Thursday, 18 August 2016

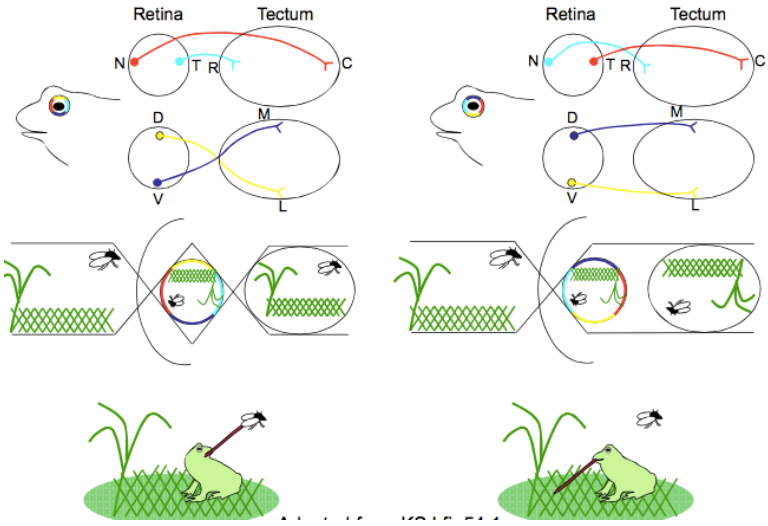
8 Cathy - Central Pathfinding

History of Growth Cones

- Cajal: GC's were first described:
  - has sensitivity: can sense things in its env.
  - its ends can more around quickly
  - whole structure has motif force that allows it to be propelled along
  - has destination where it is going
- harrison: observed growing tips of axons move and wiggle in culture
  - saw no evidence of specificity
  - randomness of moving
  - influenced development of theories
- weiss: observed that the cultured growing axons followed mechanical substrates
  - functionalist view: neural outgrowth is a random process (opposite to what we think about it today)
- sperry: tested the association between function and connectivity

<u>Sperry's eye rotation experiments</u>

- cut the optic nerve of the frog: the axons will regenerate in the same pattern that the connections would have normally formed
  - nasal axons always project to caudal tectum
  - temporal axons always project to rostral tectum
  - dorsal axons always project to lateral tectum
  - ventral axons always project to medial tectum
- dorsal part of visual field —> falls on ventral retina —> maps to medial part of tectum and vice versa
- cut the optic nerve and rotated the eye 180 degrees:
  - what was ventral is now at dorsal part
  - ventral and dorsal have flipped



- nasal and temporal have flipped

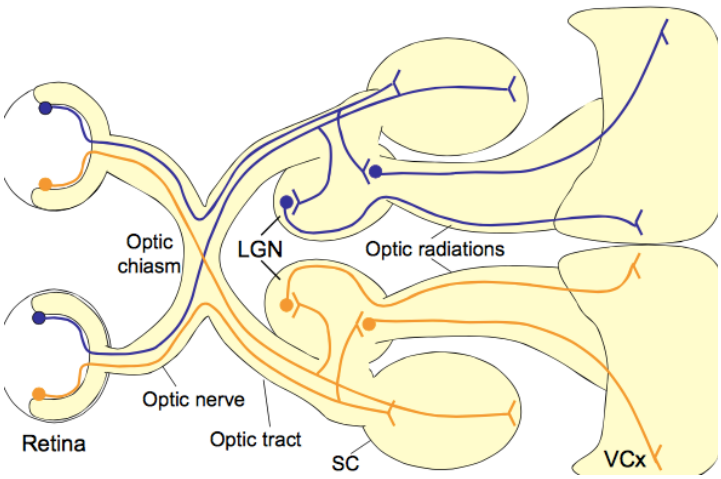
- let the axon grow in the tectum
- found that nasal axons that are now on the temporal side are still projecting to caudal tectum
- they are in a diff location and getting input from a diff part of the visual field still hasn't affected their connections
- space or input doesn't effect them but some intrinsic factor makes them project to specific part of the tectum
- impact on frog: frog was unable to respond appropriately as everything was 180 degrees different
- shows that there is some intrinsic factors that determine connectivity of molecules in the retina to the tectum

Sperry's Chemoaffinity Hypothesis: cells and fibres of the brain and cord must carry some carry some cytochemical identification tags which are distinguished from one another

Key Players in process of establishing connectivity in the CNS

- membrane bound OR extracellular molecules
- netrin
  - soluble ligand
  - binds to DCC receptor - attractive interaction
  - attraction can be modulated by co-receptor UNC5
  - presence of DCC & UNC5 causes the attraction to become repulsion instead
  - in order for DCC to have attractive response to netrin - need high levels of cGMP
- slit
  - bind to robo receptors
- semaphorins
- ephrins

- A and B types depending on the way they connect to the membrane
- bind to Eph receptors

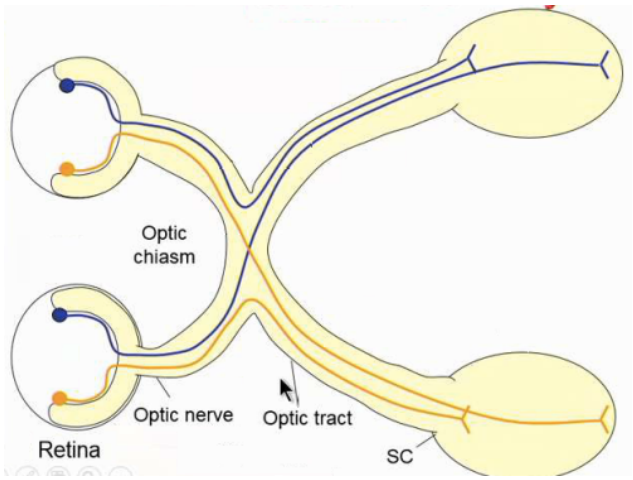


Mammalian Visual Pathway

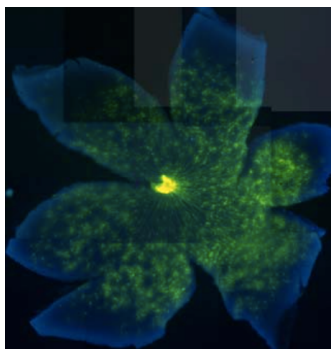
Retina—> optic disk —> optic nerve —> optic chiasm —> partial decussation (ipsilateral temporal retina + contralaleral nasal retina) —> optic tract —> branch into LGN and also continue to SC —> LGN goes to primary visual cortex

Decision points along the retinotectal pathway

- focusing on lower level of pathway
- 5 key points along the pathway and the diff mechanisms that regulate the growth and guidance of axons as they pass
  - what do they do in retina, which direction the grow in
  - how they get through optic disk
  - how they travel through optic nerve
  - partial decussation at optic chiasm
  - guidance along optic tract
  - terminating at SC

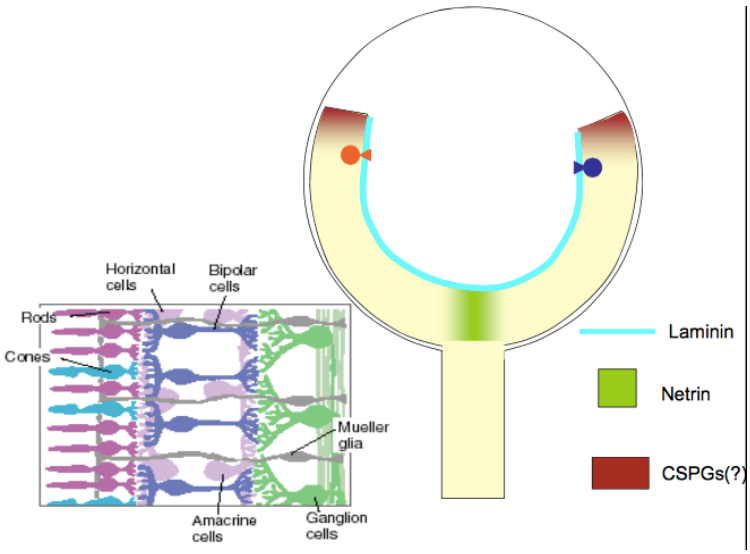


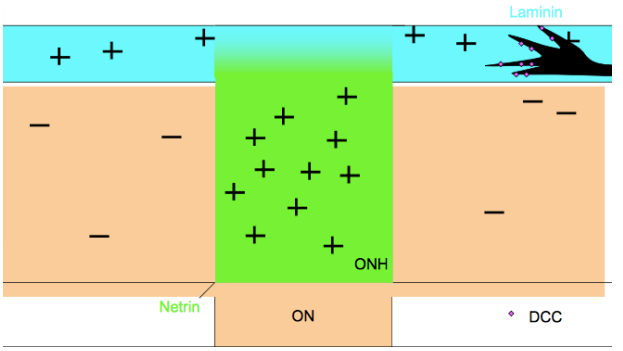
Radial lines: going directly from retinal ganglion cells towards optic disc —> tells us that something is directing them towards the exit point of the retina



Early part of pathway

- cross-section through retina
- different cell types in retina making lamina pattern
- ganglion cells: send axons out to optic disc
- glial cells have foot processes: secrete laminin - extracellular matrix molecule important for the guidance of ganglion cell axons to optic disc
- sheath of laminin demarcates where the optic fibre layer is going to form - forms adherence substrate for ganglion cells axons to grow along
- glial cells also produce inhibitory molecules - inhibitory to the axons but not the dendrites —> causes axons to grow in specific path
- optic disc: where axons exit the retina
- netrin expressed in optic disk: important attractant molecule for axons (thats what people thought)
- knockout netrin: axons were still growing towards the optic nerve head/optic disc BUT they did not diverge like they're supposed to, instead they kept growing straight
- radial direction of ganglion cells in retina towards optic disc occurs because the retina is growing centre out
- peripheral part of the retina expresses CSPGs: inhibitory molecules that have an inhibitory ring peripheral to where ganglion cells are being generated —> drives the axons radially down towards the optic nerve head
- axons are growing radially towards the optic nerve head which contains a large source of netrin



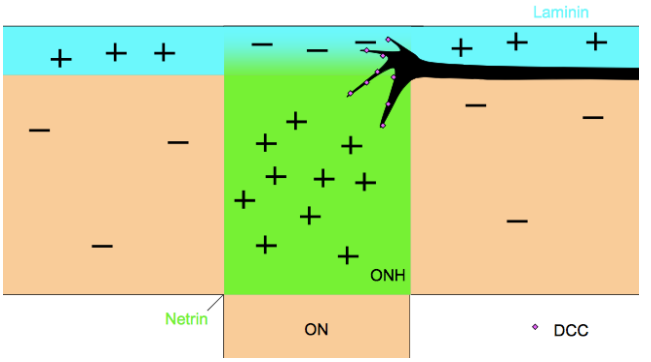


Laminin promotes axon growth in optic fibre layer

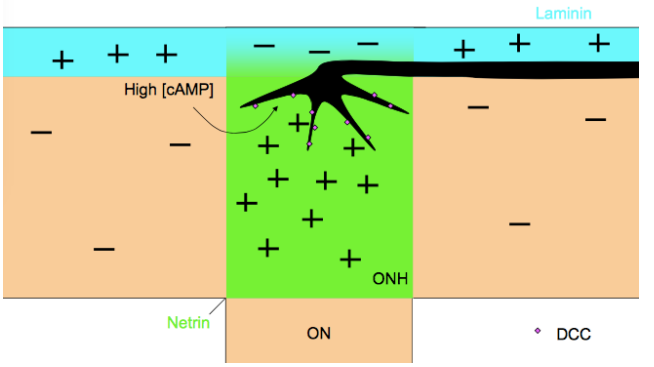
- axons grow along the surface of laminin (adherence substance)

Laminin and Netrin Interact to cause Turning

- knockout netrin: ganglion cells don't bend 90 degrees through optic disc (like they normally would), but they keep growing straight



- netrin: important for turning of axons
- as the axons are growing along, they express the DCC receptor for netrin
- netrin is attractive molecule
- presence of netrin + laminin —> becomes inhibitory
- axon grows along laminin but parts of the growth cone that are more superficial will encounter inhibitory interaction —> makes the superficial parts of the GC collapse while the deeper parts are in a pure netrin (attractive) env
- makes the GC undergo a 90 degree bend down into the pure netrin env and away from the netrin + laminin (inhibitory env.)

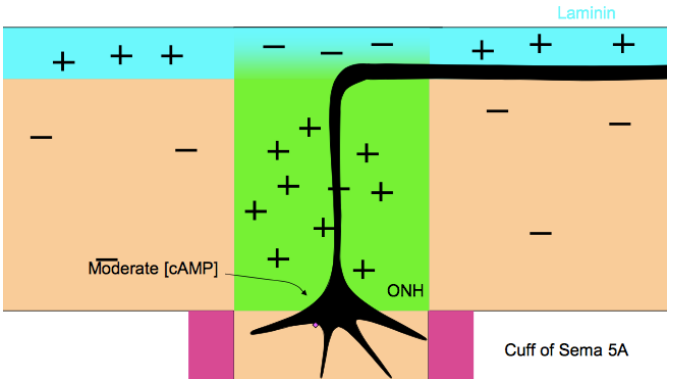


Netrin induces RGC axons to enter the Optic Nerve Head

- pure netrin env also contains high levels of cAMP —> makes it very attractive

Attraction to Netrin is decreased after passing through Optic Nerve Head

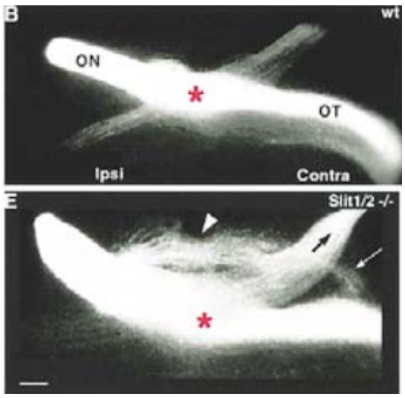
- once the axon has turned to enter the optic disc, we need to get it to keep moving along:
  - receptor expression of DCC is downregulated
  - cAMP in growth cone drops



—> this causes netrin to no longer attractive so GC grows out of the rich netrin env and enters the optic nerve proper

- in the optic nerve, cuff of Sema5A that forms a ring: prevents the axons from straying, keeps them going straight

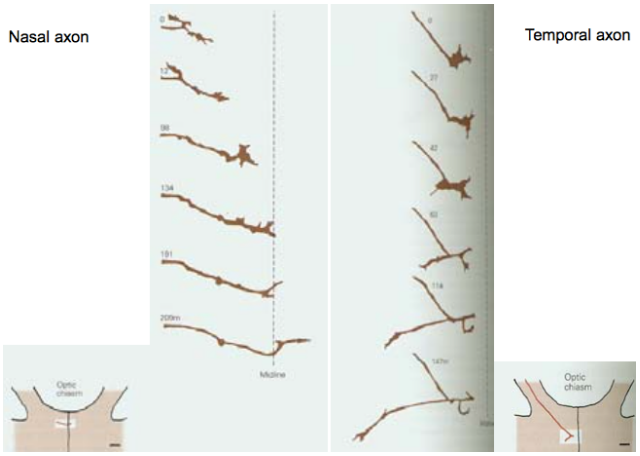
SLITS prevent premature crossing of the midline



- SLITS: guidance molecules expressed in a clump around the optic chiasm
- they constrain the point where the crossing can occur
- knockout SLITS1 and 2: optic chiasm forms more anteriorly, most axons leave the optic chiasm too early —> in appropriate crossing
- make sure that the optic chasm is constrained to one small location

Temporal and Nasal axons at optic chiasm

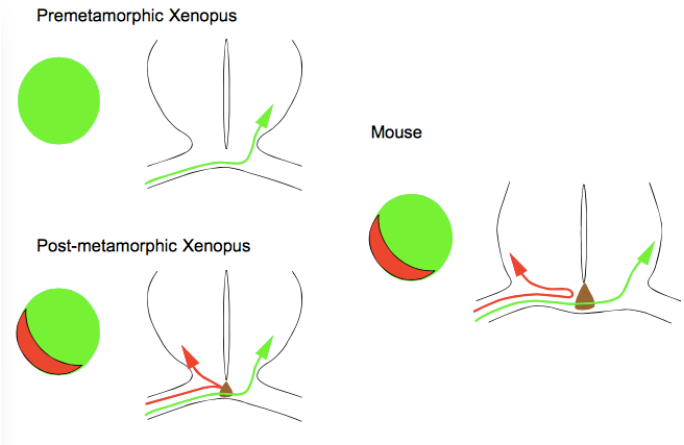
- time lapse recording of ganglion cell axon growing towards chiasmatic midline
- as the axon grows closer to the midline —> time starts increasing
- GC is slowing down and has become more complex —> this is typical when GCs get to decision points
- nasal axon: slows down, becomes more complex —> grows past the midline and starts growing faster again
- temporal axon (dont cross over): slows down, becomes more complex —> doesn't cross over —> part of GC that was closest to optic chiasm collapses and the other side sprouts and continues to grow in the other direction



- evidence of chemical cues that were differentially sensed by nasal and temporal axons

Ephrin B2 at the Xenopus Chiasm

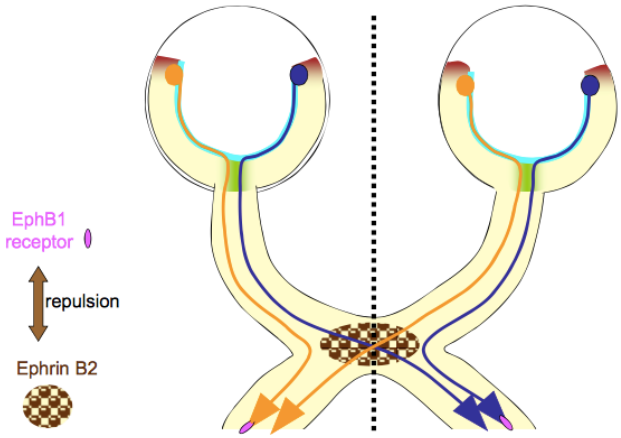
- pre-metamorphic frogs: all axons go contralaterally, dont have an ipsilateral projection
- metamorphosis: eyes move anteriorly so there is now an ipsilateral projection for binocular vision
- at the time of metamorphosis - there is up regulation of Ephrin B2 at the chiasmatic midline

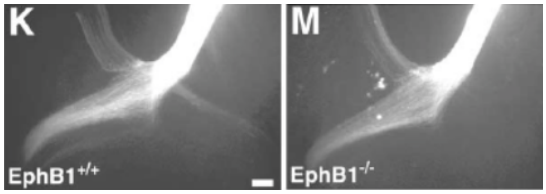


- same thing occurred in mice: expression of Ephrin B2 unregulated right around the time the ipsilateral projections are forming at the optic chiasm

<u>Eph B1 receptors are required for the development of the ipsilateral projection</u>

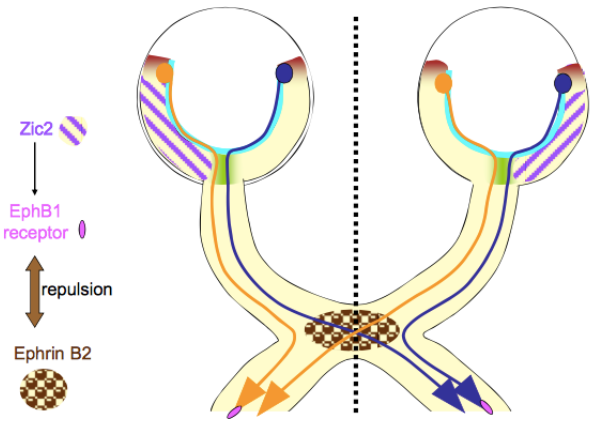
- embryonic day 17, when ipsilateral projections are forming in mouse retina
- knockout of EphB1 in the retina: ipsilateral projections no longer formed
- they form due to repulsive interaction between EphB1 ligand and EphrinB2
- mediates the differential response of nasal vs temporal axons at the optic chiasm
- also found in humans: 40% of retinal ganglion cells go ipsilaterally (temporal part) —> EphB1 expression correlates the ipsilateral projection in humans





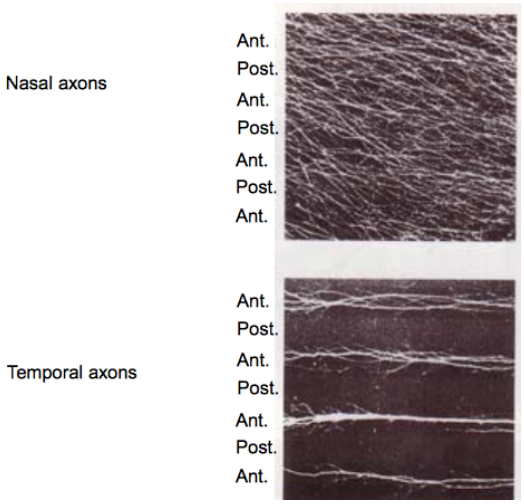
<u>Zic2 is expressed in the VTC and is required for the formation of the ipsilateral projection</u>

- Zic2: transcription factor upstream of EphB1
- high expression of Zic2 in ventrotemporal retina which is where the EphB1 expression occurs
- knockout of Zic2: ipsilateral projections no longer form



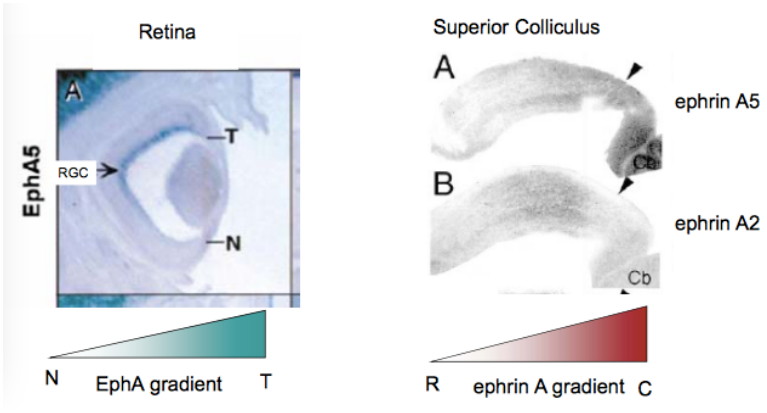
- not sure what molecules guide the axons through optic tract
- different species: axons go to diff places

Membrane stripe assay



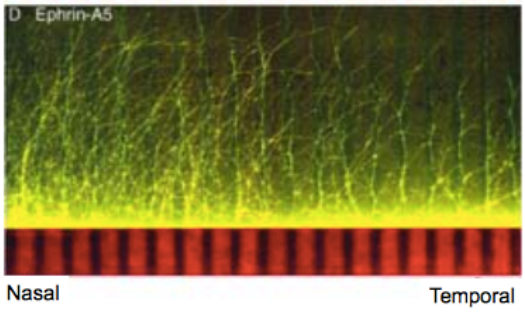
- strip of nasal or temporal axons —> cultured them —> made stripes: they either came from anterior/rostral or posterior/caudal tectum
- temporal axons: showed strong bias to only grow on membranes which originated from anterior part of SC, didn't grow at all on posterior
- nasal axons: grew in both anterior and posterior parts
- there is some chemical that is differentially repelling the temporal axons but not the nasal axons

Eph A receptors and their ligands are expressed in reciprocal gradients across in retina and SC

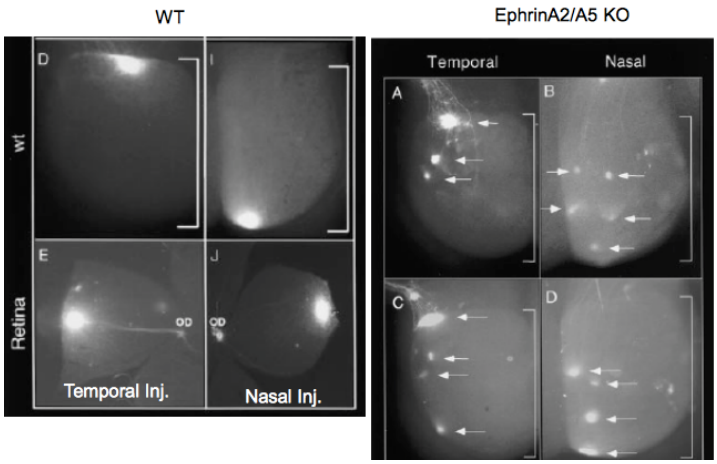


- eph A receptors are important for setting up the mapping along the rostral and caudal axis of the superior colliculus
- high expression of EphA5 receptor in temporal part of the retina which diminishes gradually to very low levels as you go to the nasal retina
- ligands of these receptors have corresponding pattern in the superior colliculus: EphrinA2 and EphrinA5 are low at rostral side and get higher as you move towards the caudal side

Ephrin As repel temporal retinal axons in vitro



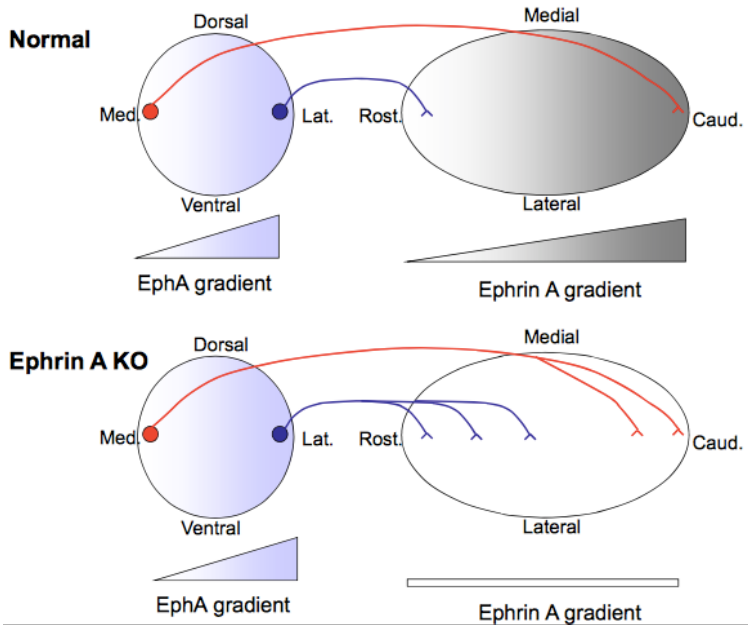
- Ephrin A ligands can induce differential behaviour between nasal and temporal axons
- nasal axons grow all over the place but temporal axons dont grow where the EphrinA ligand is present



In vivo experiment

- wildtype mouse:
  - temporal retina always projects to rostral part of superior colliculus
  - nasal retina always projects to caudal parts of superior colliculus
- ephrinA2 and ephrinA5 knockout mouse
  - projections are no longer as nicely mapped
  - temporal retina terminates in same spot as before but there are other extra termination zones more caudally in the tectum as well
  - nasal retina termiantes in caudal part but also projects to more rostral areas

—> evidence that the interaction between Eph receptors and Eph ligands are critical for making maps of the visual field in the optic tectum/superior colliculus

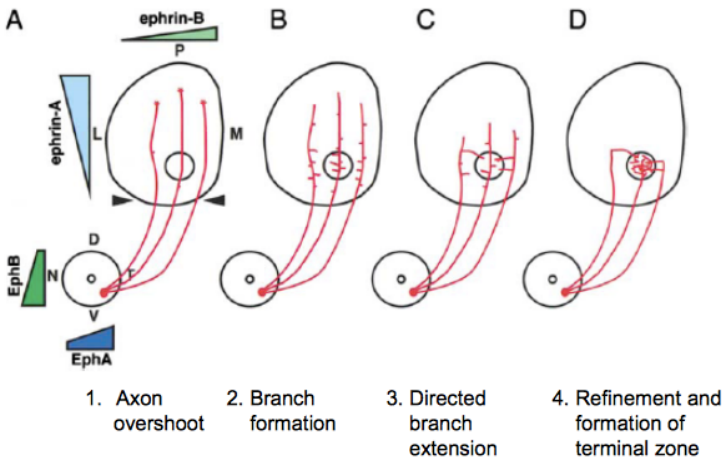


- medial = temporal
- lateral = nasal
- repulsive interaction: high level of Eph receptor is mapped to the part with a low level of Eph ligand
- knockout of the ligand or receptor: map of visual field no longer formed properly

Stages in formation of a topographic map

—> gradients of molecules that help set up connectivity

- Ephrin A ligand (low rostral - high caudal in superior colliculus): undergoes repulsive interactions with Eph A receptor (low nasal - high temporal in retina)
- Ephori B ligand (low lateral to high medial gradient in superior colliculus): undergoes attractive interactions with Eph B receptor (low dorsal - high ventral gradient in retina)
- early on (1): axons/GCs grow straight past the termination zone (circle in the middle)



- then branches come out of the axons (2): bias towards forming more numerous/longer branches closer to the termination zone
- (3) EphB ligand helps axons to grow up or down the gradient in towards the termination zone
- with further development (4): the overshoots go away to end up with a termination zone in the right place —> this process is not just dependent on guidance molecules but also interactions with activity

<u>Correlated firing of retinal neurons is disrupted in mice deficient for the B2 subunit of nicotinic AChR</u>

- recording spontaneous activity of ganglion cells in the retina —> can record patterns of activity
- the pattern of activity is occurring in the retina (ventral —> lateral): correlated bursts of activity in the diff parts of the retina
- wildtype: the closer together 2 recording sites are - the better correlated the firing will be
- if pattern of activity is disrupted - removing B2 subunit of nAChR effects coordination of firing via amacrine cells —> no longer any correlation between the different cells (random firing patterns)
- although the guidance molecules are in tact in the mice —> due to the disruption of activity: normal topographical map in the retina is no longer formed
- refinement process has been unable to occur
- interaction of guidance molecules + firing patterns —> leads to final pattern of connectivity