
**Lamination of RGC inputs is preserved in ephrin-A mutant mice.** Parasagittal SC sections stained with anti-GFP antibody to visualize RGC axons labeled in DRD4-GFP (A–C), TRHRGFP (D–F), and CB2-GFP (G–I) transgenic mouse lines. Quantification of GFP fluorescence across the RGC recipient layer of the SC (uSGS) is shown in the graphs in panels C, F and I. Scale bar: 250 mm.