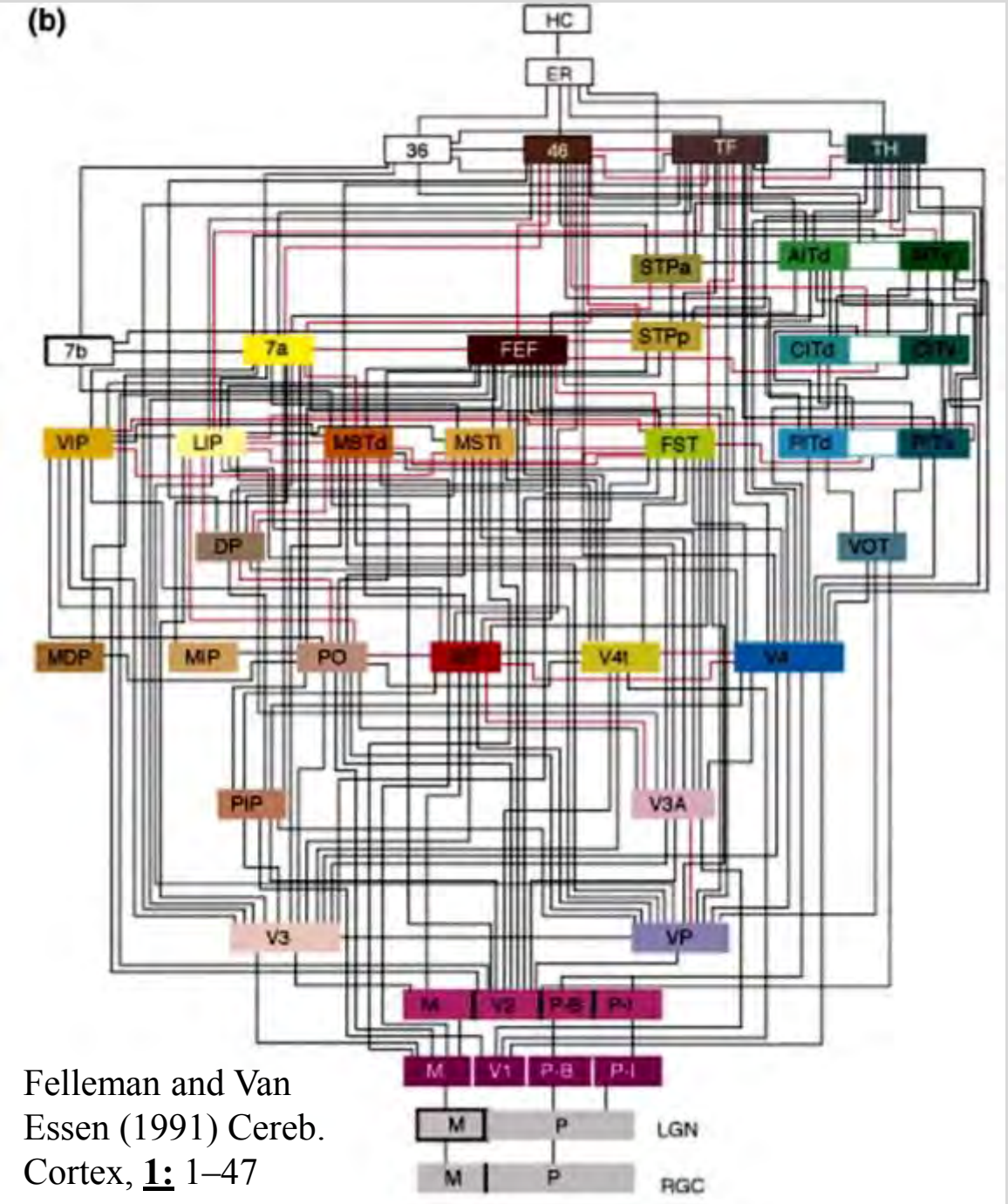
“Finding the Right Partner – Growth Cone Extension and Pathfinding in Developing Axons and Dendrites”

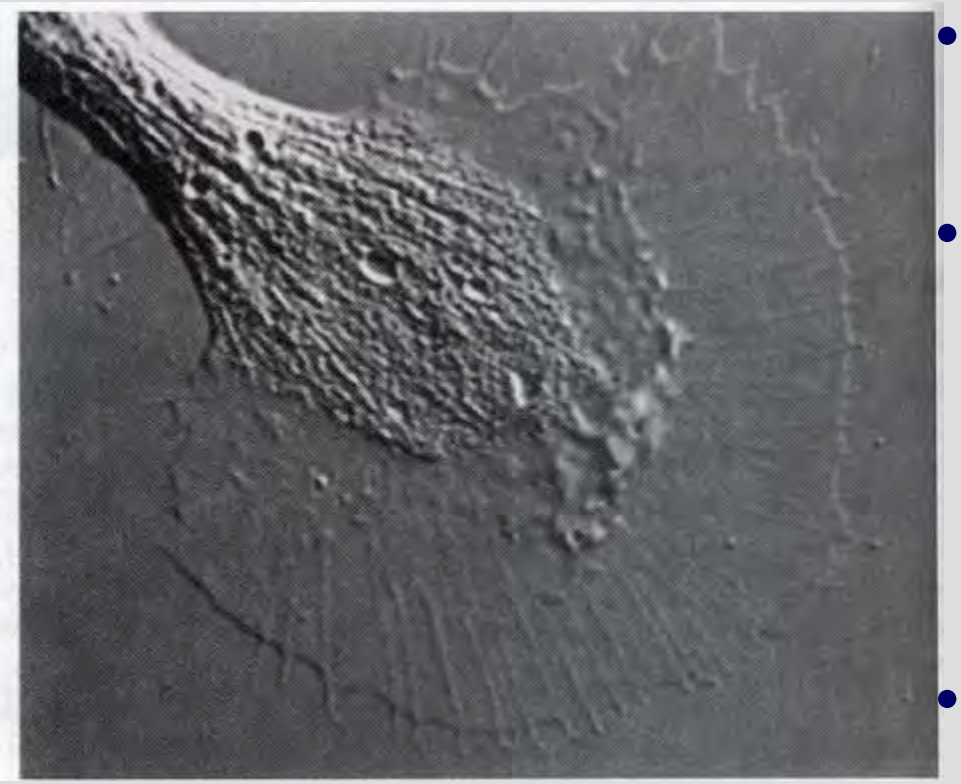
A "Projectome" (Lichtman and Sanes, 2008)



Felleman and Van Essen (1991) Cereb. Cortex, **1:** 1–47

Hierarchy of visual areas. This hierarchy shows 32 visual cortical areas. These areas are connected by 187 linkages, most of which have been demonstrated to be reciprocal pathways.

The Growth Cone



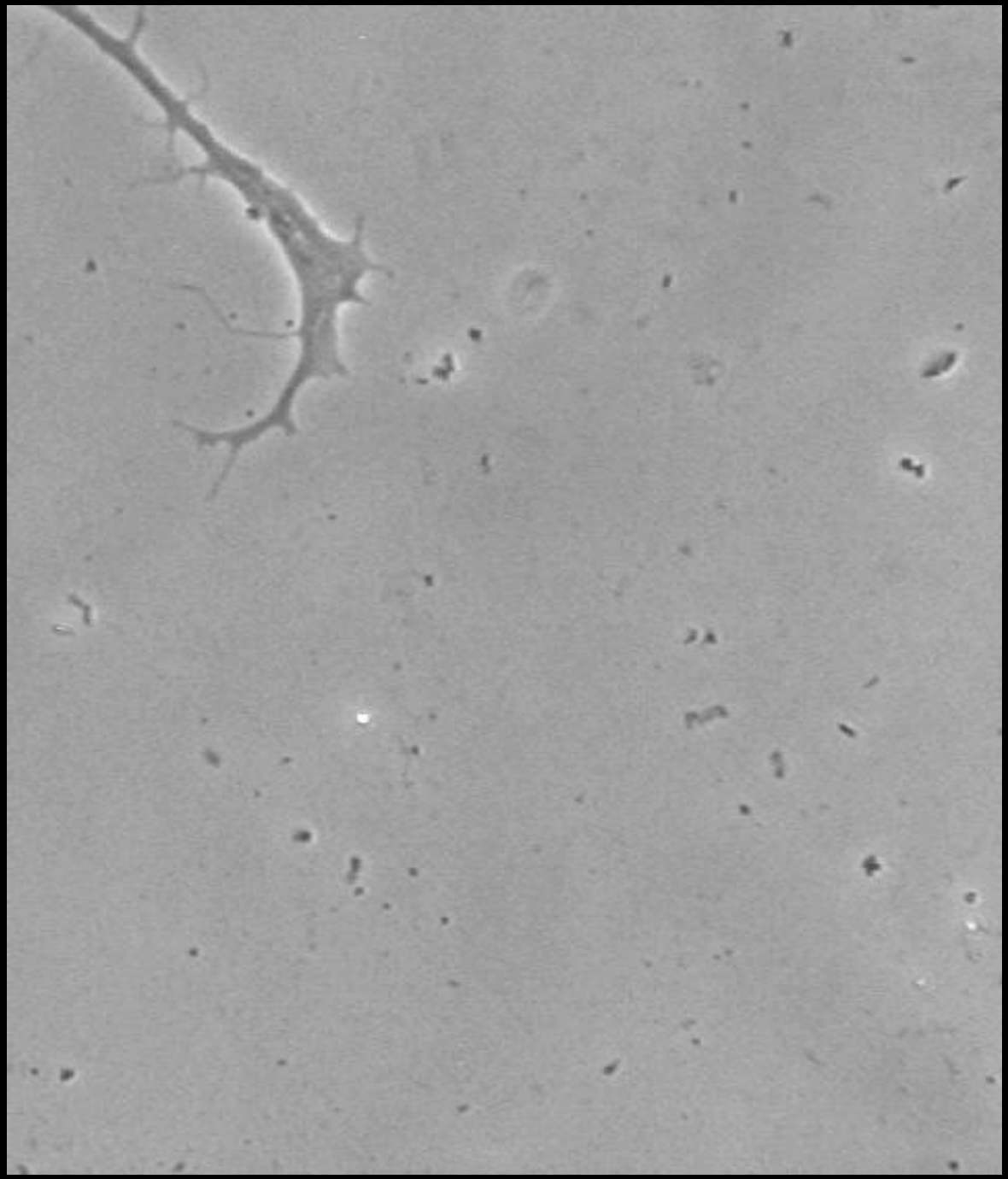
- Lamellipodia
- Filopodia
- contractile filaments (actin and myosin)
- Recognizes complex molecular pattern of guidance cues in its environment and can react to cues by changing direction of growth
- Leading to directed growth towards the target area
- On axons and dendrites

The Growth Cone Pulls the Axon

- The growth cone is highly mobile and can exert force on the axon
- Grows with a speed of 20 - 120 μm per hour
- Growth requires a substrate for adhesion
- Antibodies that prevent the adhesion can inhibit axonal growth
- Tension of a growth cone (PC12 cells): ~200-400 pN (Dennerll et al. (1988) *J. Cell Biol.* **107:** 665–74)
- Force required to detach a growth cone from the substrate: 100–200 pN μm^{-2} (Zheng et al. (1994) *J. Cell Biol.* **127:** 2049–2060)
- a single filopodium exerts a force not exceeding 3 pN
- The force exerted by lamellipodia ranges up to 20 pN

Growth Cone Biophysics

- How many neurons do you need to lift a “Weisswurst” (100 gram) against gravity?
- One kg will weight ~10 Newton
- One growth cone develops a force of 200 pN
- About 500 million neurons with one growth cone each

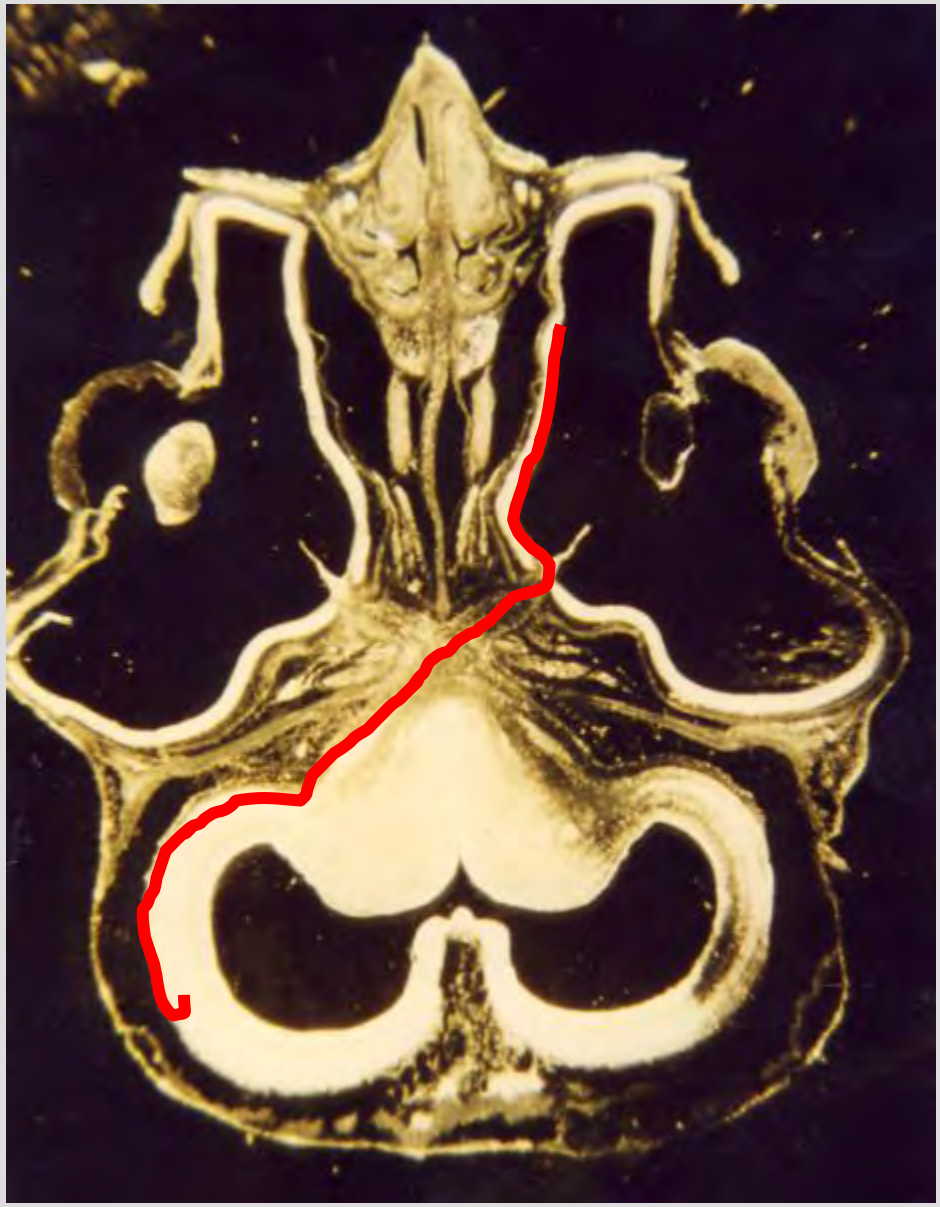


one frame
per second

Distances that have to be Bridged by Axonal Growth

- How long is the path the growth cones have to bridge?
- Distance to grow vary but generally short since embryo is small
- Growth cone has size of ~10 μm
- Assuming that distance is 1 cm (equivalent for visual system in birds), it would correspond to ~ 2 km distance for a human

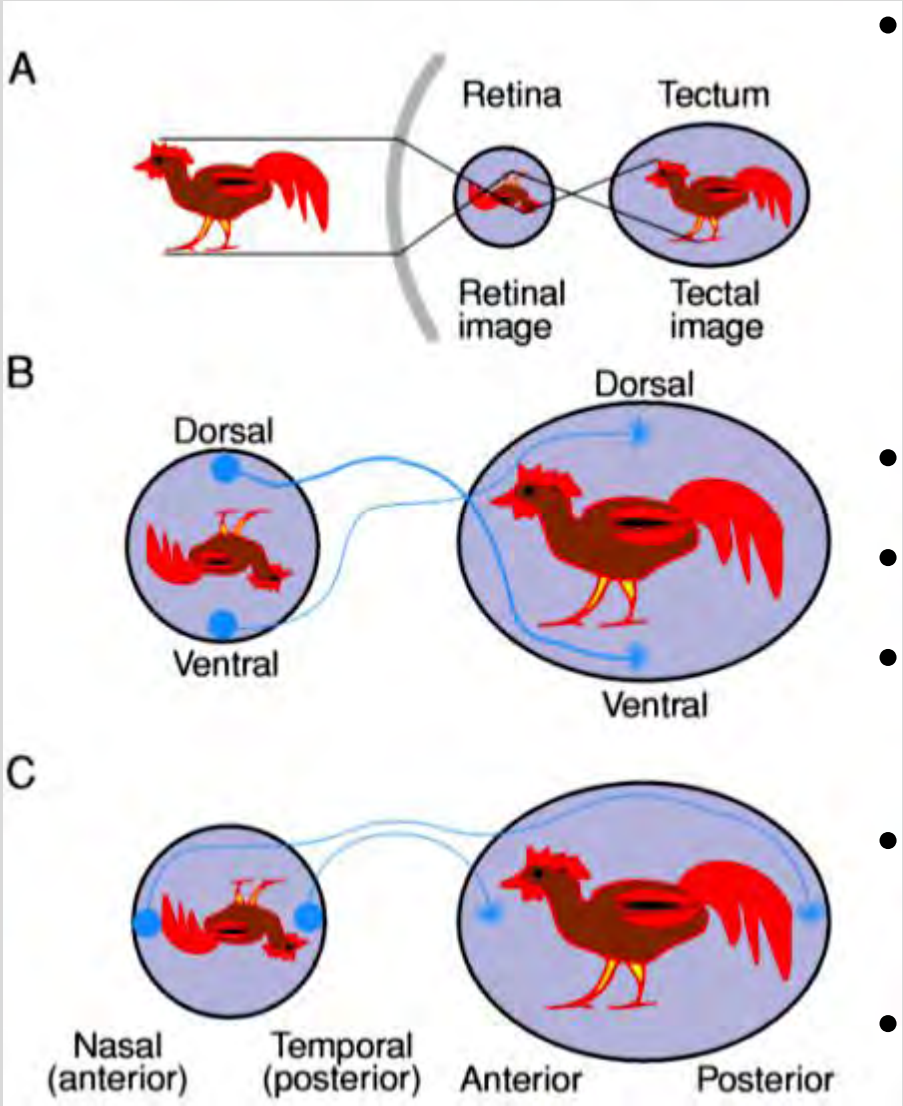
Downtown Riga



The Retino-Tectal System

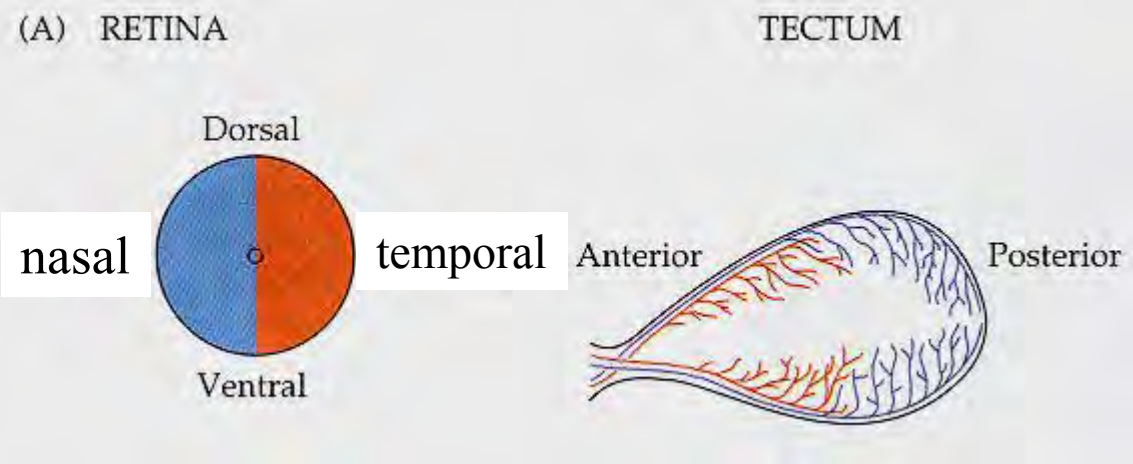
- Growth and pathfinding in the visual system of birds and amphibians
- 1 mio retinal ganglion cells grow within the retina to the optic nerve head
- Through the optic nerve and tract (100 % contralateral)
- Innervate optic tectum (mesencephalon) in **topographic** order

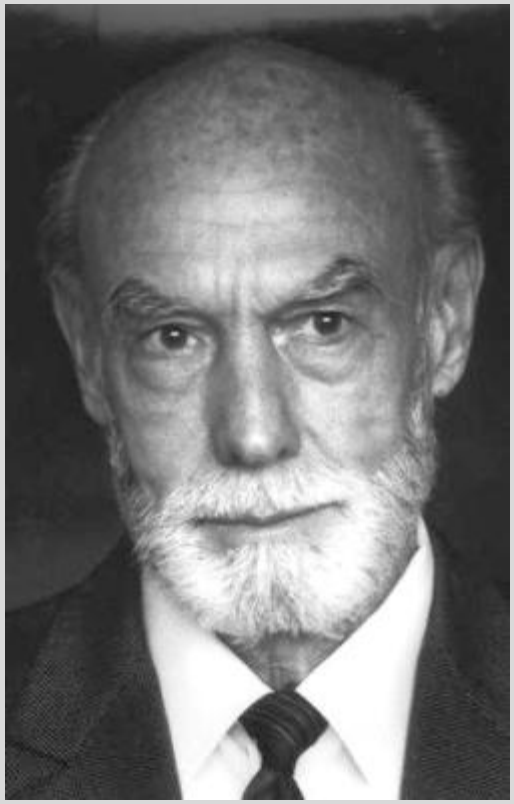
Topographic Connectivity: The Retino-Tectal Projection



- Neighboring cells in the retina are connected to neighboring target cells in the optic tectum (mesencephalon/equivalent to LGN)
- Retinotopy
- Similar: tonotopy, somatotopy
- Nasal axons project to posterior tectum etc.
- Classic system to investigate axon growth and pathfinding
- Why temporal axons not in posterior tectum?

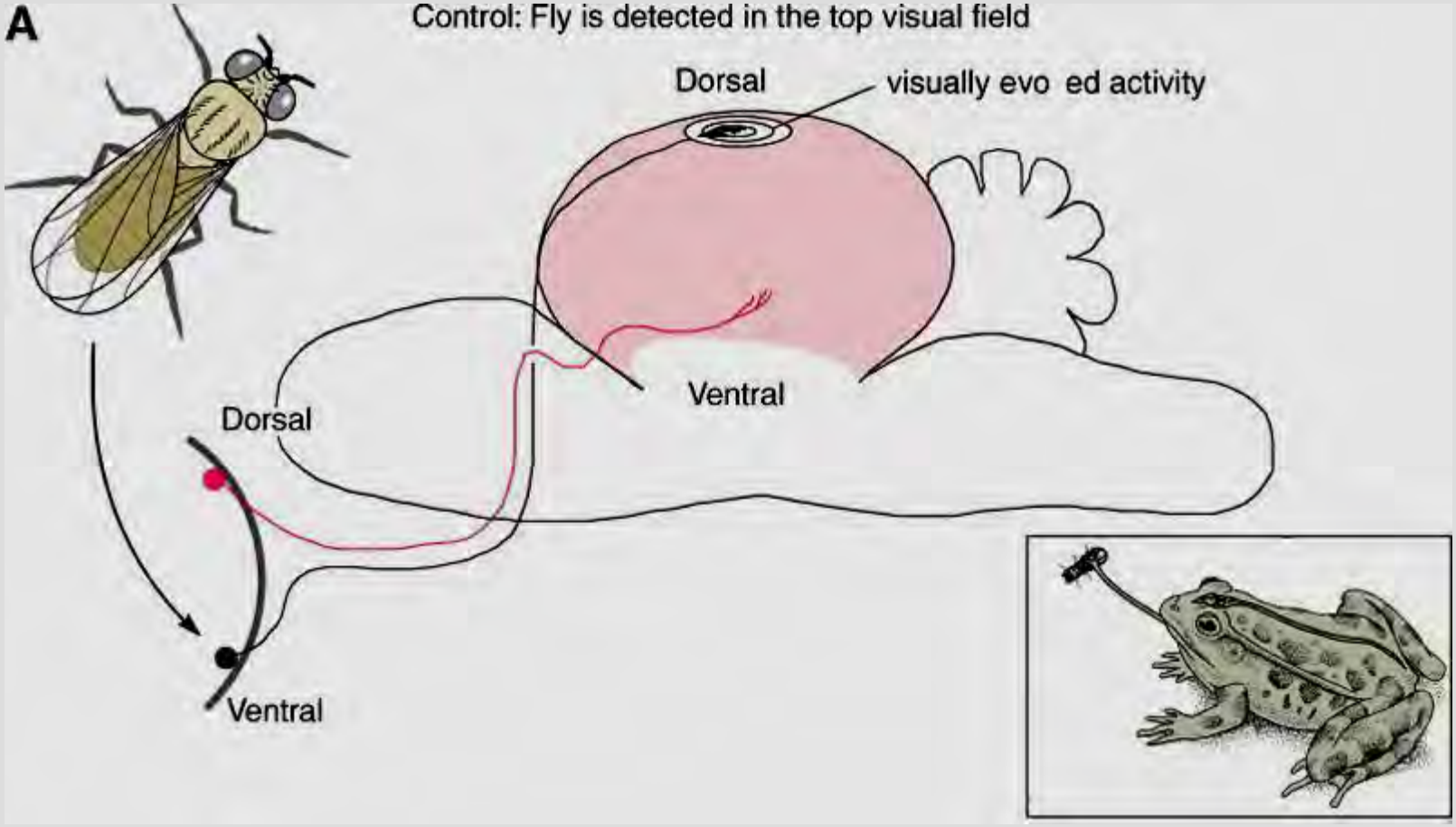
The „Chemospecificity Hypothesis“ (R. Sperry ~1960)





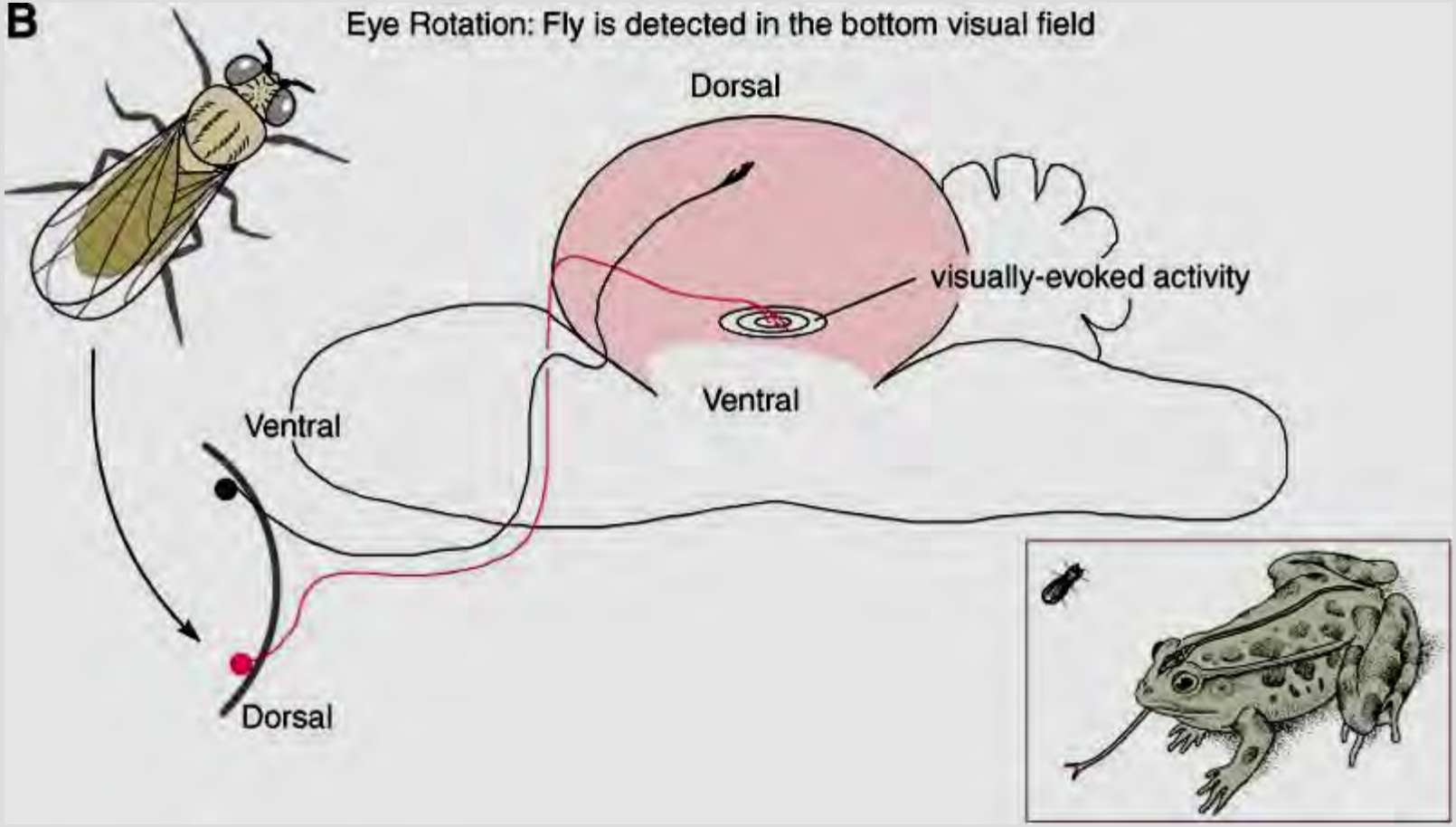
- Cut optic nerve – regeneration in frogs and amphibia possible
- Regenerated axons have topographic projection
- Optic nerve cut **AND** eye turned 180°.
- Question: is the topographic projection restored according to the function of the neuron, or according to environmental information read by growth cone?

The Sperry Experiment (~1950)



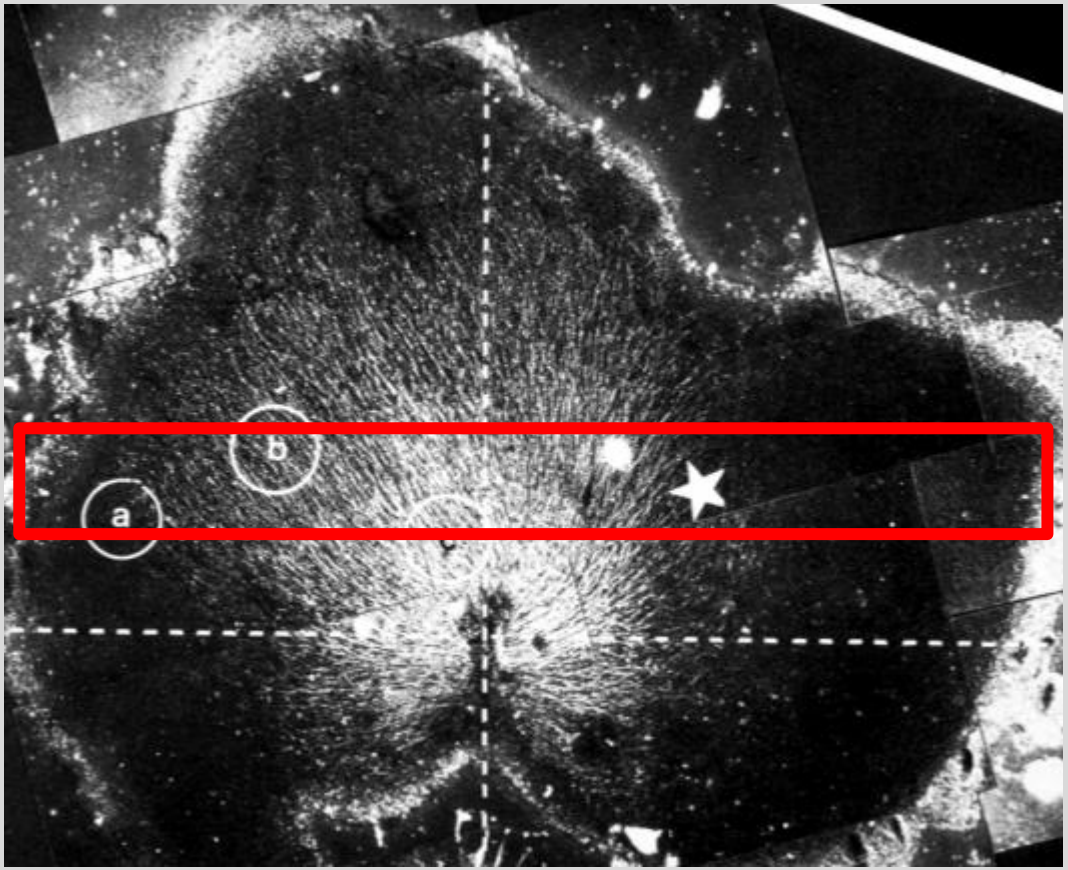
- When eye is rotated, the regenerating retinal ganglion cells have the choice of either growing according to their (new) position in the retina (information of visual field) or according to their previous position, i.e. reacting to signals available to them in the optic tectum

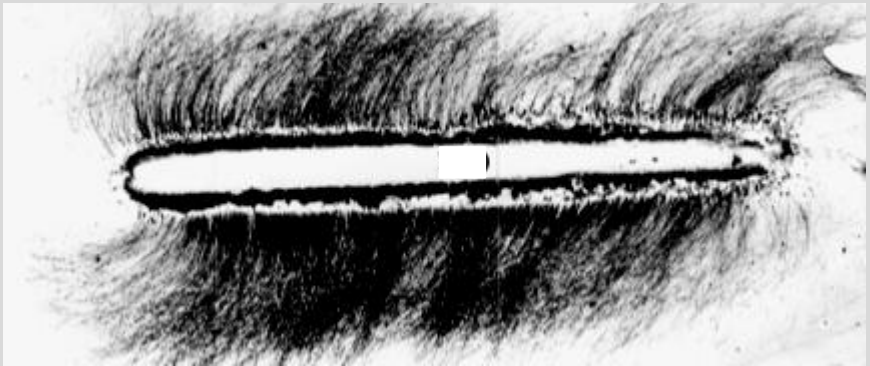
The Sperry Experiment (~1950)



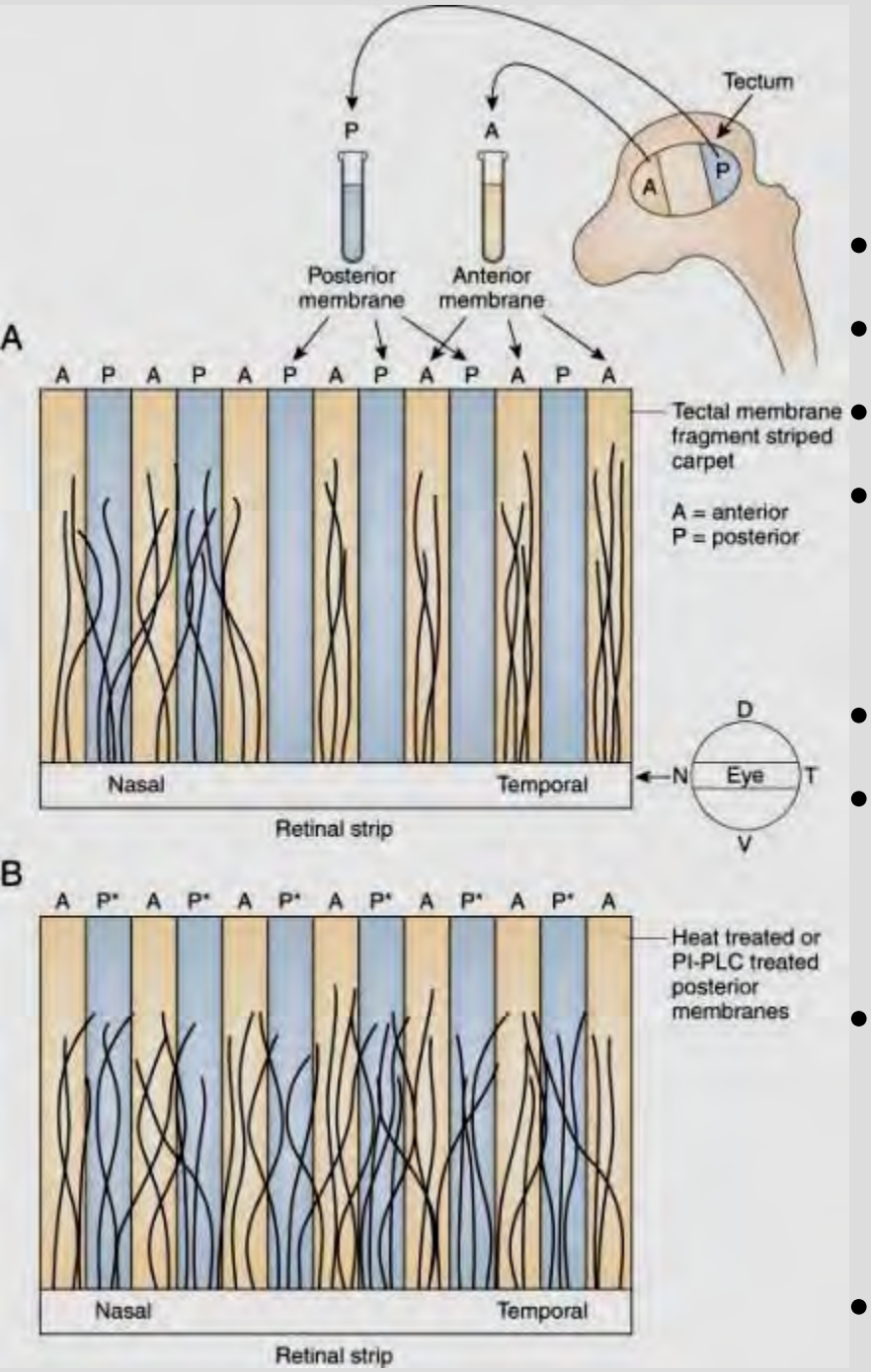
- „the animal struck downward in front of them and got a mouthful of mud and moss“ (R. Sperry, 1943)
- RGC grow according to signals provided by the tectum and read by the axon
- Perpendicular tags/gradients across retina and tectum

Growth of Retinal Ganglion Axons within the Retina





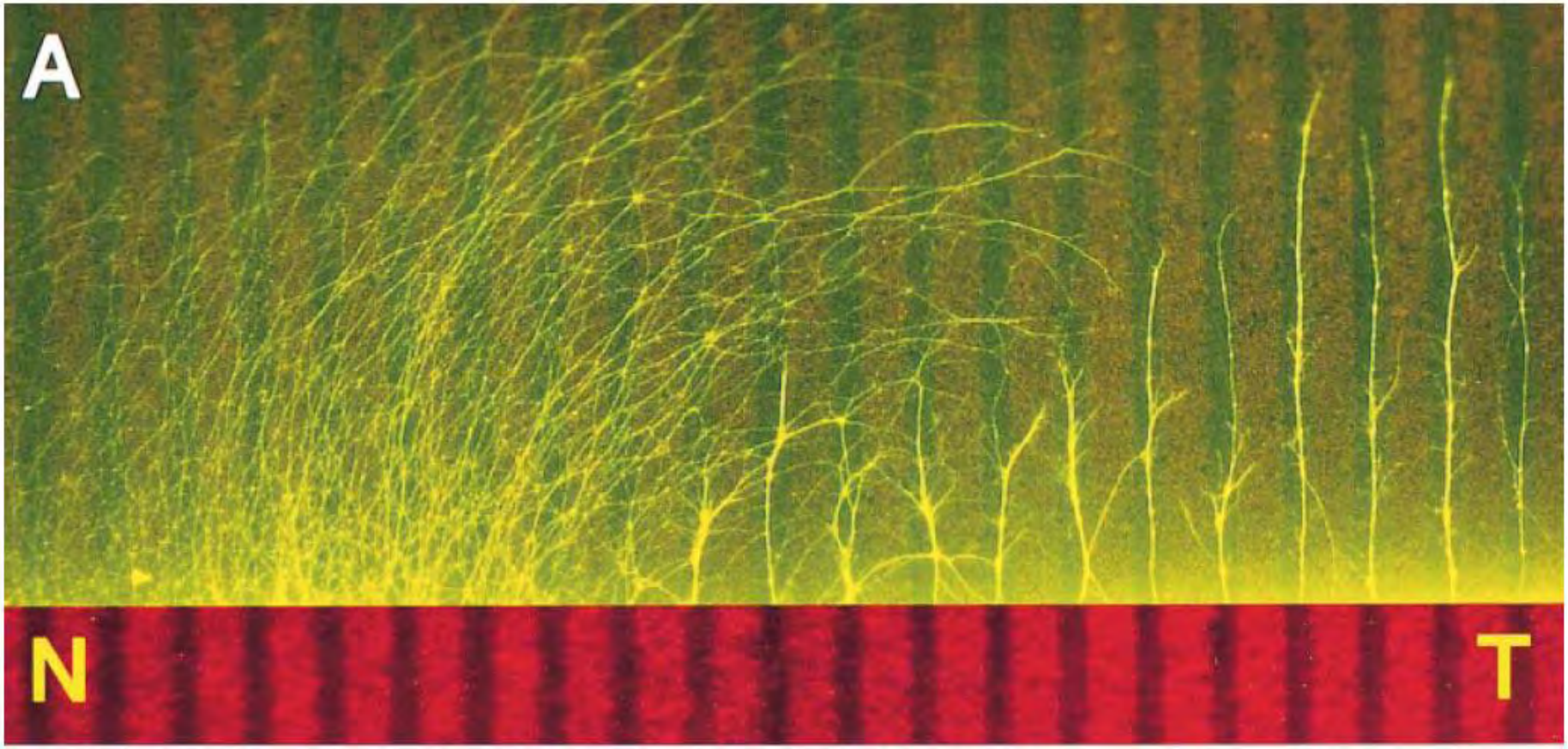
- Retinal flat mount
- Selective staining of ganglion cells
- Growth of axons towards the optic nerve head



The „Stripe Assay“

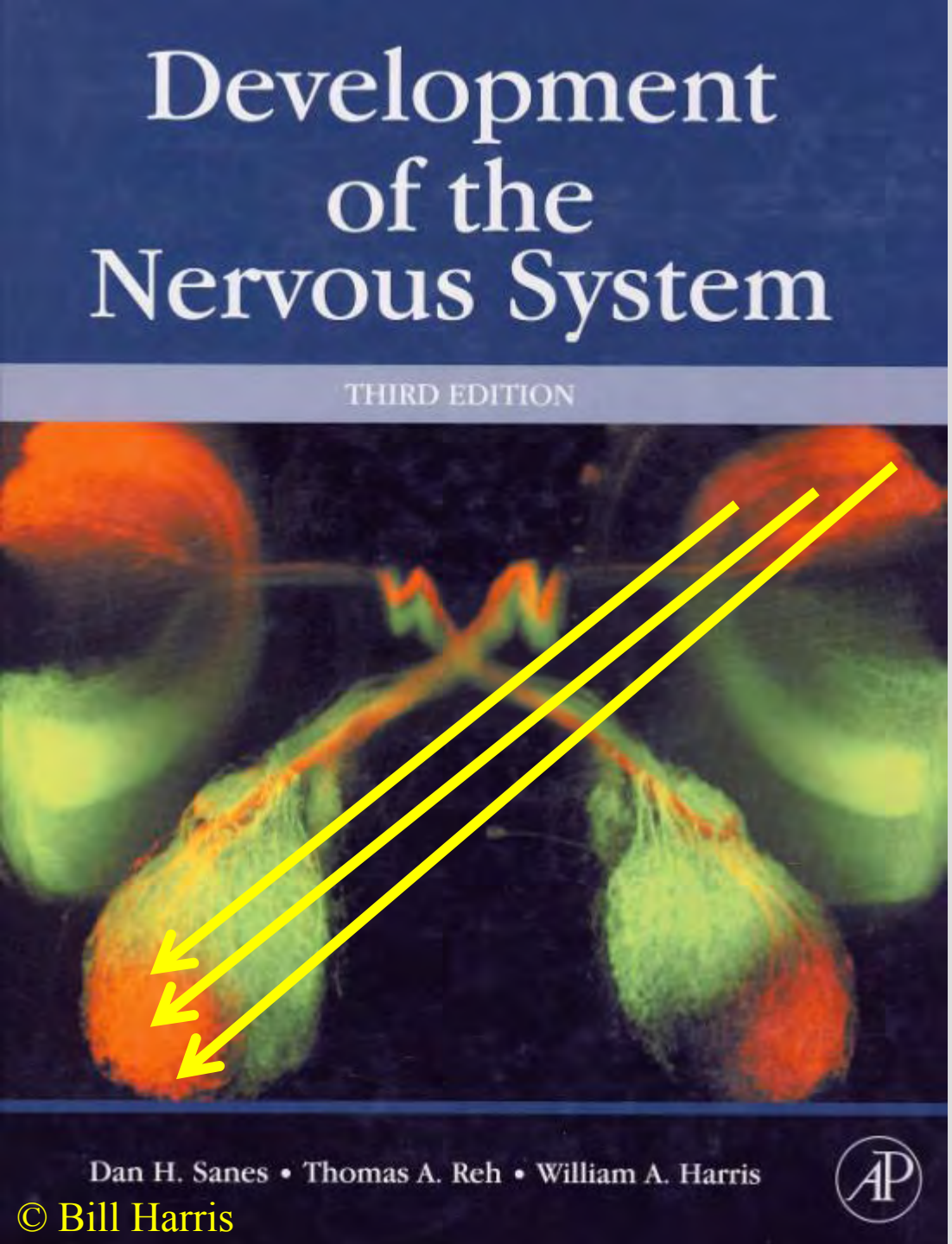
- F. Bonhoeffer (~1980)
- Retino-tectal system of birds
- Completely crossed projection
- Retinal nasal axons project to the anterior tectum, temporal axons to the posterior tectum
- Stripe assay allows binary decision
- All axons grow on membrane preparations from all parts of the tectum
- However, **when given the choice**, temporal axons will prefer anterior vs. posterior plasma membranes (Walter et al., 1987)
- “avoidance factor” is heat-inactivatable and GPI-anchored

The „Stripe Assay“



Monschau et al., 1997

- Nasal axons grow equally well on anterior or posterior membranes
- Temporal axons prefer to grow on anterior membranes and avoid posterior membranes (red)



The Retino-Tectal System

- Nasal axons (red) and temporal axons (green) project to the anterior and posterior part of the tectum in frogs
- Temporal RGC axons do not grow into posterior tectum because they are inhibited to do so

Molecules That Influence Axonal Growth: The „Carrot and Stick“ Model

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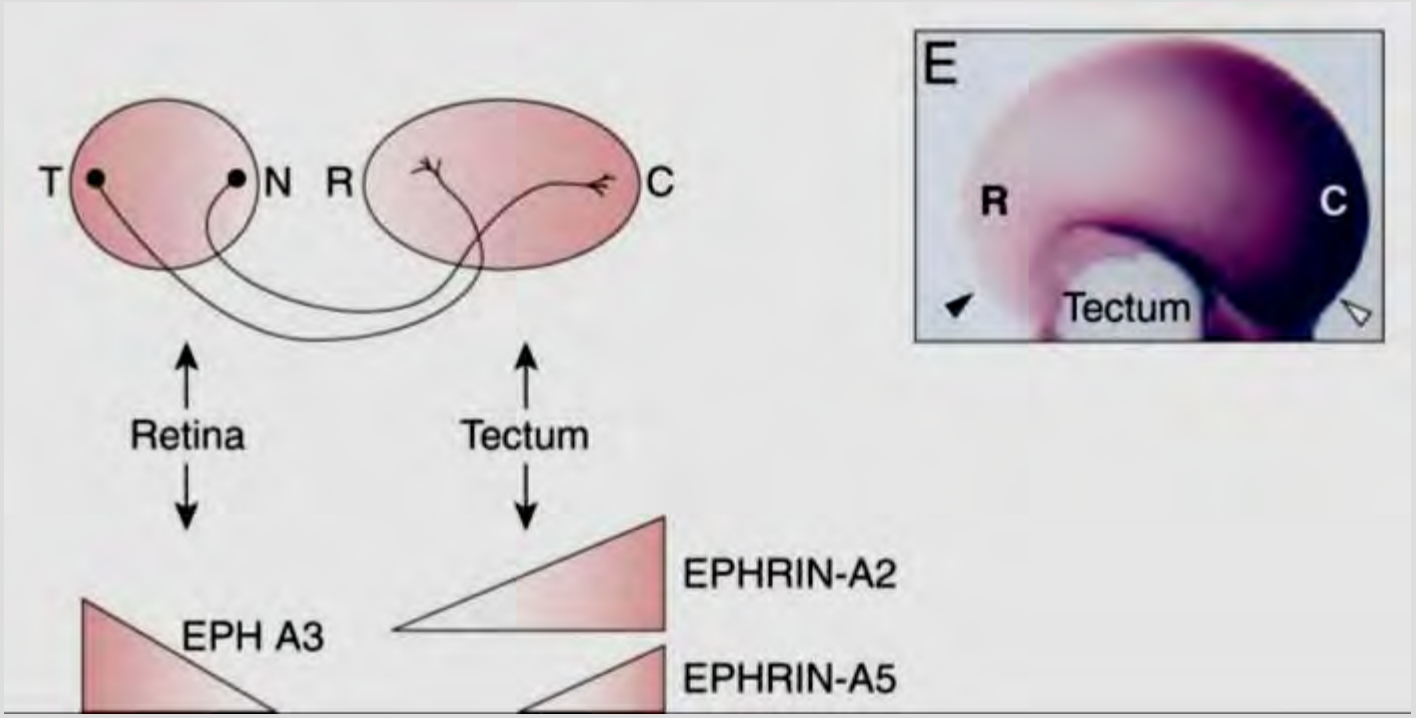
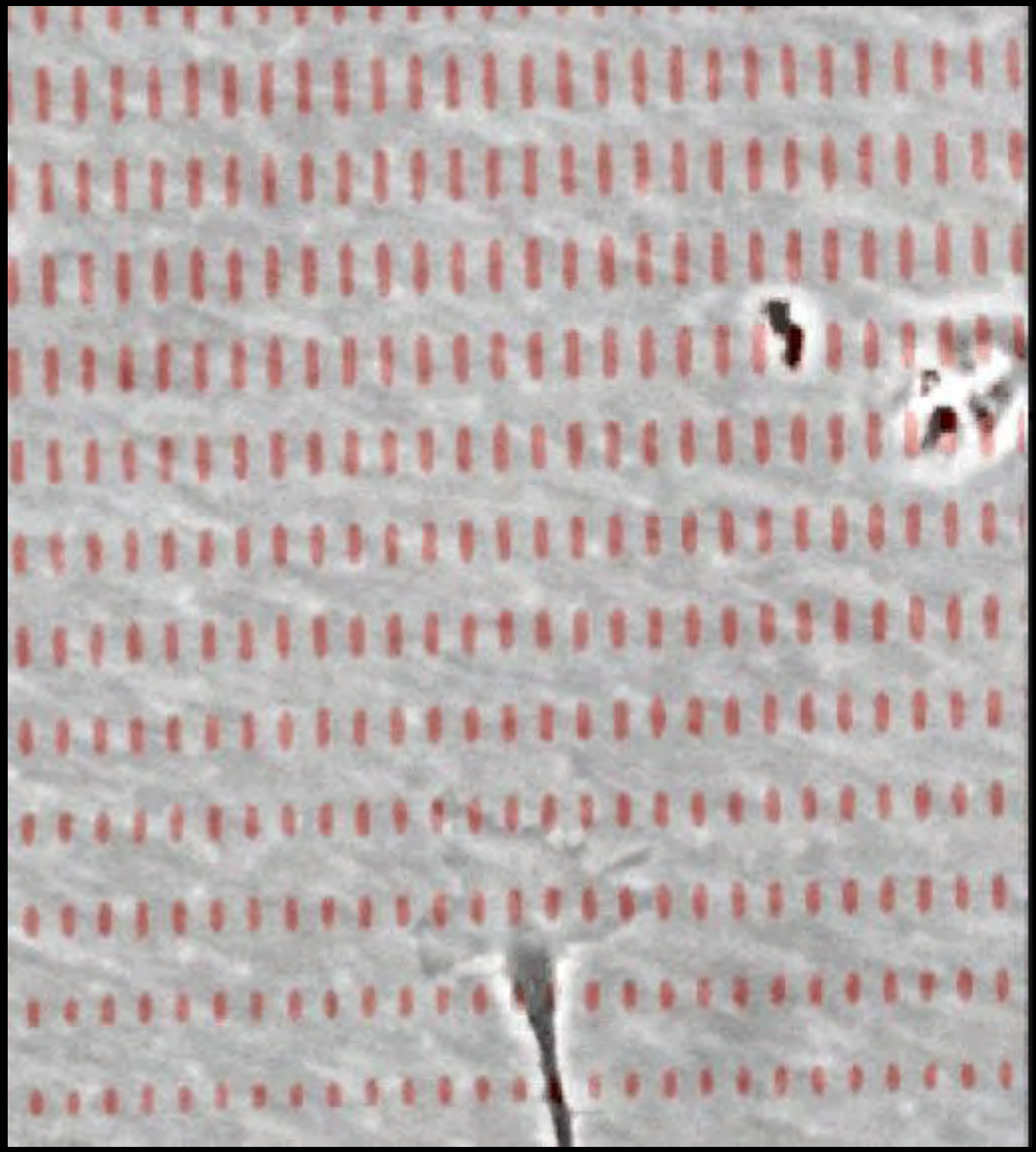
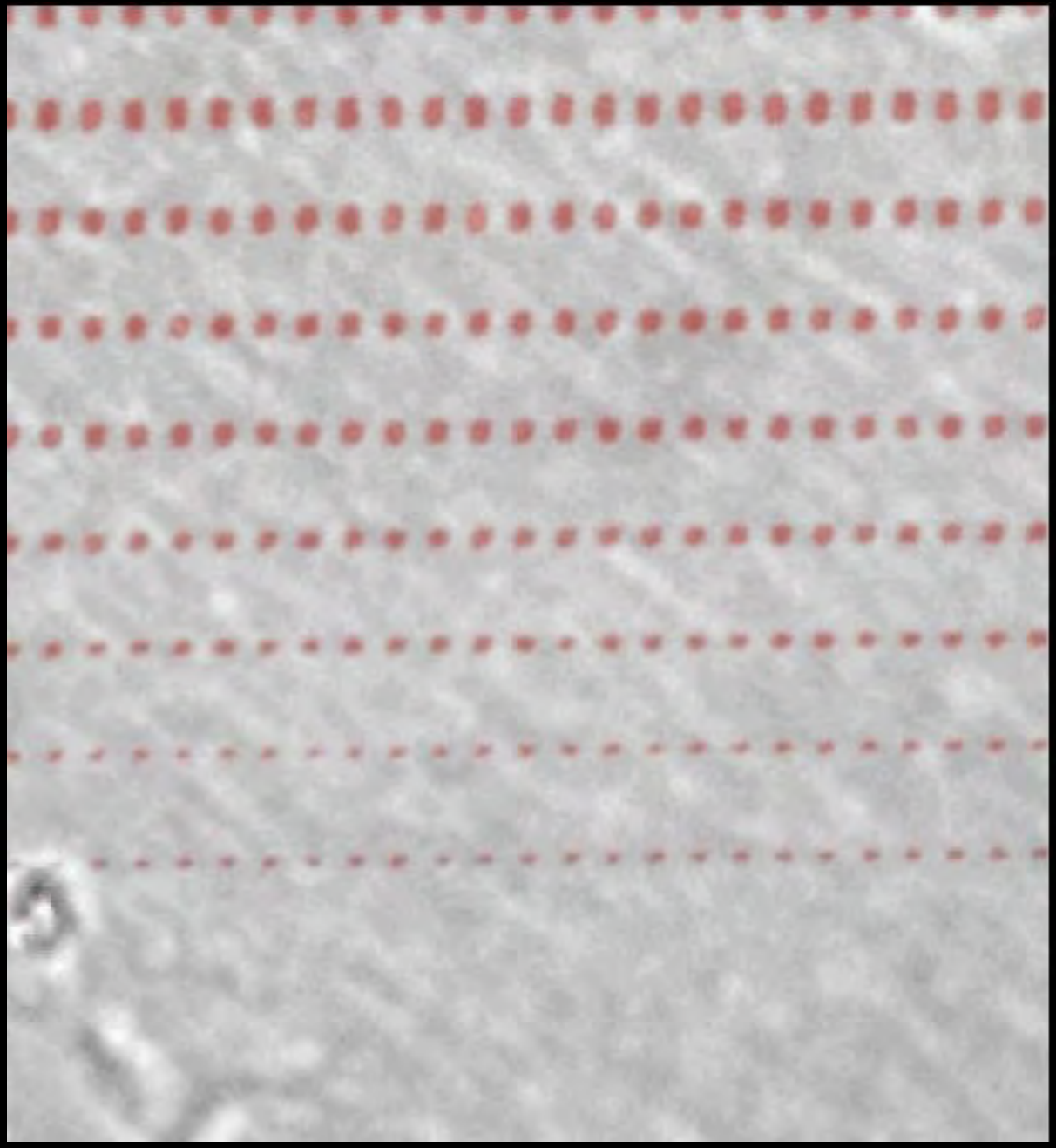
Ephrins/Eph Receptors in the Retino-Tectal System



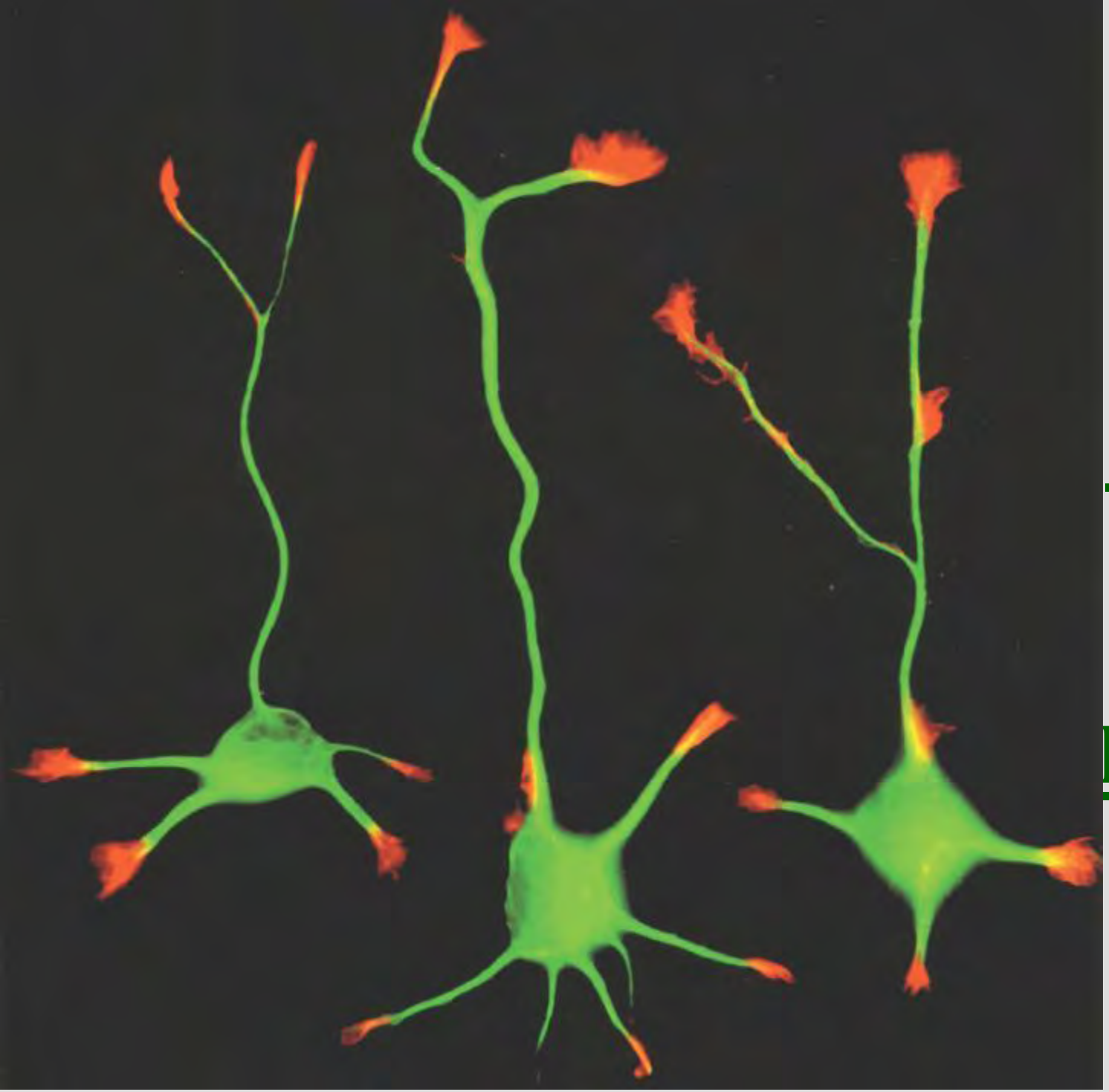
- Avoidance factor are Ephrins, detected by Eph Receptors on retinal ganglion cell growth cones
- Opposing gradients of ephrin-A5 and -A2 and their receptors (EphA3) are expressed in gradients in retina AND tectum – can explain topographic projection
- Ephrins and Eph receptors have same effect on RGCs in stripe assay as the membranes
- Assay does not reproduce all facets of the retino-tectal projection



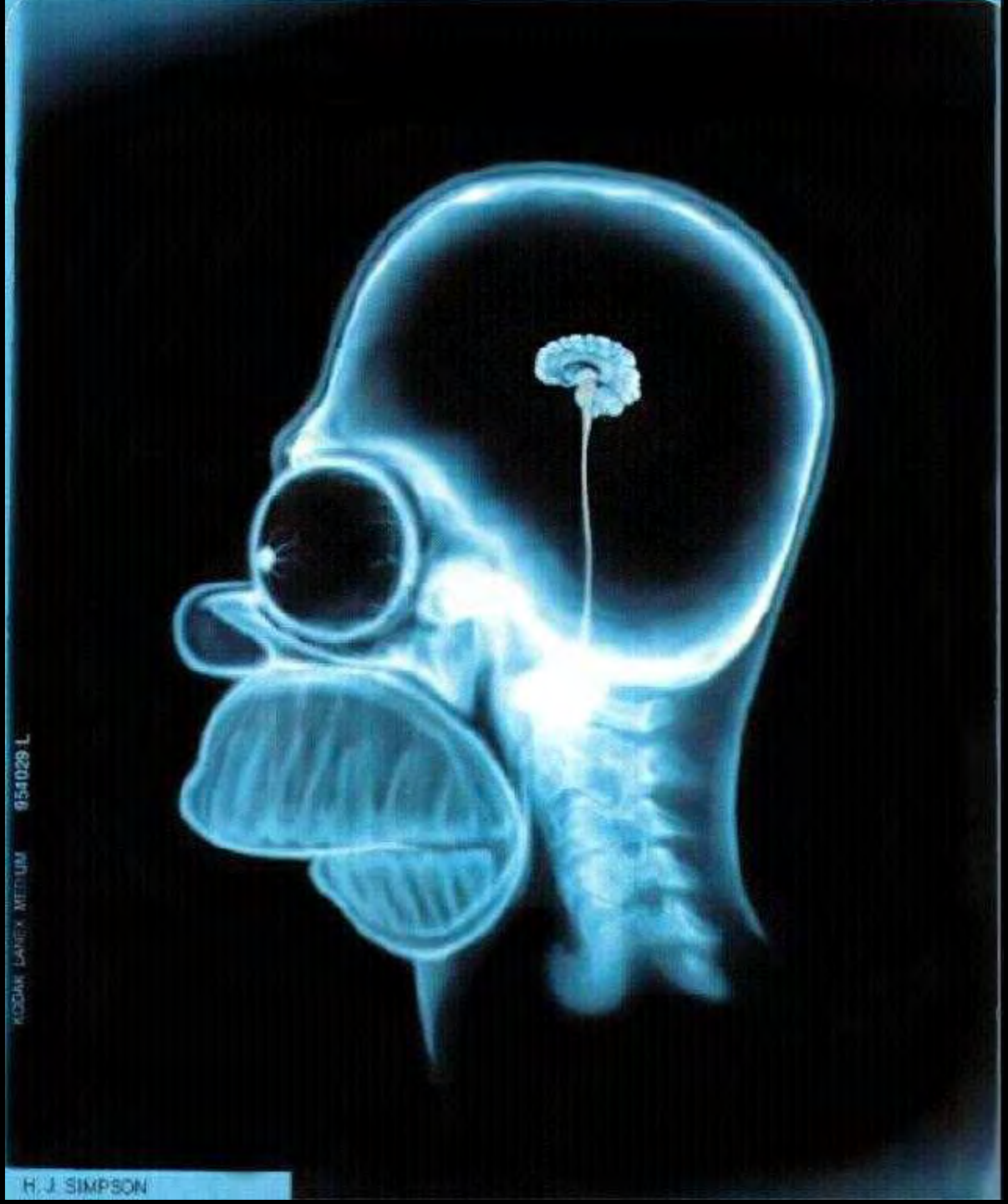
Nasal growth cone on laminin substrate with ephrin-A5 dots



Temporal growth cone on laminin substrate with ephrin-A5 dots



The Growth Cones on Axons AND Dendrites



Not All Brains are Identical !

Number Synesthesia

55555555555555	55555555555555
55555552555555	55555552555555
55555525255555	55555525255555
55555255525555	55555255525555
55552555552555	55552555552555
55525555555255	55525555555255
55255555555525	55255555555525
52525252525252	52525252525252