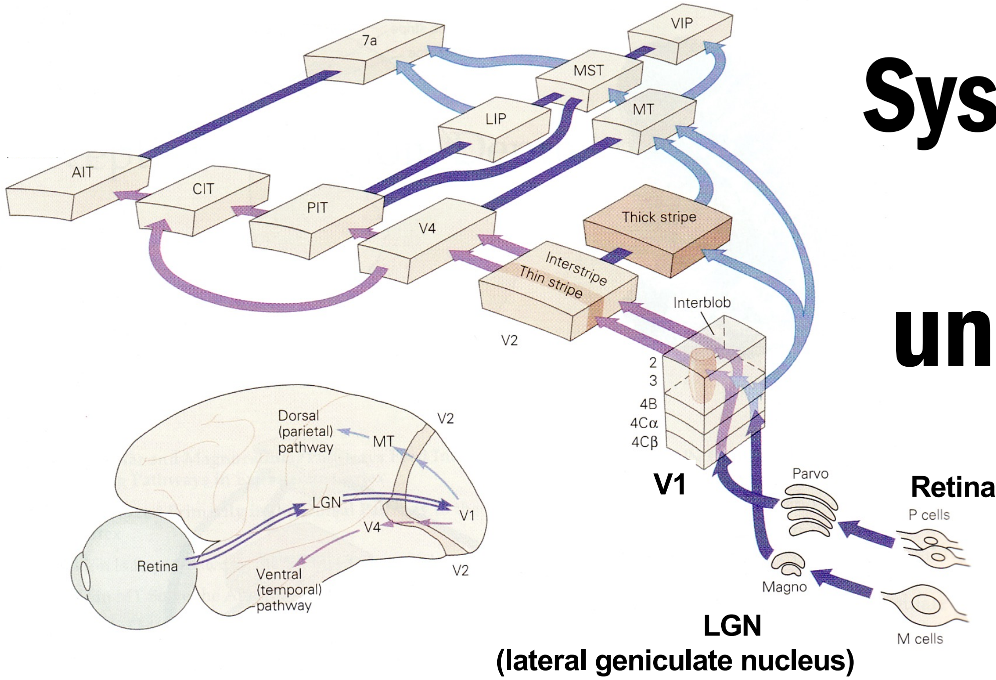
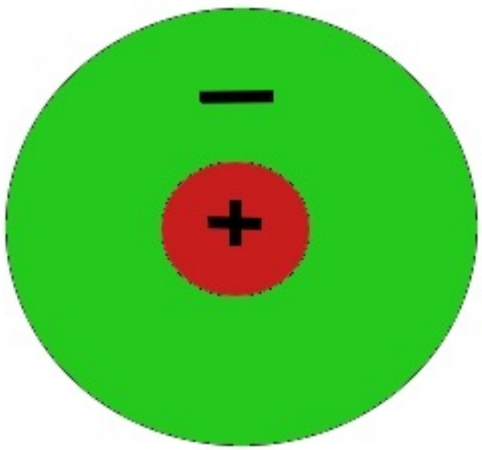


Systems neuroscience approach to understanding vision

Mackenzie Mathis, PhD &
Thomas Sainsbury, PhD

The beginning of the visual system: retinal ganglion cells and the Center-Surround Model



Center-Surround Receptive Fields
The center-surround model is based on the organization of the RGCs' receptive fields, which are the specific areas of the retina where light stimuli can influence the firing rate of the cell. These receptive fields are structured in a center-surround arrangement, consisting of two distinct parts:

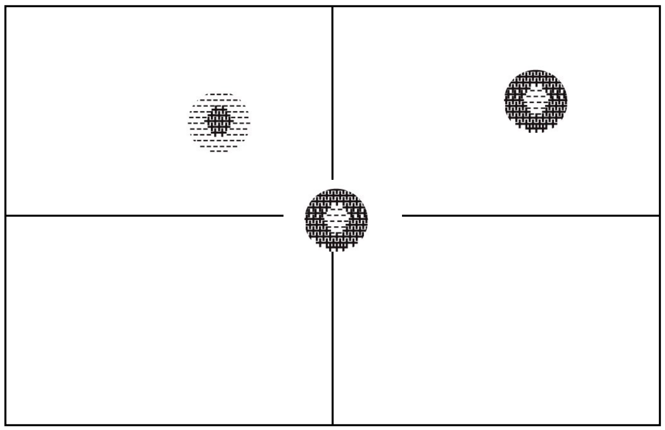
•**Center**: The central part of the receptive field can either be excitatory (increasing the cell's firing rate when stimulated by light) or inhibitory (decreasing the cell's firing rate when stimulated by light).

•**Surround**: The surrounding part of the receptive field has the opposite effect to the center. If the center is excitatory, the surround is inhibitory, and vice versa.

Image adapted from https://muse.union.edu/visualmotion/center-surround/

The beginning of the visual system: retinal ganglion cells and the Center-Surround Model

Field of view:



If you record from a retinal ganglion cell (RGC).
They fire APs with generally two types of responses:

"ON-center":
↑ AP- frequency
in the center of the receptive field (RF)

"OFF center"
↓ AP-frequency
in the center of the RF

Adapted from BIO-311

What The Frog's Eye Tells The Frog's Brain

- one of the most cited papers of the era!

1940 PROCEEDINGS OF THE IRE November

What the Frog's Eye Tells the Frog's Brain*

J. Y. LETTVIN†, H. R. MATURANA‡, W. S. McCULLOCH‖, SENIOR MEMBER, IRE, AND W. H. PITTS‖

Summary—In this paper, we analyze the activity of single fibers in the optic nerve of a frog. Our method is to find what sort of stimulus causes the largest activity in one nerve fiber and then what is the exciting aspect of that stimulus such that variations in everything else cause little change in the response. It has been known for the past 20 years that each fiber is connected not to a few rods and cones in the retina but to very many over a fair area. Our results show that for the most part within that area, it is not the light intensity itself but rather the pattern of local variation of intensity that is the exciting factor. There are four types of fibers, each type concerned with a different sort of pattern. Each type is uniformly distributed over the whole retina of the frog. Thus, there are four distinct parallel distributed channels whereby the frog's eye informs his brain about the visual image in terms of local pattern independent of average illumination. We describe the patterns and show the functional and anatomical separation of the channels. This work has been done on the frog, and our interpretation applies only to the frog.

it moves like one. He can be fooled easily not only by a bit of dangled meat but by any moving small object. His sex life is conducted by sound and touch. His choice of paths in escaping enemies does not seem to be governed by anything more devious than leaping to where it is darker. Since he is equally at home in water and on land, why should it matter where he lights after jumping or what particular direction he takes? He does remember a moving thing providing it stays within his field of vision and he is not distracted.

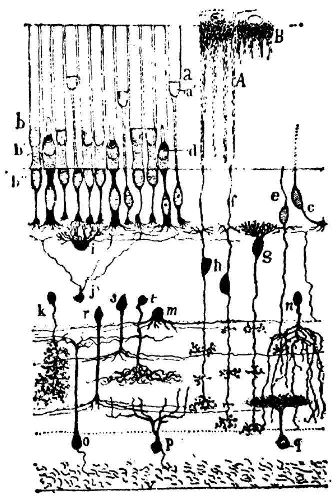
Anatomy of Frog Visual Apparatus

The retina of a frog is shown in Fig. 1(a). Between the rods and cones of the retina and the ganglion cells,

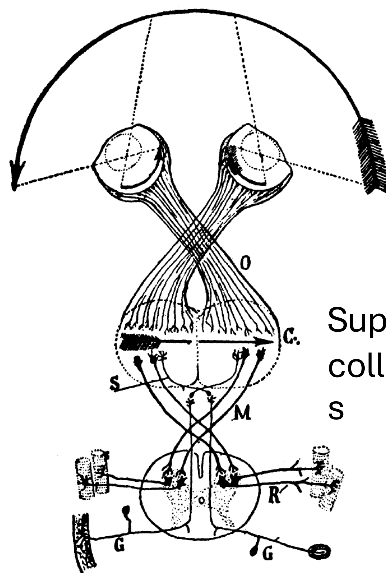
Jerome Lettvin (at MIT; Wikipedia)

1950s: On the hunt for feature detectors ...

"The assumption has always been that the eye mainly **senses light,** whose local distribution is transmitted to the brain in a kind of copy by a mosaic of impulses," – J. Lettvin



(a)



(b)

Superior colliculu s

Ramon y Cajal

Anatomy-guided logic: "Clearly, such an arrangement would not allow for good resolution were the retina meant to map an image in terms of light intensity point by point into a distribution of excitement in the optic nerve."

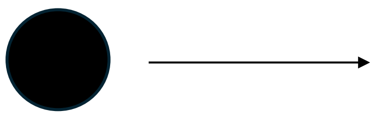
- the center-surround model felt incomplete
- Could retinal ganglion cells encode additional features such as contrast and motion?

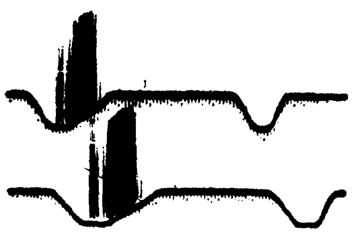
The experiments:

"First, we should find a way of recording from single myelinated and unmyelinated fibers in the intact optic nerve.
Second, we should present the frog with as wide a range of visible stimuli as we could, not only spots of light but things he would be disposed to eat, other things from which he would flee, sundry geometrical figures, stationary and moving about, etc. From the variety of stimuli we should then try to discover what common features were abstracted by whatever groups of fibers we could find in the optic nerve. Third, we should seek the anatomical basis for the grouping."

Image via GPT-4

The experiments:





(a)

(b)

(f)

This record is from a single fiber in the optic nerve.
A: The fiber discharge to movement of the edge of a 3^0 black disk passed in one direction but not to the reverse movement. (Time marks, 20 per second.)
B: The same fiber shown here giving a continued response when the edge stops in the field.
F: disk moves, stops, moves, stops, moves

What The Frog's Eye Tells The Frog's Brain

The eye tells the brain much more than just light intensity!

Lettvin's work identified different types of RGCs that responded selectively to specific features of the visual environment, such as:

•**Edge detectors**: Cells that responded to edges or contours within the visual scene.

•**Motion detectors**: Cells that were particularly sensitive to moving objects.

•**Dimming detectors**: Cells that detected decreases in light intensity, which could indicate the presence of a predator.

What The Frog's Eye Tells The Frog's Brain

"Bug Perceivers"
One of the most famous findings from Lettvin's work.

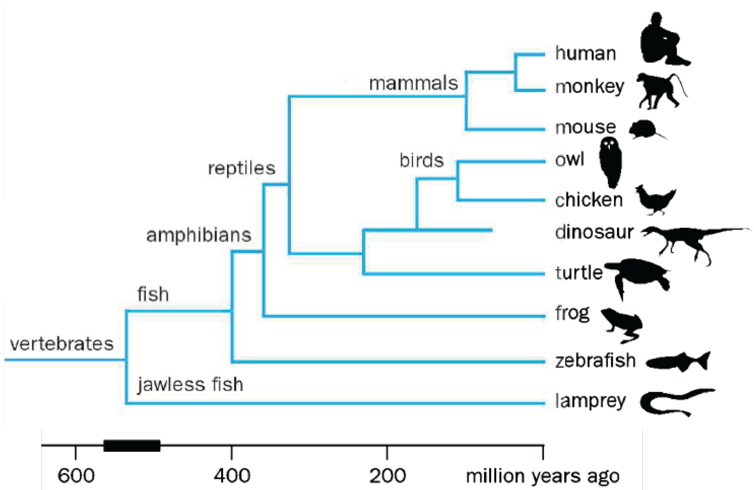
These were neurons in the frog's retina that responded optimally to small, dark objects moving against a lighter background—essentially, the types of stimuli that resemble prey such as flies.

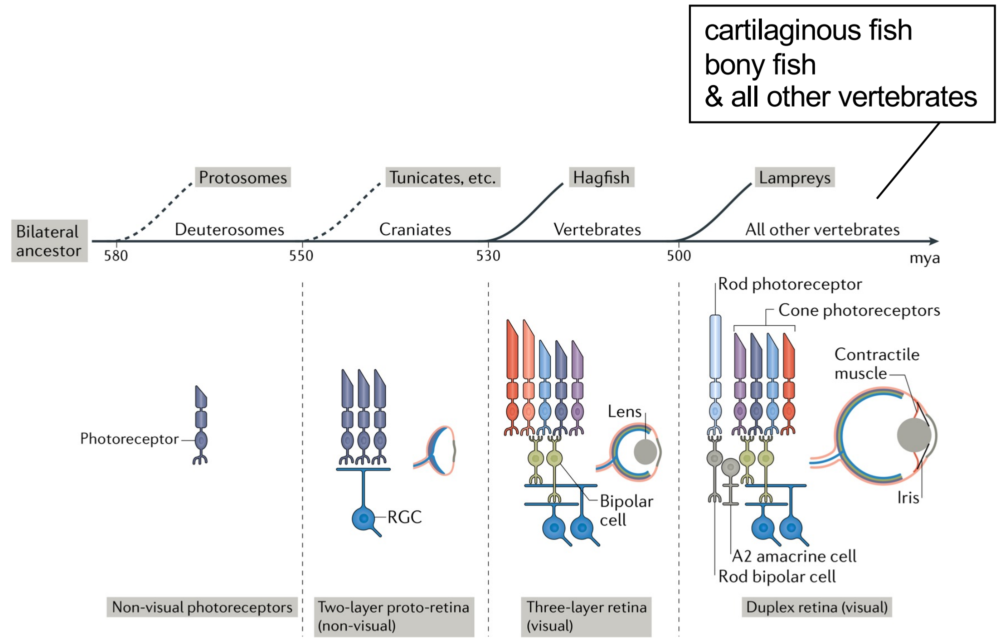
This discovery suggested that the frog's visual system – even at the level of the retina -- was finely tuned to detect and respond to specific stimuli that were crucial for survival, such as food and threats!

"Could one better describe a system for detecting an accessible bug?"

Image via GPT-4

The retina has evolved in the predecessors of fish & all other vertebrates ~ 500 - 530 Mya

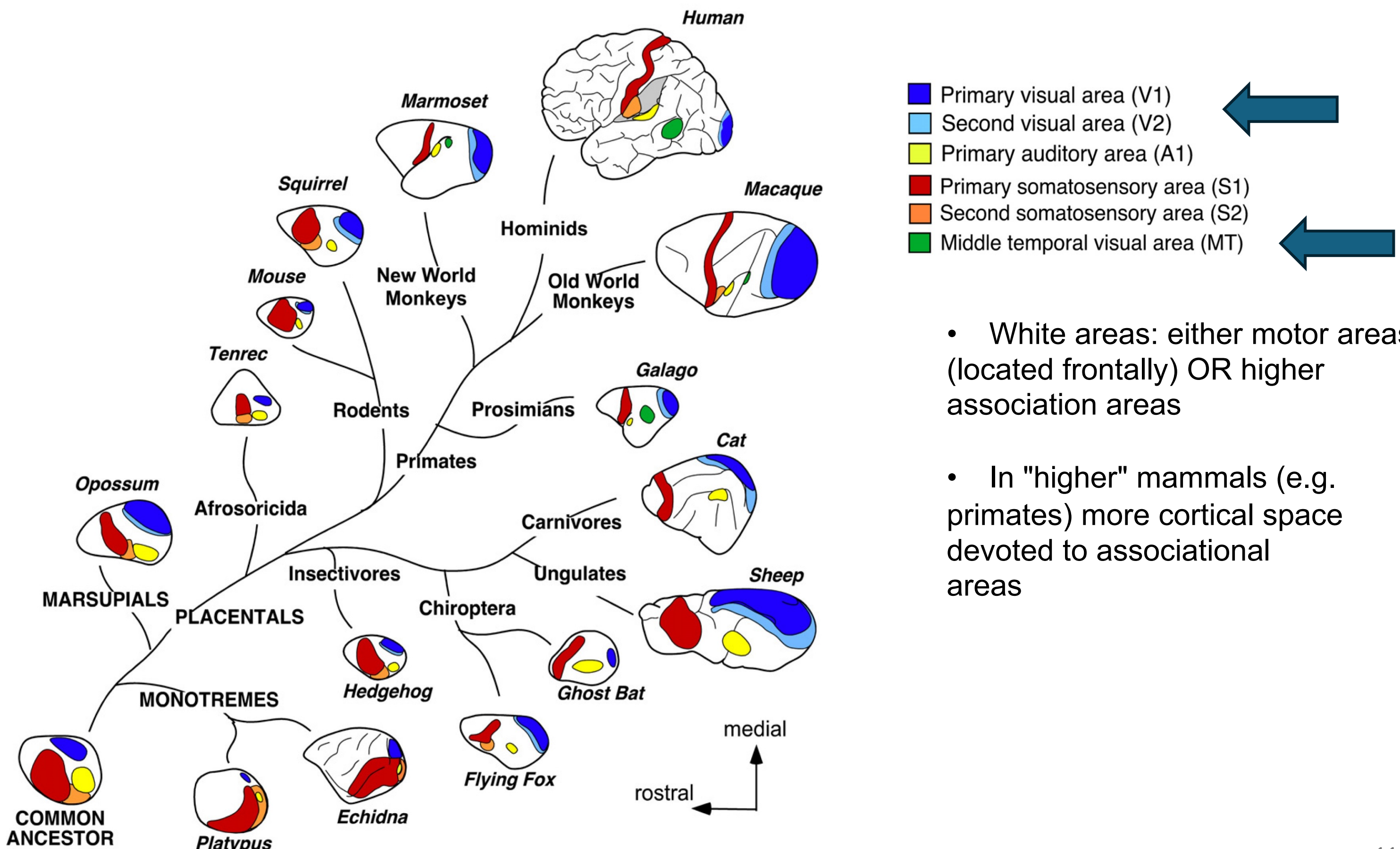




Luo, Fig. 12-2:

Baden et al. 2020
Nature Reviews Neuroscience

Visual areas have evolved & persisted across many species

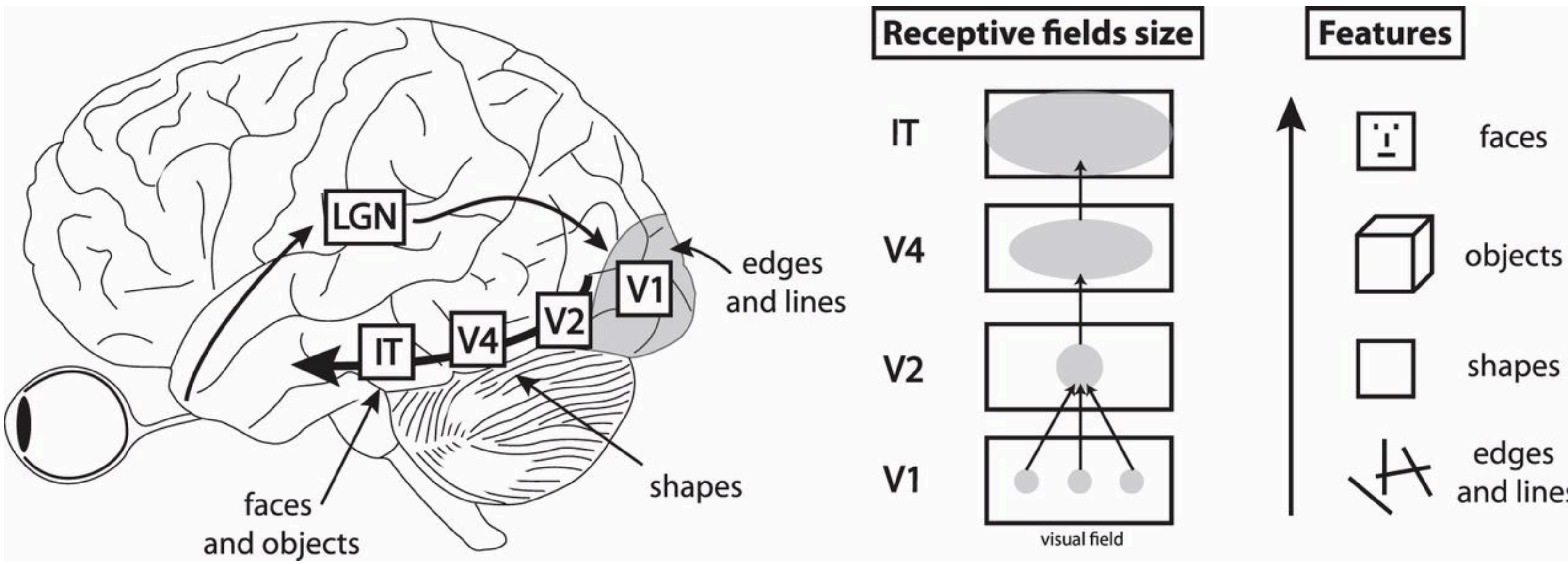


- White areas: either motor areas (located frontally) OR higher association areas

- In "higher" mammals (e.g. primates) more cortical space devoted to associational areas

Leah Krubitzer 2007, *Neuron*

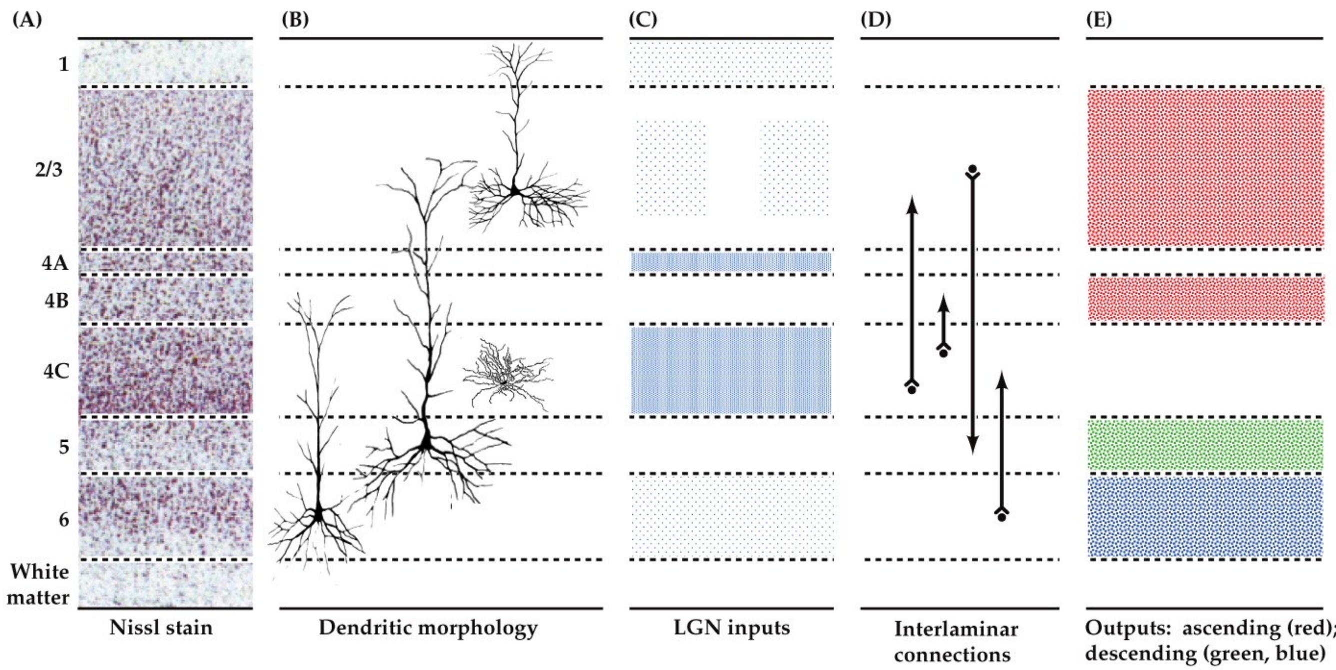
Hierarchical visual processing



- V1 neurons are most sensitive to low-level features, such as edges and lines.
- In higher visual areas, like V4 and IT, receptive fields are larger, and neurons are sensitive to complex features, such as shapes and objects.
- Responses of high-level neurons are fully determined by the neural firing of lower-level neurons. For example, the neural firing to a square is determined by the neural firing for two vertical and two horizontal lines.

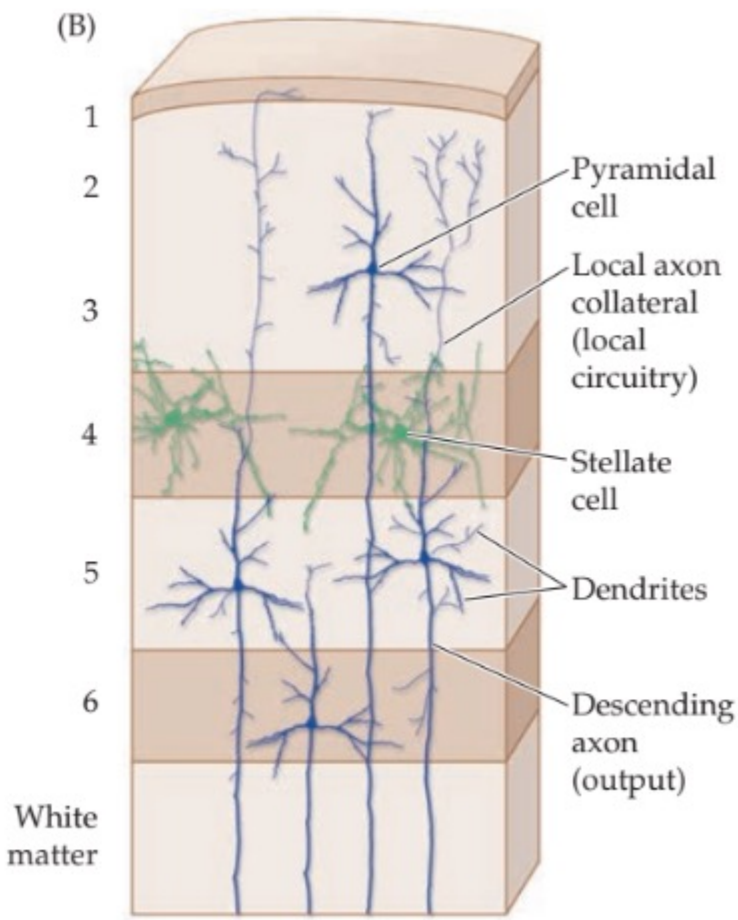
From: When crowding of crowding leads to uncrowding
Journal of Vision. 2013;13(13):10. doi:10.1167/13.13.10

Organization of the visual cortex (a typical primary sensory cortex)



- **inputs from LGN: to Layer 4**, and "patchy" to L2/3, L1
- in-between layer connections: **L4 → L2/3** **L2/3 → L5**
- outputs:
    - L2/L3 "ascending" ; "associational"; that is to higher cortical areas (e.g. V2)
    - L5 "descending" to superior colliculus (!)
    - L6 "cortico-thalamic" back to visual thalamus

Organization of the visual cortex (a typical primary sensory cortex)



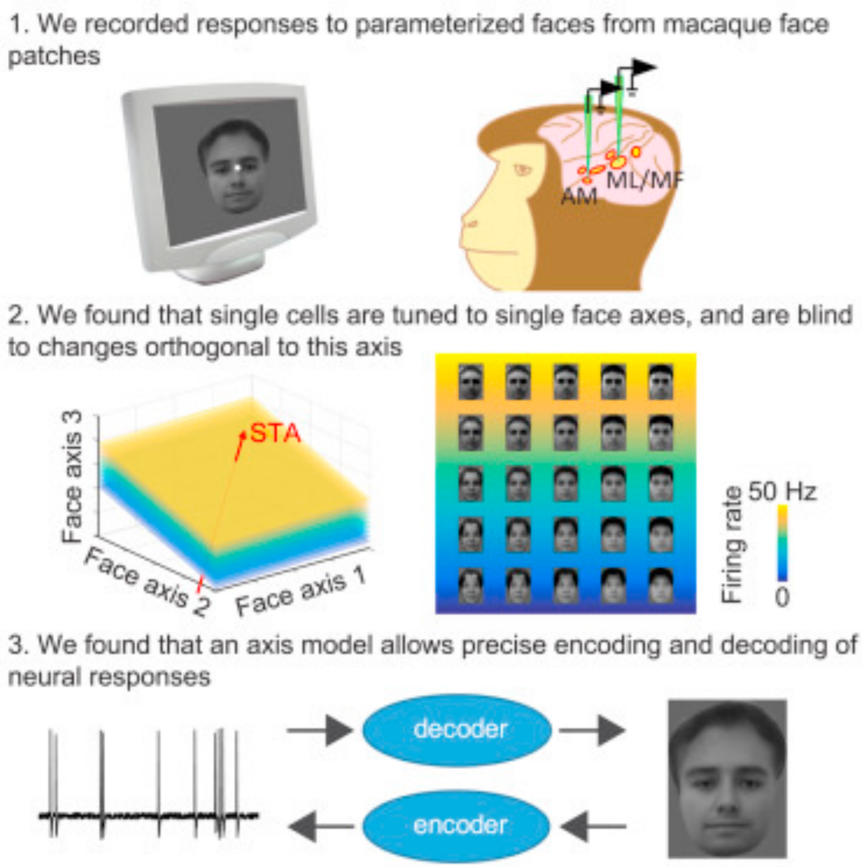
- **inputs from LGN: to Layer 4**, and "patchy" to L2/3, L1
- in-between layer connections: **L4 → L2/3**
  L2/3 → L5
- outputs:

- L2/L3 "ascending" ; "associational"; that is to higher cortical areas (e.g., V2)
- L5 "descending" to superior colliculus (!)
- L6 "cortico-thalamic" back to visual thalamus

2 broad classes of neurons:

- Spiny (pyramidal & stellate) GLUT
- Aspinous (smooth) GABA

Higher visual areas in primates: face patch neurons in inferotemporal (IT) cortex



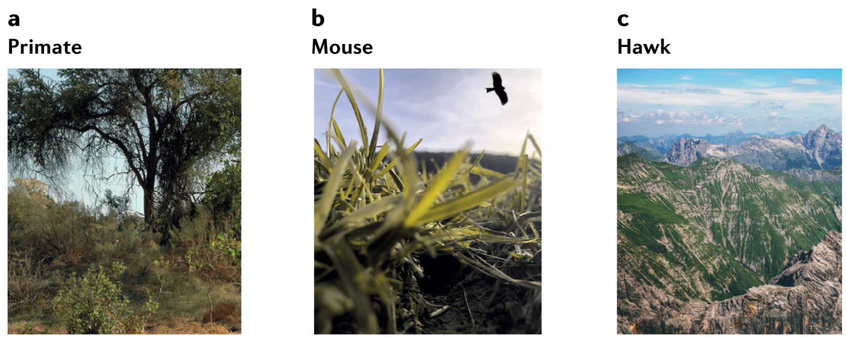
The Code for Facial Identity in the Primate Brain

Le Chang[1,*] and Doris Y. Tsao[1,2,3,*]
[1]Division of Biology and Biological Engineering, Computation and Neural Systems, Caltech, Pasadena, CA 91125, USA
[2]Howard Hughes Medical Institute, Pasadena, CA 91125, USA
[3]Lead Contact
*Correspondence: lechang@caltech.edu (L.C.), dortsao@caltech.edu (D.Y.T.)

http://dx.doi.org/10.1016/j.cell.2017.05.011

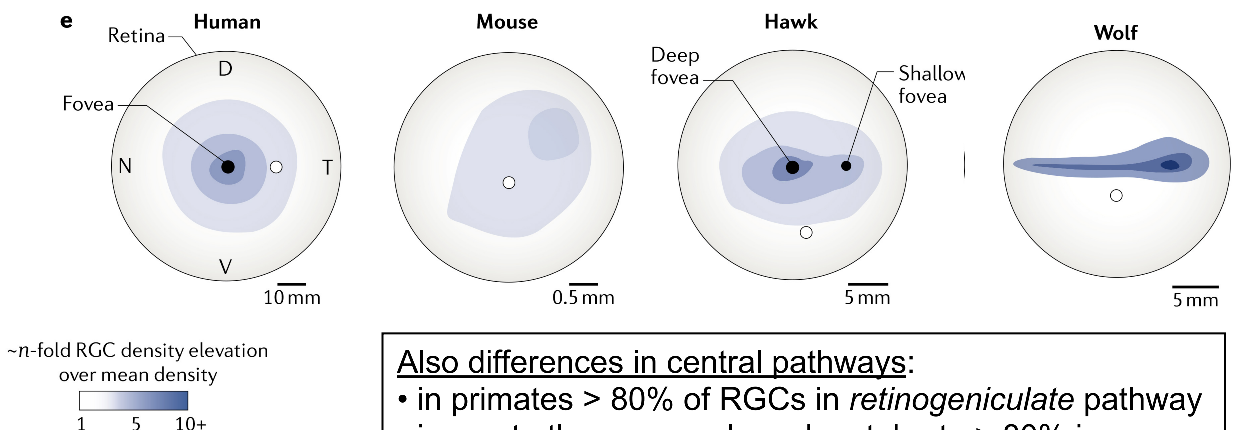
•Facial images can be linearly reconstructed using responses of ~200 face cells

Across species, there are evolutionarily-drivers to the visual pathway



Across vertebrates species, retina organization is different according to the requirement of the ecological niche

Baden et al. 2020 Nat. Reviews:
"Understanding the retinal basis of vision across species"

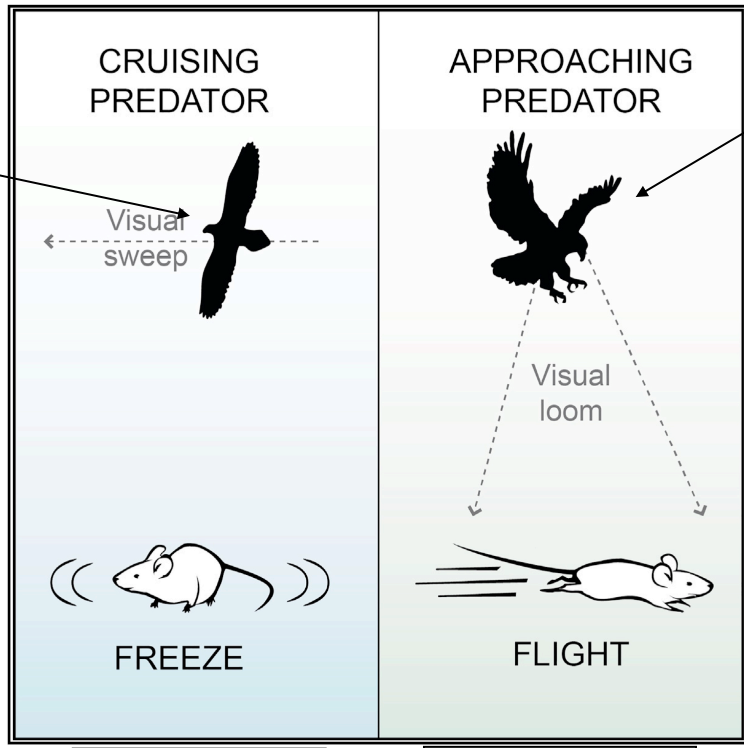


<u>Also differences in central pathways</u>:
- in primates > 80% of RGCs in *retinogeniculate* pathway
- in most other mammals and vertebrate > 80% in *retinotectal* pathway!

Prey / predator relations as a drive for evolution

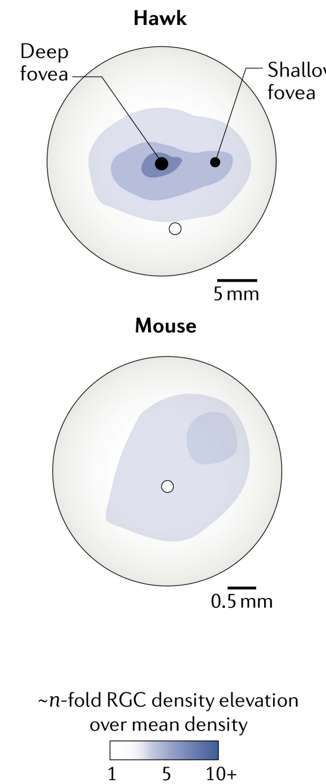
<u>Example</u>: hawk (predator) and mouse (prey)

<u>Buzzard</u>:
- high visual acuity
- motion detection!



<u>Buzzard</u>:
- Hunting behavior

<u>Mouse</u>:
- appropriate defense behavior
(depending on visual input information!)

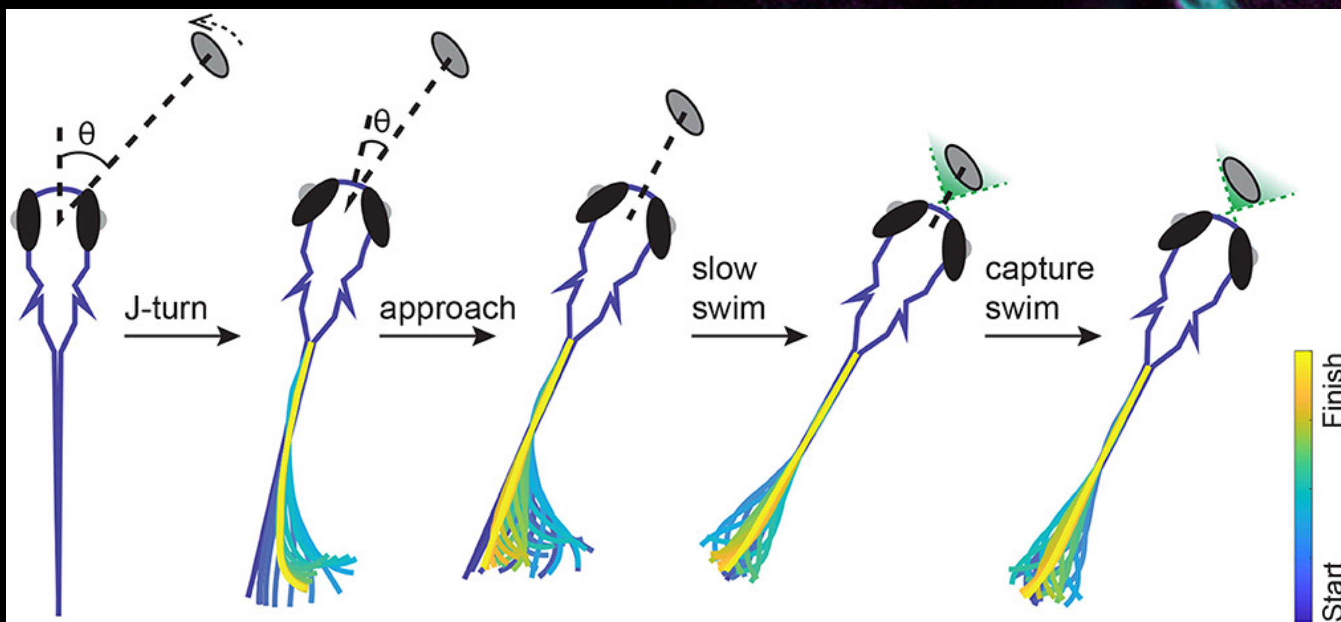


- Freeze (to avoid motion detection!)

- Flight / escape (when predator comes close)

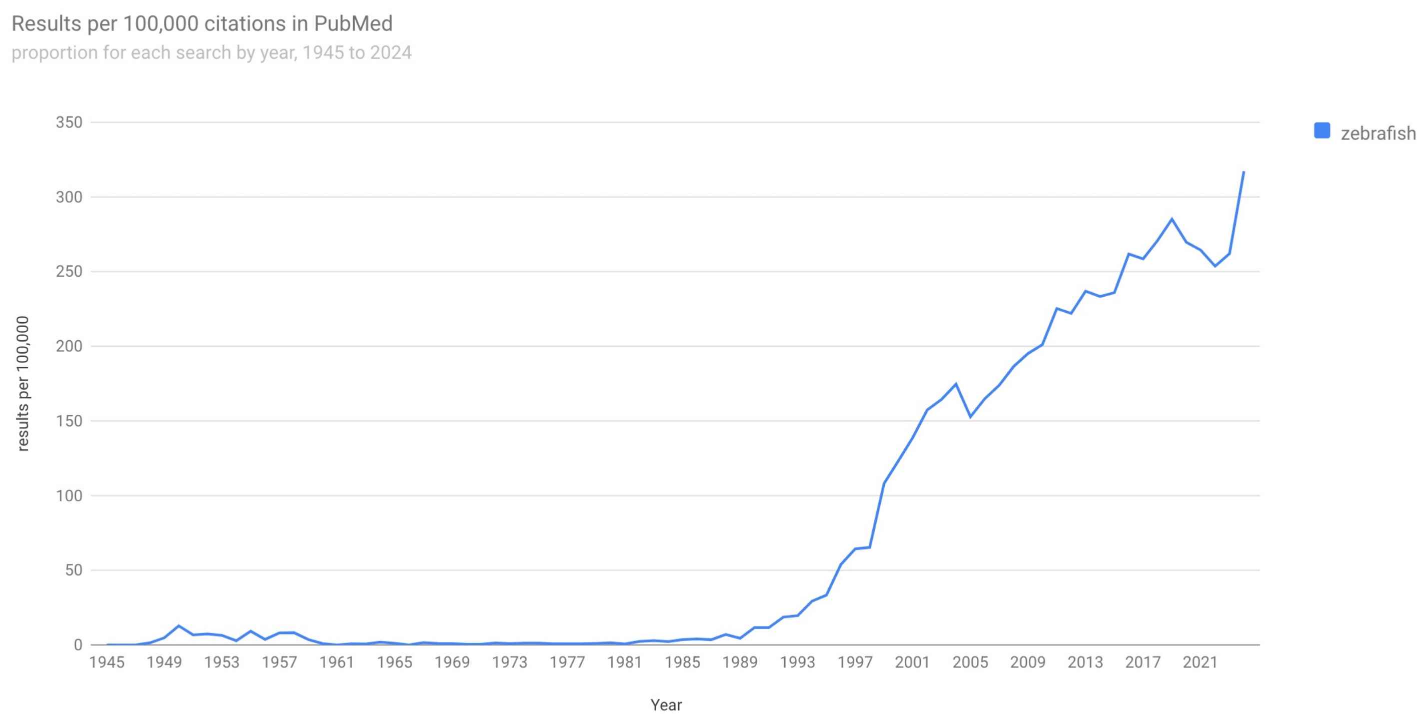
from: De Franceschi et al. 2016, Curr. Biology

Zebrafish as a model organism in systems neuroscience: whole brain access during prey capture & predator avoidance ...

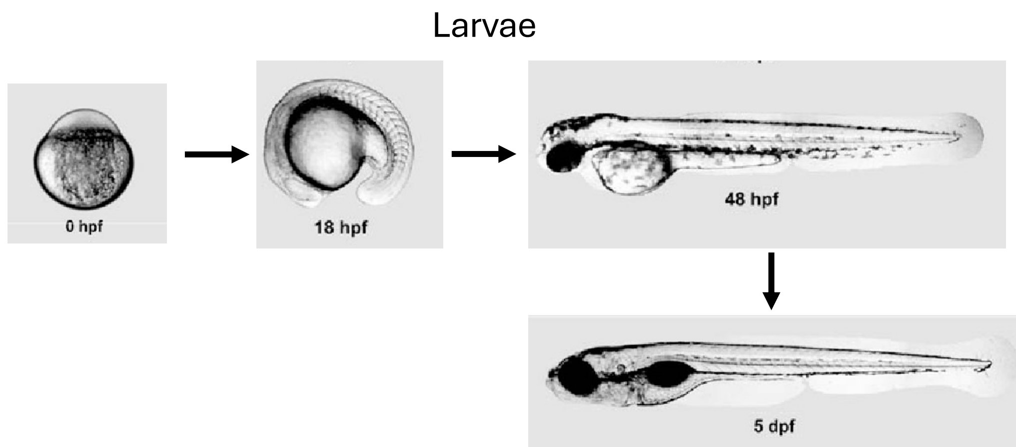


https://doi.org/10.3389/fncir.2023.1087993

Using zebrafish as a model organism in neuroscience



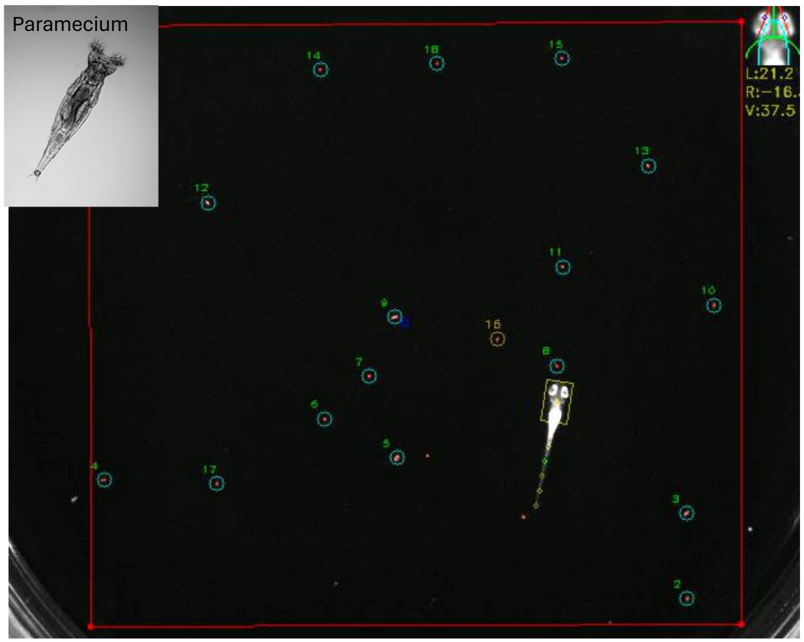
The larval zebrafish



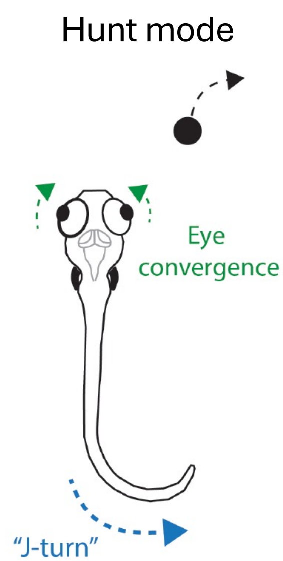
- Visual research in zebrafish typically is done on the larval zebrafish
- Quick development: single cell to a fully formed fish that is capable of visually guided behavior in just 5 days
- Very small: at 5 days post fertilization (dpf) approximately 4 mm long with a brain 100,000 neurons
- Protected animals from 5 days onwards

(video courtesy of Kristian Herrera)

The larval zebrafish uses vision to hunt

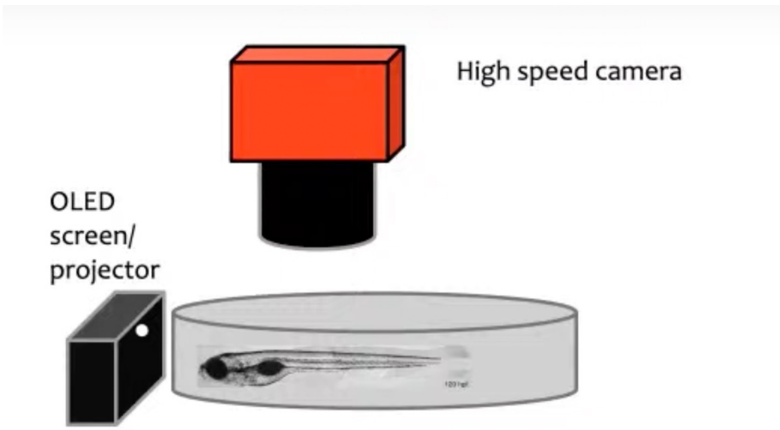


(Lagogiannis et al., 2020)



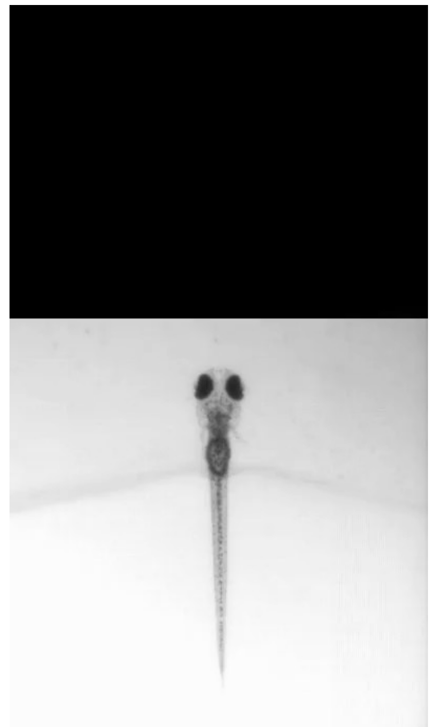
- Hunting is characterized by first converging their eyes and then making small "J-turns" to orient them towards the prey.
- Zebrafish are much worse at hunting in the dark (Ghatan et al., 2005).
- Why do the fish converge their eyes?

Immobilized fish try to hunt stimuli that look like paramecium



Fish can be placed in a blob of agarose – a hydrogel which holds the fish in place when it hardens

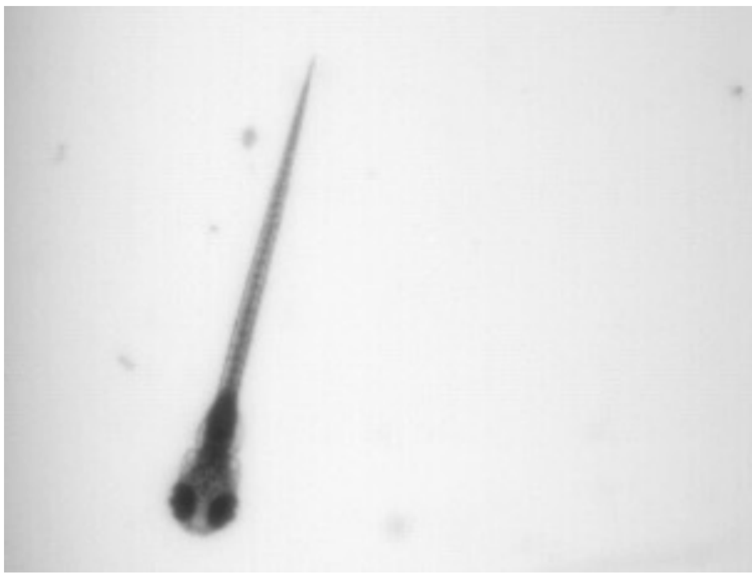
Agarose can then be cut away from around the tail to allow it to move whilst fixing the head in position



(Semmelheck 2014)

Visually evoked visual escapes

Escape response

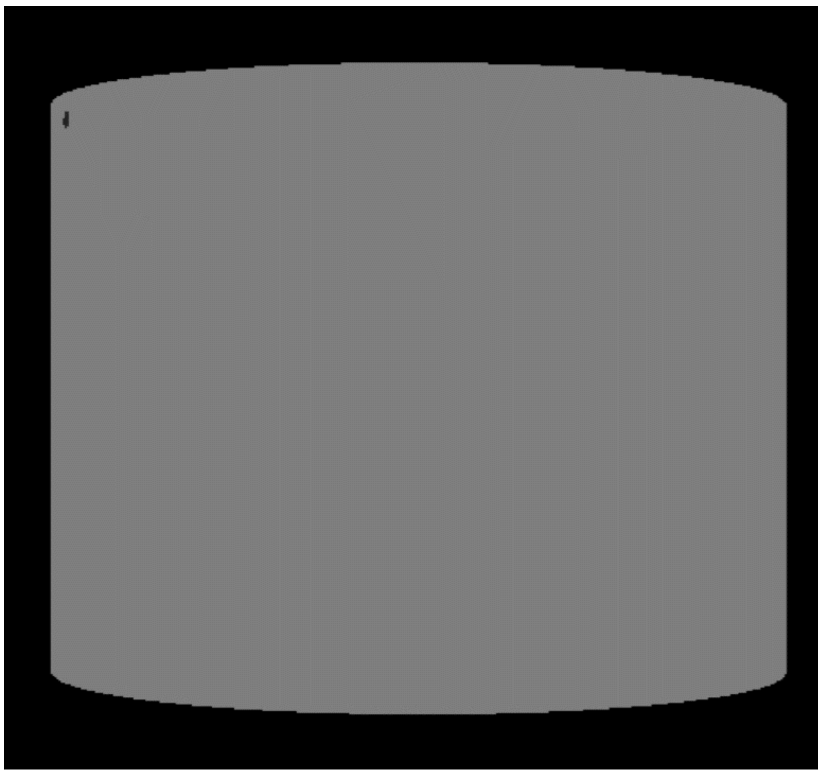


(Temizer et al., 2015)

A C-start is a turn where the moves to a high angle forming a c shape, this moves the fish away from the predator so that it can then swim away

Can also be induced by loud auditory stimuli

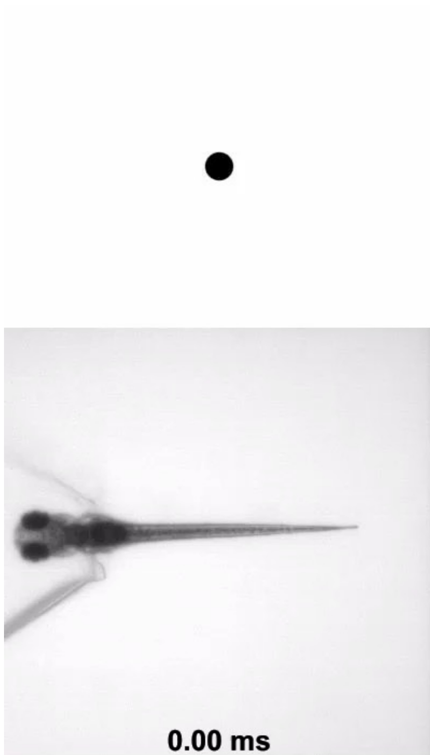
The looming stimulus: a visual representation of a predator striking

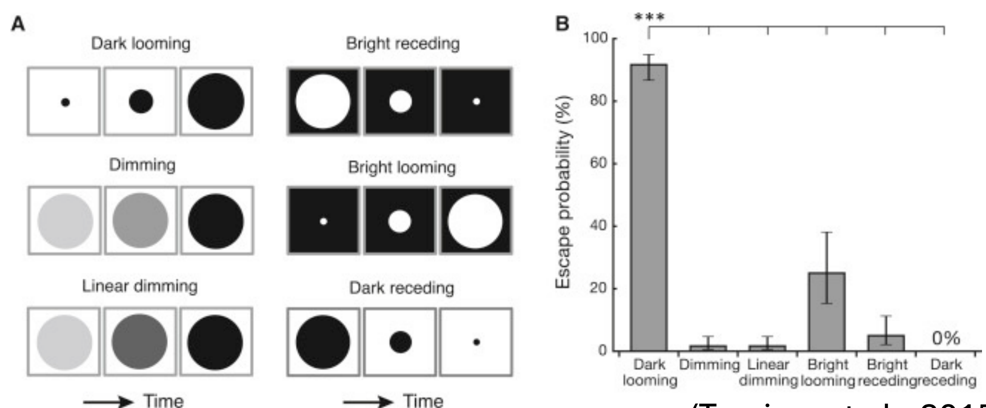


- The looming stimulus looks like an object coming towards you at high velocity (like a predator)
- Reactions to looming stimuli are very well conserved in evolution from flies to mammals
- even human children will cover their face when they are shown a loom.

(Ball et al., 1979; Sun et al., 2008; Temizer et al., 2015; Maier et al., 2004)

Looming stimulus elicits escape responses





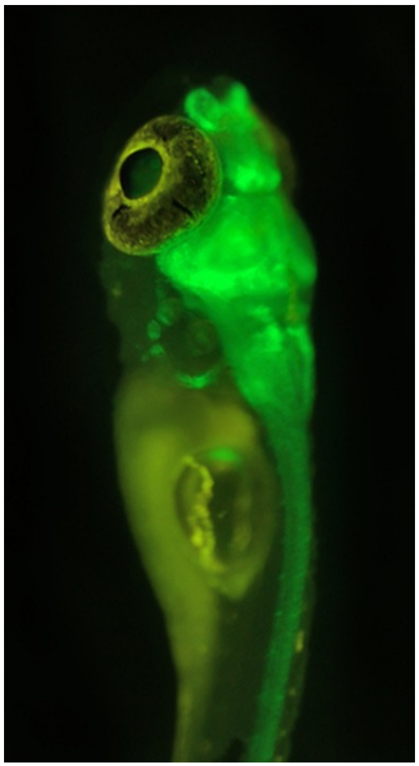
(Temizer et al., 2015)

Escape responses only happen for a dark loom

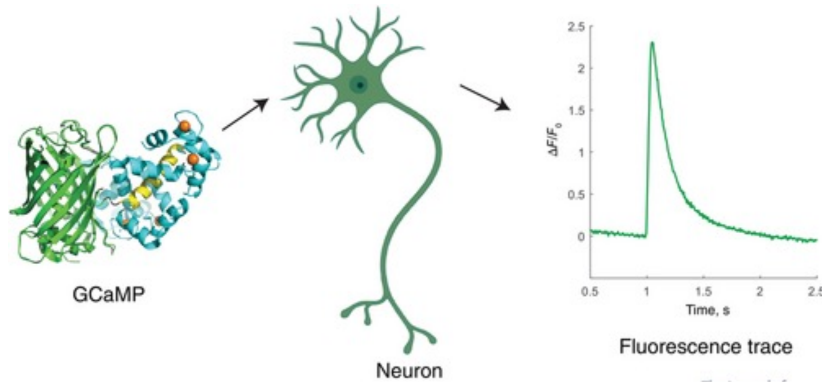
1. How does the visual system extract this information?

2. Where in the visual system does this happen?

Zebrafish are optically transparent - ideal for calcium imaging with GCaMP



HuC:H2B-GCaMP6s



- Genetically encoded calcium indicator (GECI)
- Fusion of GFP and calmodulin
- When a neuron fires Ca^{2+} floods into the neuron and binds to the GCaMP this causes the protein to fluoresce green light

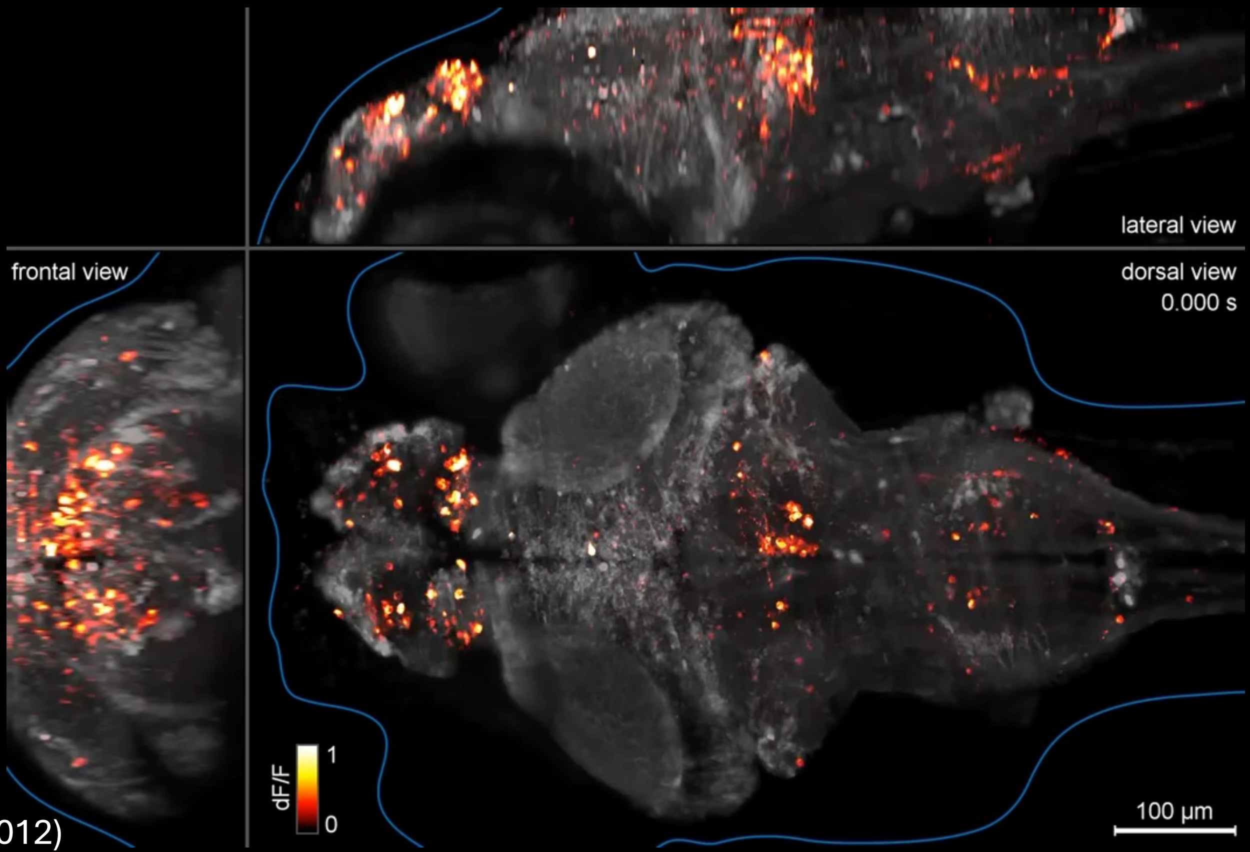
(Zang and Looger 2023)

What is ΔF/F?

$$\Delta F/F = (F - F_0)/F_0,$$

F_0 = baseline Fluorescence

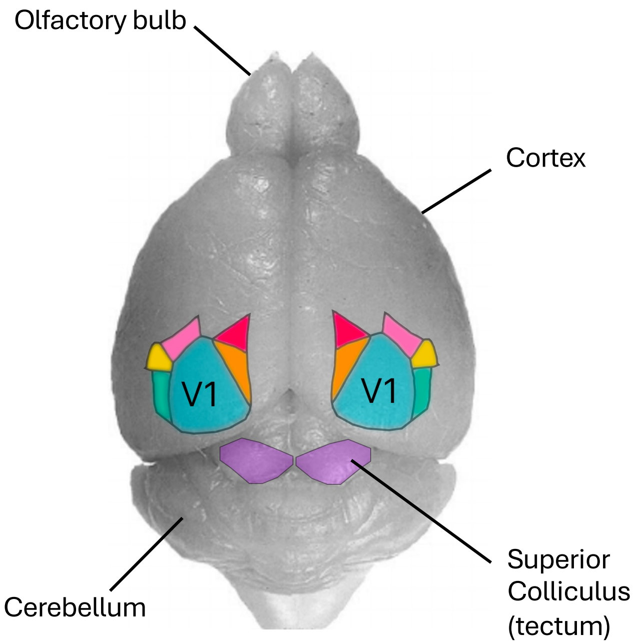
Recording from nearly every neuron in the brain!



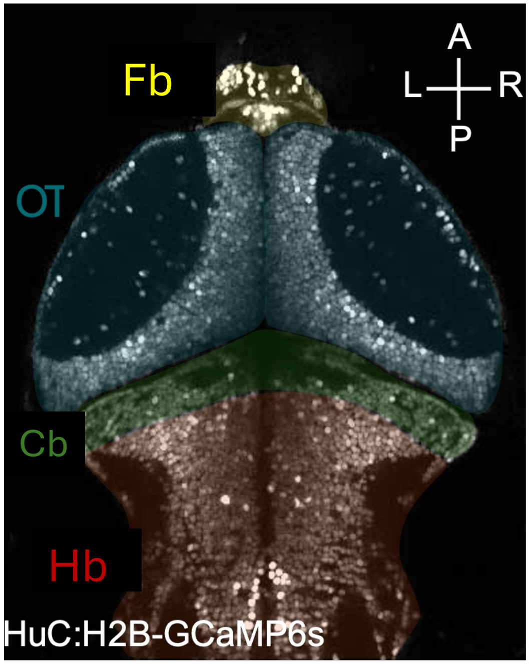
(Ahrens et al., 2012)

Zebrafish general neuroanatomy

The mouse brain



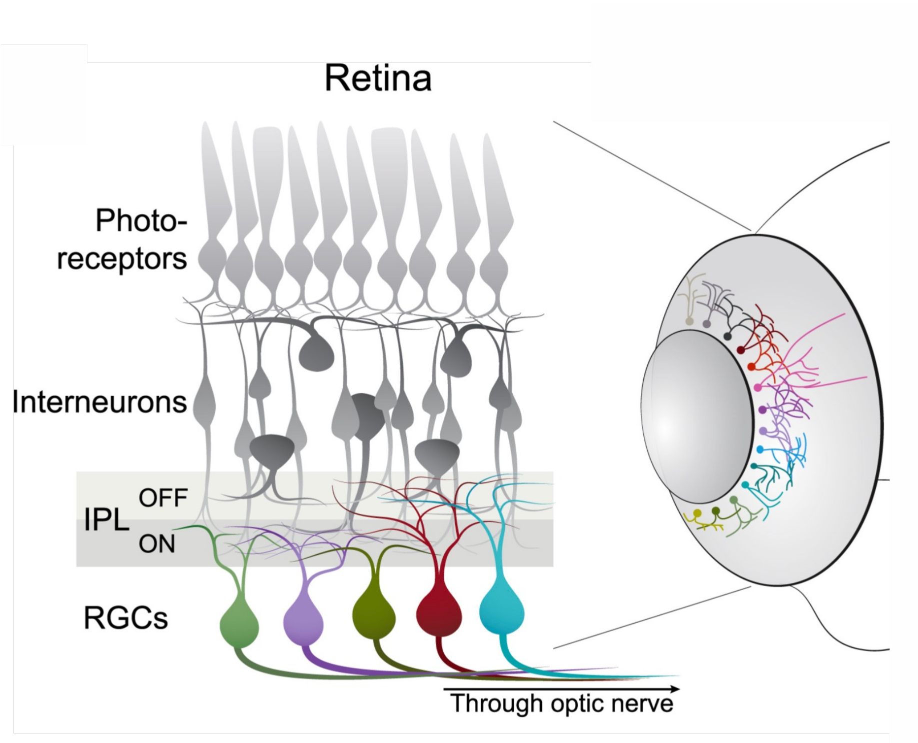
Zebrafish brain



Fb = forebrain
OT = optic tectum
Cb = cerebellum
Hb = Hind brain

- No cortex!
- Optic tectum is the main visual area and sits on the dorsal surface of the midbrain
- The optic tectum is homologous to the superior colliculus in mammals
- Contains a large neuropil region where neurons from the retina provide visual input.

The retina of fish has similar structure to mammals



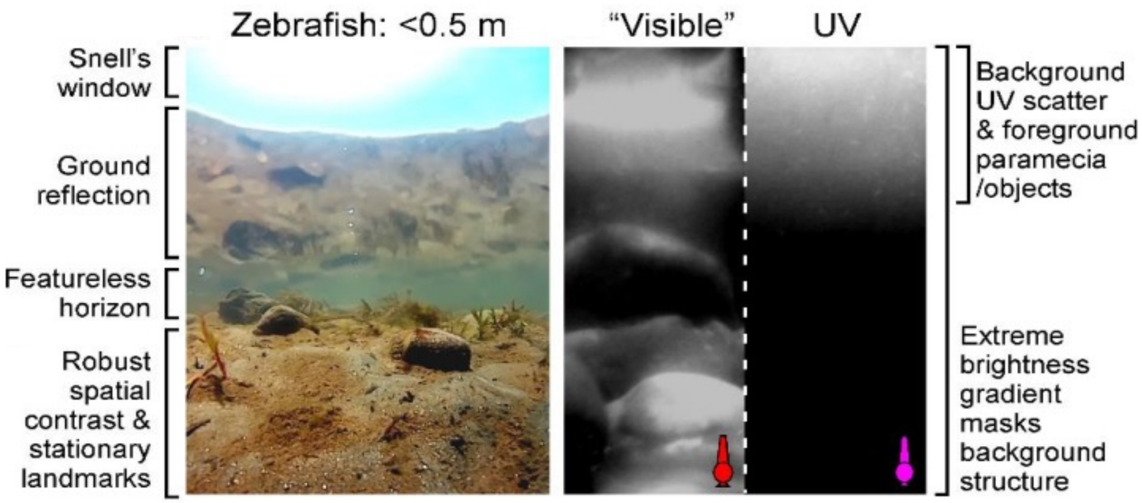
Photoreceptors:
Rods and cones – translate incoming light into electrical signals

Bipolar cells:
Transmit light to retinal ganglion cells (RGCs) – ON/OFF responses

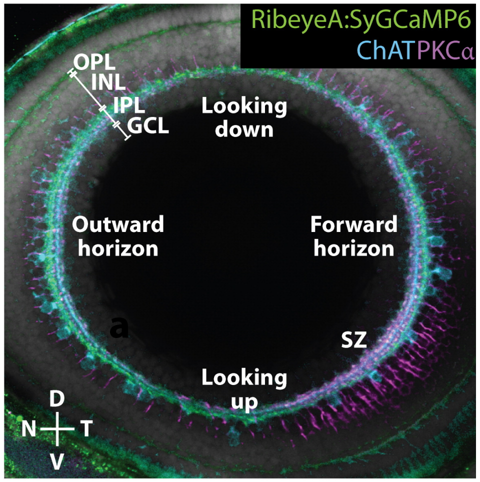
Retinal Ganglion cells:
Act as feature detectors and transmit this information to visual areas of the brain. In fish, this is primarily the optic tectum.

Fish have 4 cones (Red, Green, Blue and UV)

Why do zebrafish have UV cones?



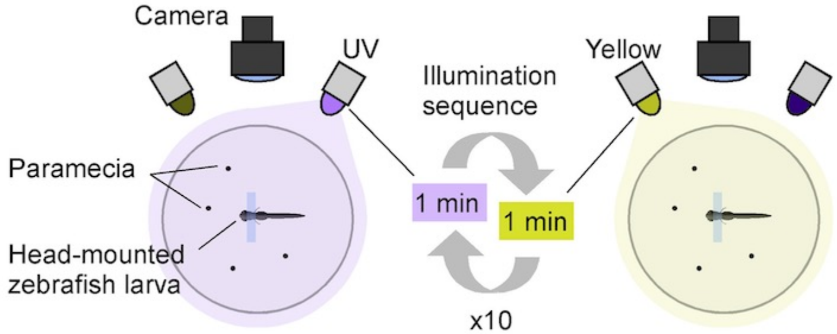
(Baden 2021)



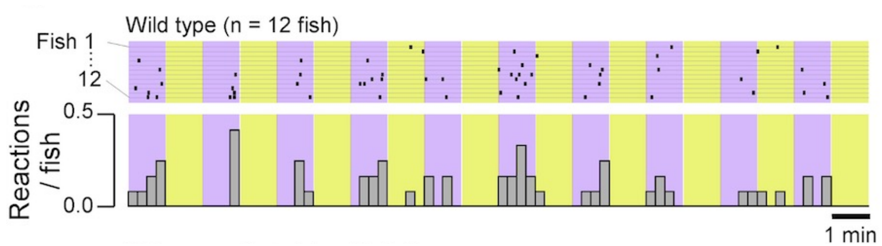
- UV light comes through the water from sunlight above but doesn't penetrate deep into the water whereas other wavelengths of light do

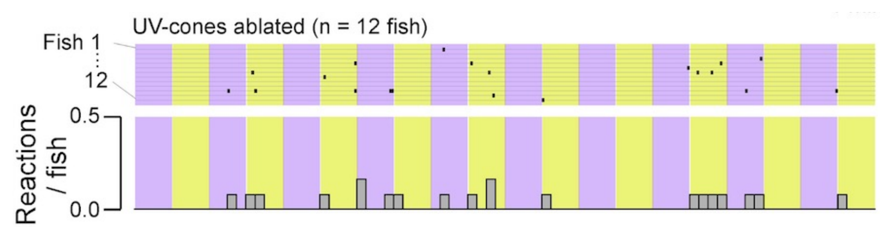
- UV cones are clustered in the ventral temporal region of the retina which corresponds to the forward upper part of the visual field

Fish hunt more in UV light than visible light



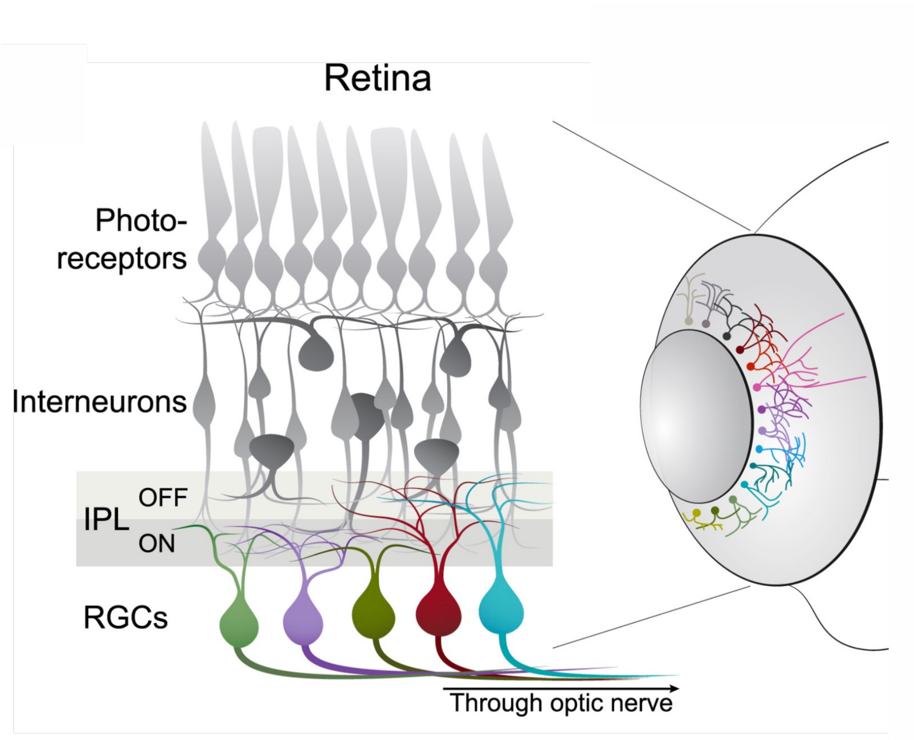
- Alternating UV and yellow light when a fish is in a petri dish full of paramecium
- The fish go into hunt mode far more frequently in the UV light
- Genetically ablating UV cones also stops the fish from hunting





(Yoshimatsu et al., 2020)

The retina of fish has similar structure to mammals



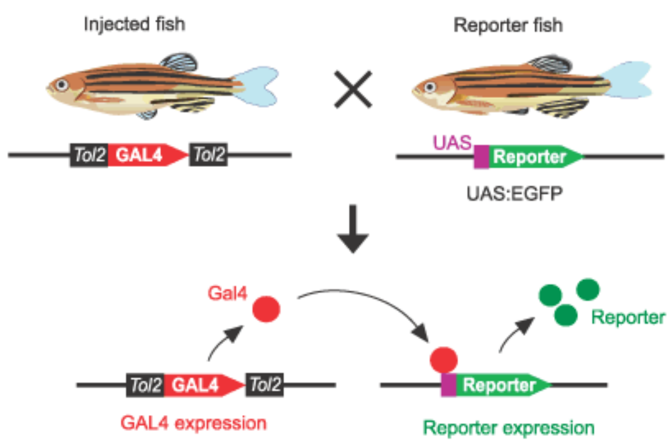
Photoreceptors:
Rods and cones – translate incoming light into electrical signals

Bipolar cells:
Transmit light to retinal ganglion cells (RGCs) – ON/OFF responses

Retinal Ganglion cells:
Act as feature detectors and transmit this information to visual areas of the brain. In fish, this is primarily the optic tectum.

Fish have 4 cones (Red, Green, Blue and UV)

Key concept: the Gal4-UAS system



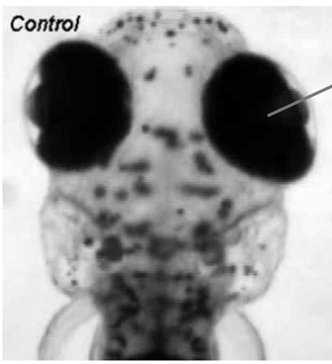
(Asakawa et al., 2008)

- The GAL4-UAS works in a similar way to the cre-recombinase system that is used in mice

- GAL4 is a transcription factor that binds to an upstream activator signal (UAS) causing transcription of the downstream reporter (such as GFP).

- If the Gal4-is placed downstream of a particular endogenous promotor then this can restrict expression to a single neuron subtype

Labeling RGC terminals with GCaMP



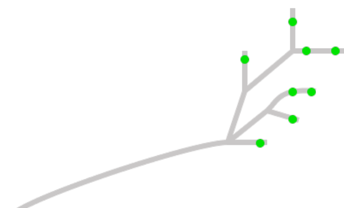
Pigment in the eye
Difficult to image

Islet2b:Gal4 x UAS:syGCaMP

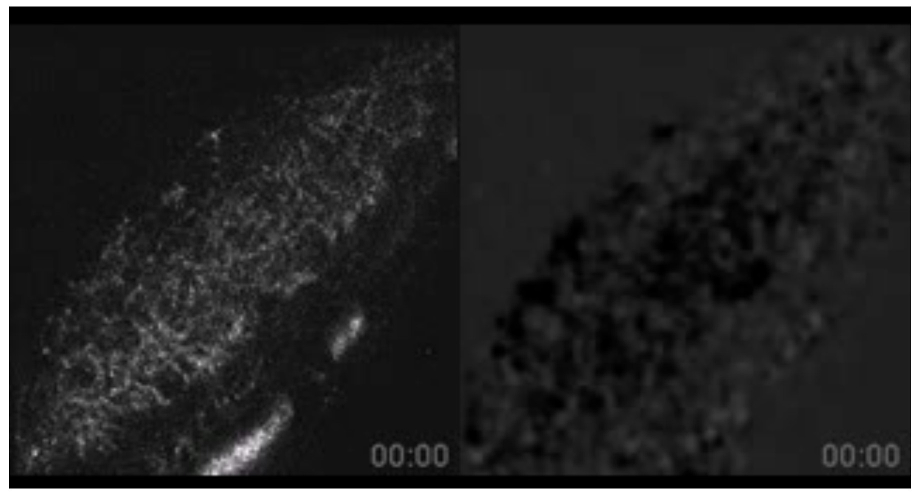
Expressed In RGCs

Synaptic protein (synaptophysin)

SyGCaMP

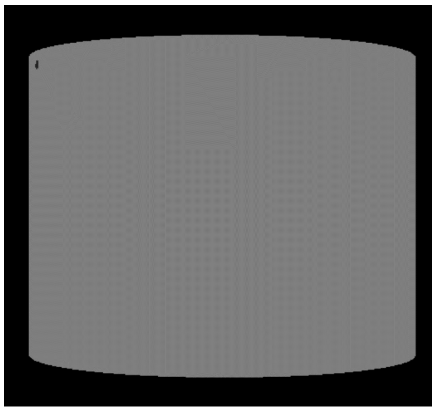


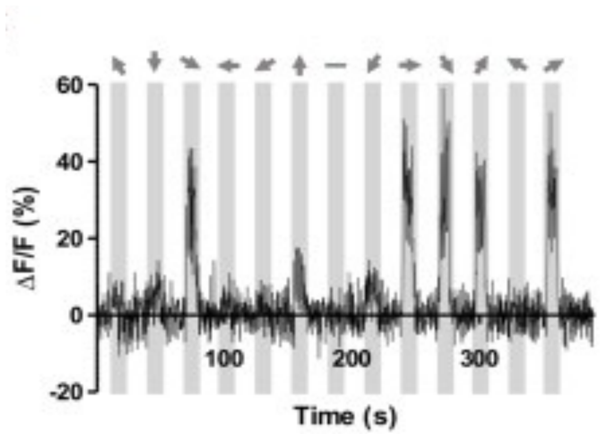
Imaging the synaptic terminals of retinal ganglion cells

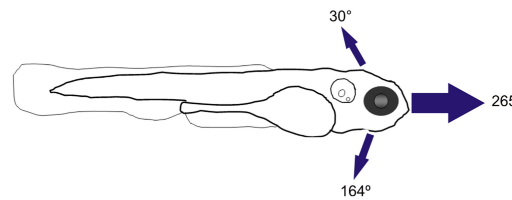


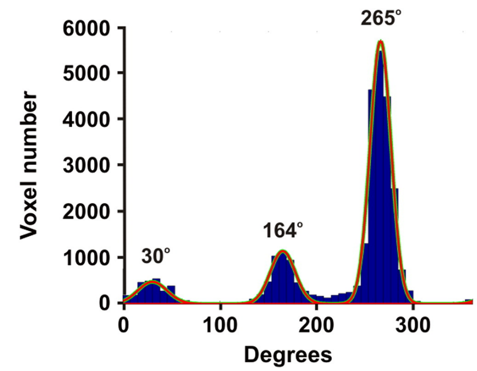
(Nikolaou et al., 2005)

RGCs are selective for directional motion

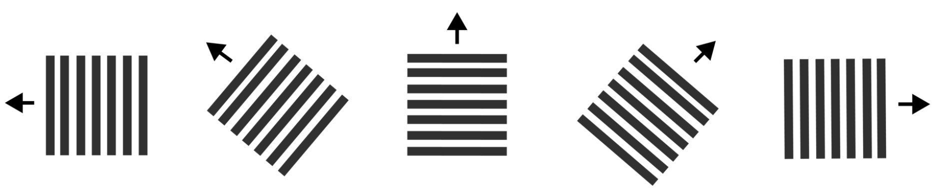








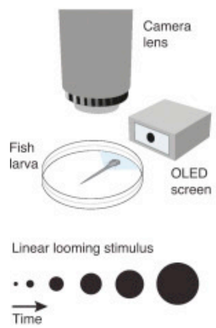
Present gratings moving in different directions

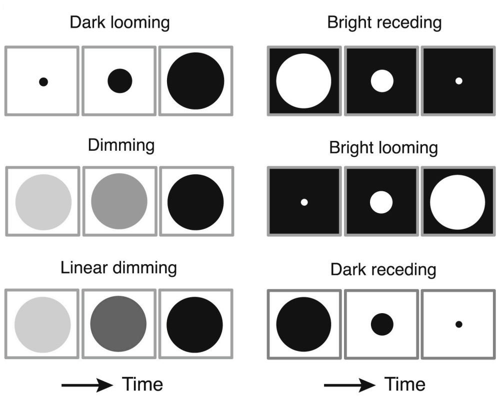


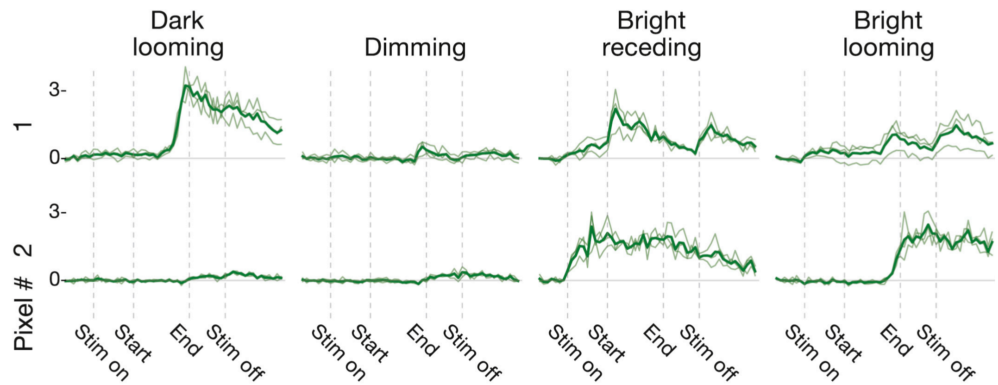
- 3 different direction selective populations
- Most are selective for the forward direction

(Nikolaou et al., 2005)

Retinal ganglion cells respond to the looming stimulus



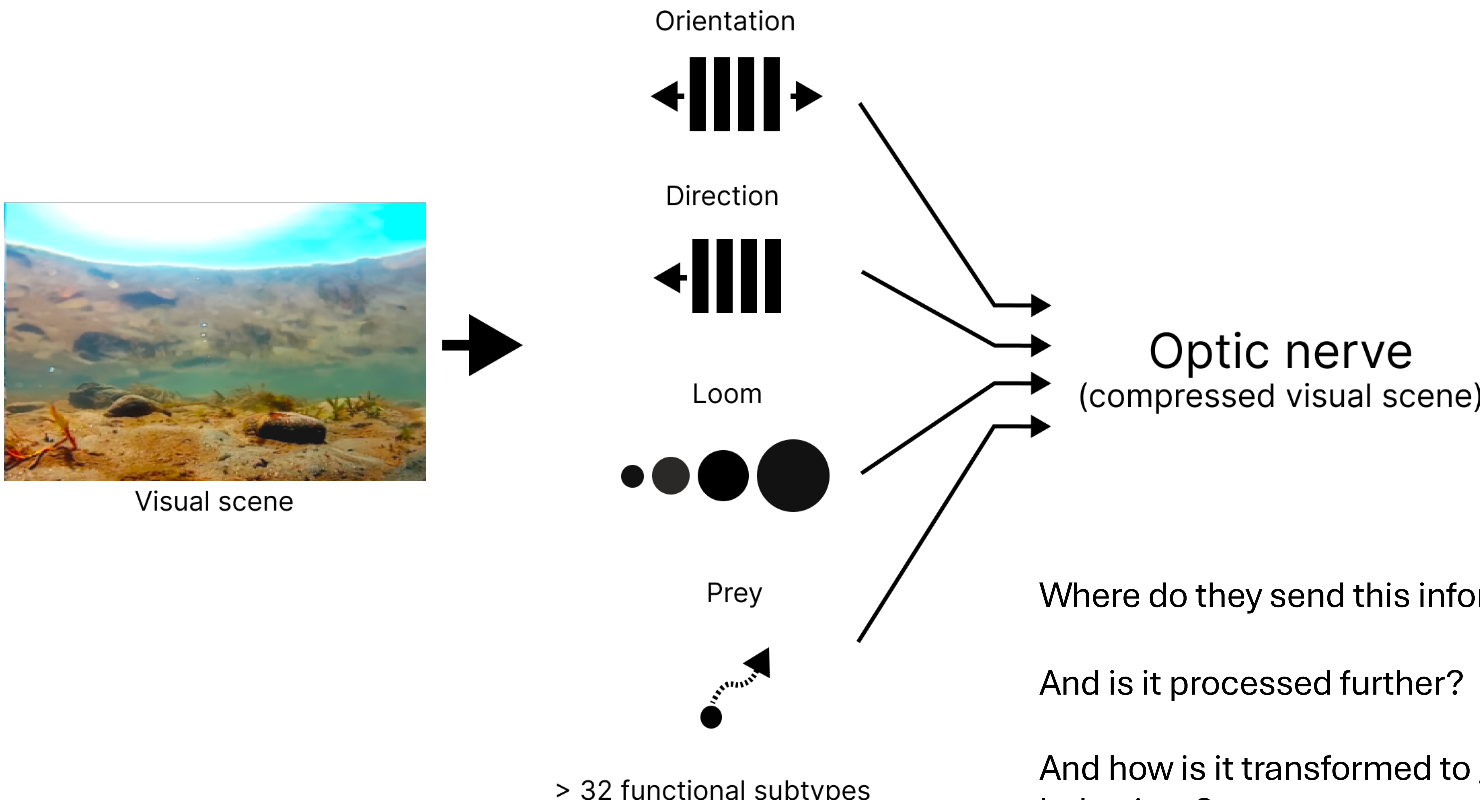




(Temizer et al., 2015)

Some RGCs show the largest responses for the dark loom over the other stimuli suggesting that they are tuned for detecting predators

Summary: Parallel processing of the visual scene by RGC types in the retina

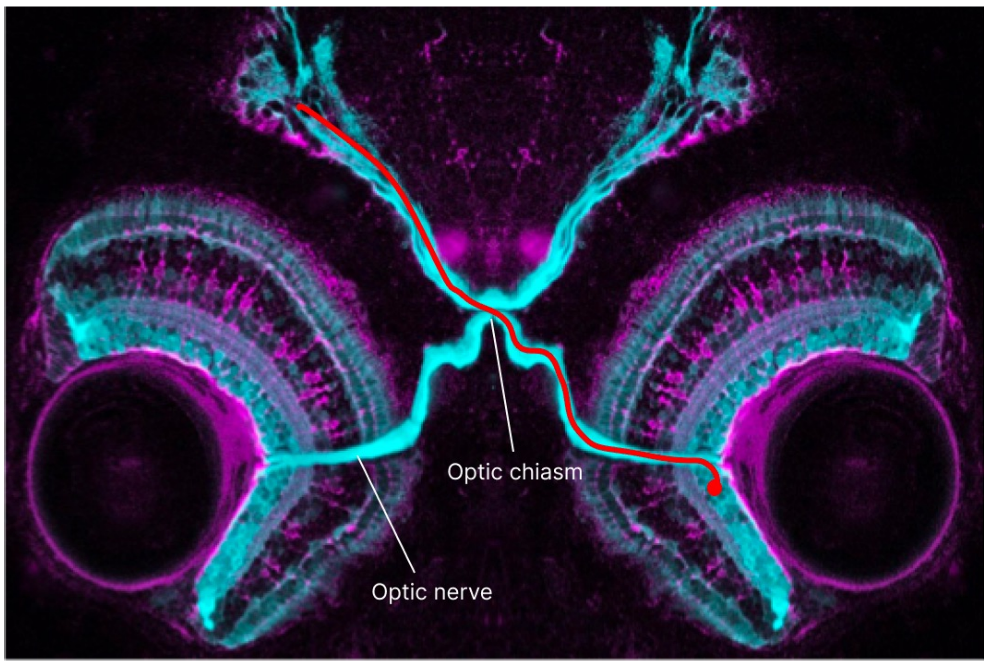


Where do they send this information?

And is it processed further?

And how is it transformed to generate behaviour?

Retinal ganglion cells fully decussate at the optic chiasm



Decussate = cross over the midline

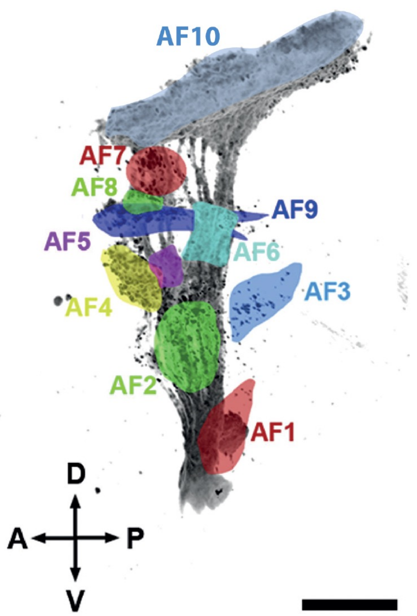
Optic chiasm: Greek letter chi = x

Unlike in mammals the RGCs fully decussate so this means that the right side of the brain only receives input from the right left

Note: this can be useful for experiments because you can manipulate neurons on one side of the visual system without affecting the other = internal control

All RGCs project to the tectum but they also synapse in other visual areas

RGC arborization fields (AF)

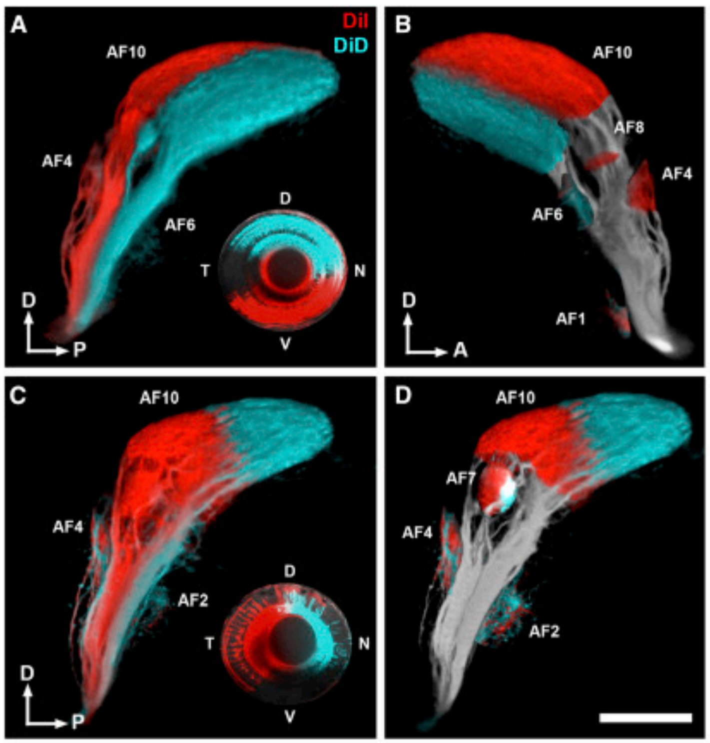


(Roubles et al., 2014)

- AF 10 – is the largest largest retinorecipient area and is known as the optic tectum
- All retinal ganglion cells target the optic tectum but they also send of collateral branches to other visual areas
- Such as the pretectum (AF4-6), which is a region that processes global motion and is important for the optomotor response

Input to the tectum is highly organized: it preserves visual space

Injections of lipophilic dyes into the retina



- Injecting lipophilic dyes is a way to trace the projections of neurons in bulk

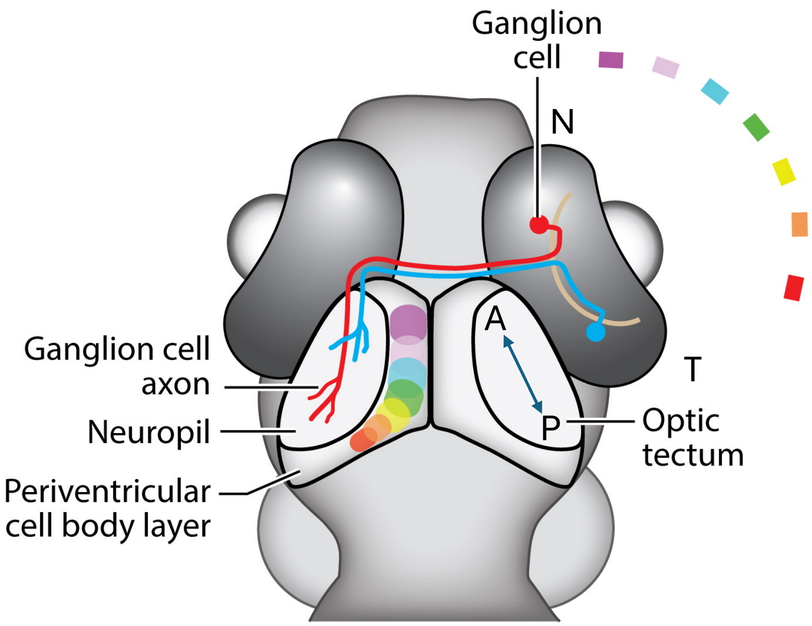
- Injecting into different regions of the retina revels that RGCs terminate in the tectum in a way that preserves visual space

- Dorsal retina projects to ventral tectum, ventral retina projects to dorsal tectum

- Temporal retina projects to anterior tectum, nasal retina projects to posterior tectum

(Roubles et al., 2014)

Retinotopy: RGCs preserve a map of visual space in the tectum

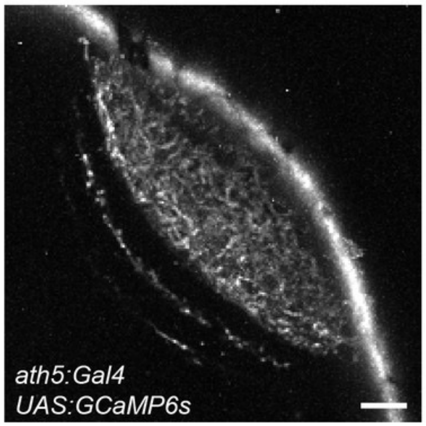


(Bollman 2019)

- This means that within the tectum there is a map of visual space (A retinotopic map)
- When neurons are active in a particular region of the tectum = possible locate the position of the stimulus in visual space

The structure of the tectum: integrates visual information and other sensory modalities

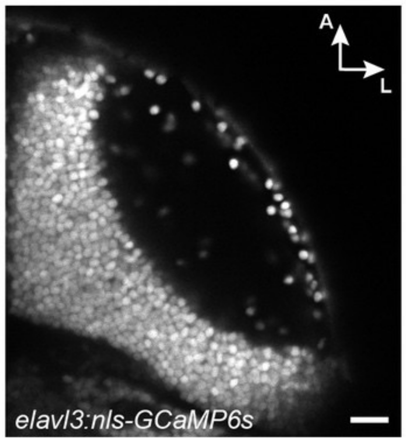
RGC terminals in the tectum

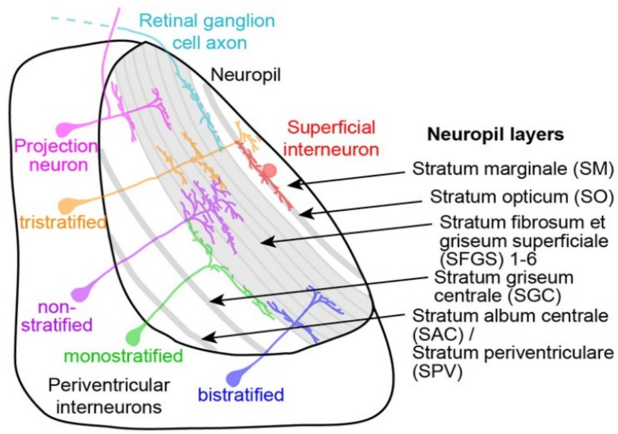


Arranged in layers

Deeper layers input from other sensory information

Tectal neurons

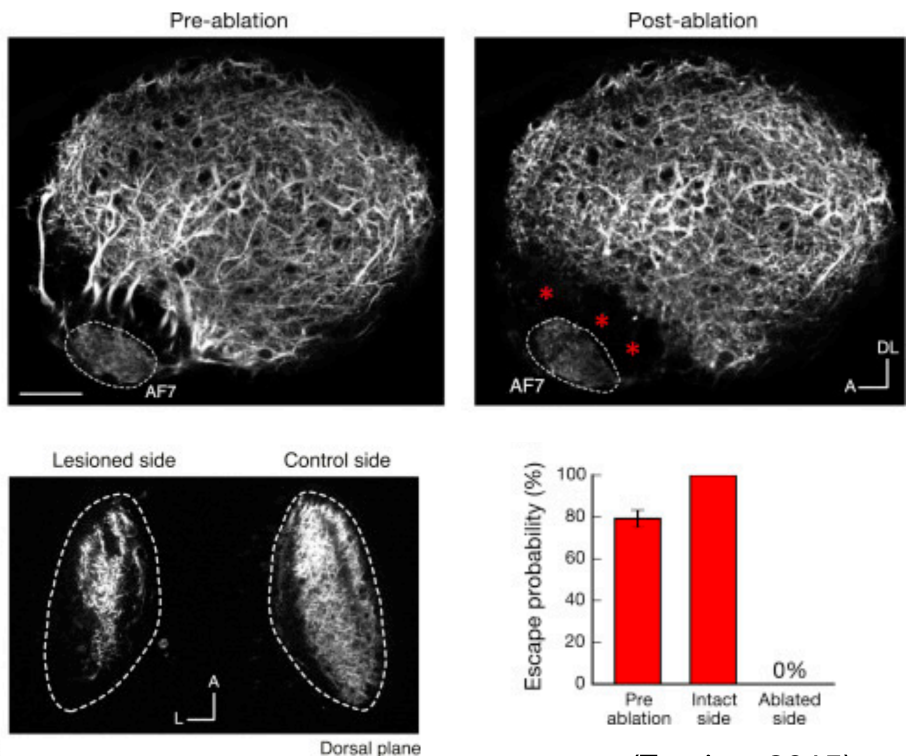




Periventicular neurons = PVNs (also known as tectal neurons)

Superfical interneuron = SINs (inhibitory interneurons)

Removing visual input to the tectum stops hunting and visual escapes

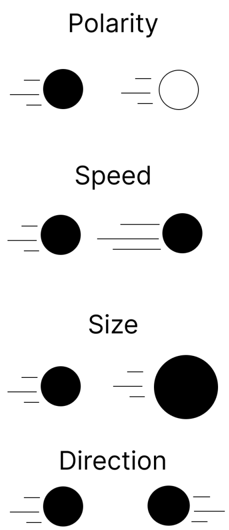


(Temizer 2015)

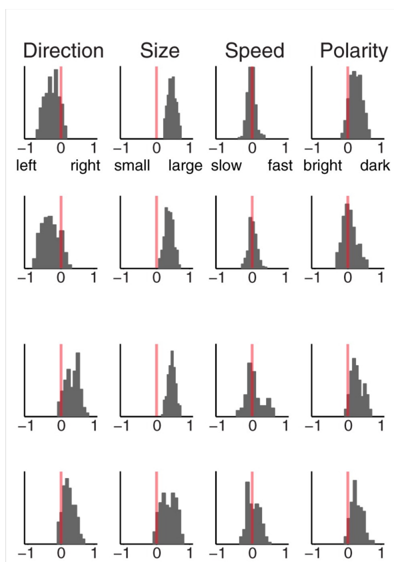
- Ablating retinal input to the tectum with a laser
- Since the RGCs fully decussate you can do this on one side and have an internal control
- Escapes are abolished on the ablated side
- This same manipulation stops fish from hunting (Ghatan et al., 2005)
- But fish can still perform the optomotor response!
- This suggests that the tectum plays a role in local motion processing but not global motion

Tectal neurons show mixed selectivity for prey like stimuli

Prey like stimuli

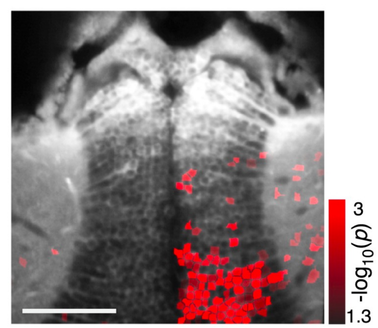


Tectal selectivity

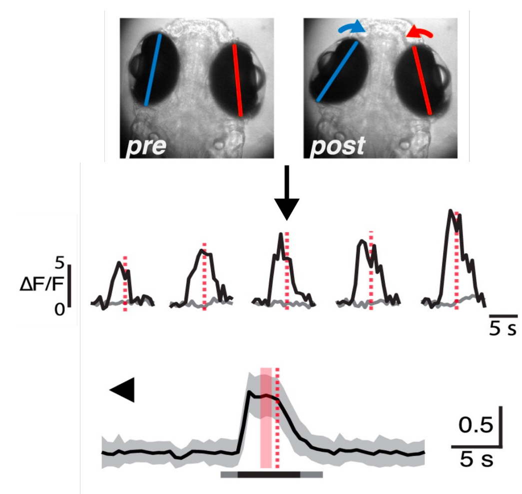


Mixed selectivity: direction, size and polarity
Prey detectors?

Tectal assembly



Neighboring neurons
Have similar tuning



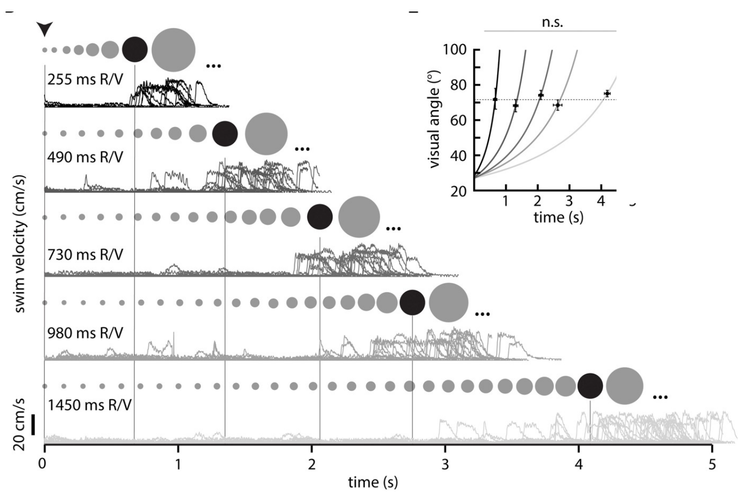
Tectal assemblies fire just before the eyes converge
Showing that they may also act as premotor neurons

(Bianco et al., 2015)

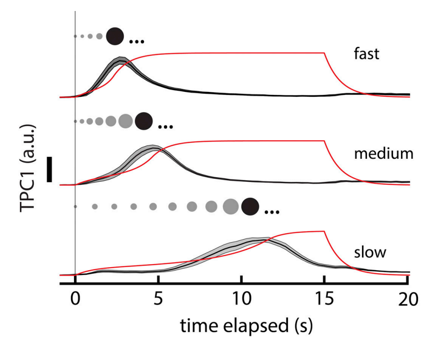
Tectal processing of the loom?

Neural Circuits Underlying Visually Evoked Escapes in Larval Zebrafish

Timothy W. Dunn • Christoph Gebhardt • Eva A. Naumann • ... Misha B. Ahrens • Florian Engert • Filippo Del Bene • Show all authors



Tectal population responses

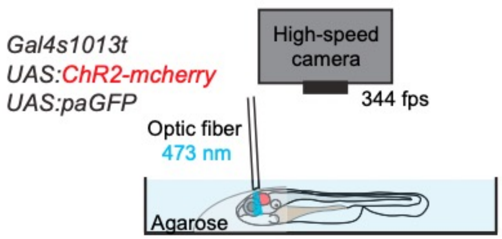


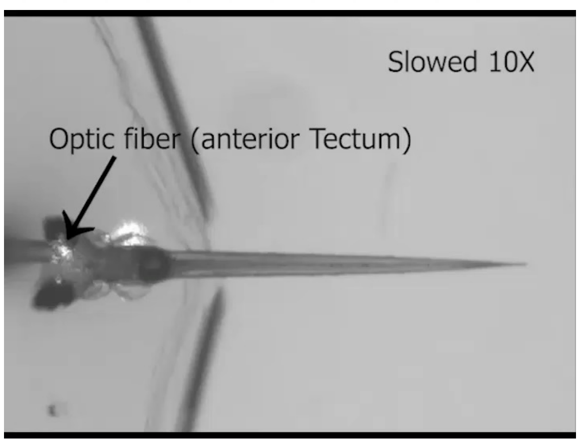
- Zebrafish perform c-starts when the loom gets to a critical size
- Tectal neurons are tuned to this critical size
- There is a defined circuit within the tectum for computing this

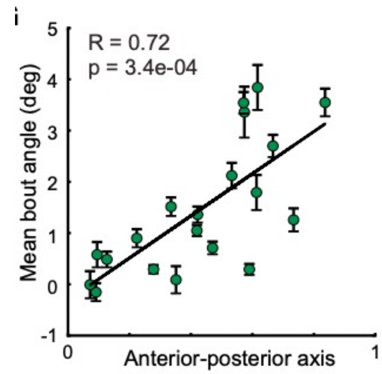
(Dunn et al., 2016)

How does visual processing lead to behavior?

The tectum also is capable of recruiting orientating behaviors



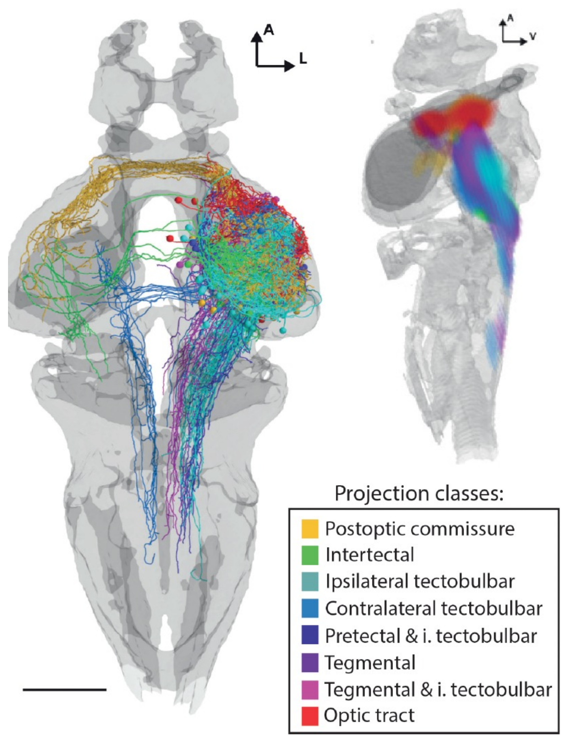




- Optogenetic stimulation of different regions of the tectum along the anterior posterior axis produces different tail movements
- Anterior stimulations look more like “j-turns” aka hunting behaviors
- Posterior stimulations look like “c-starts” aka escape behaviors
- This suggests that there is a motor map in the tectum

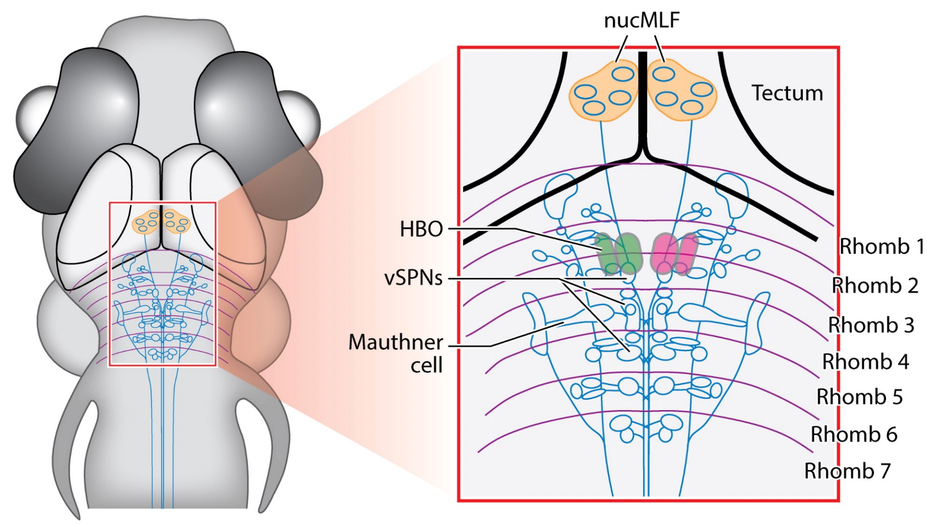
(Helmbrecht 2023)

The tectum sends visual information to motor centers in the hind brain



(Helmbrecht 2023)

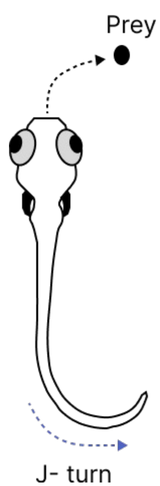
The reticular spinal system



Reticular spinal neurons descend into the spinal cord and control movement of the tail

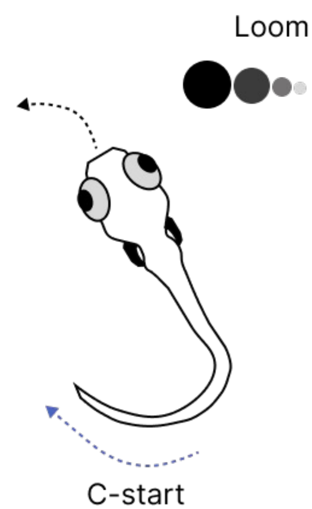
The role of the tectum: identify to position of predators and prey in local visual space

Approach



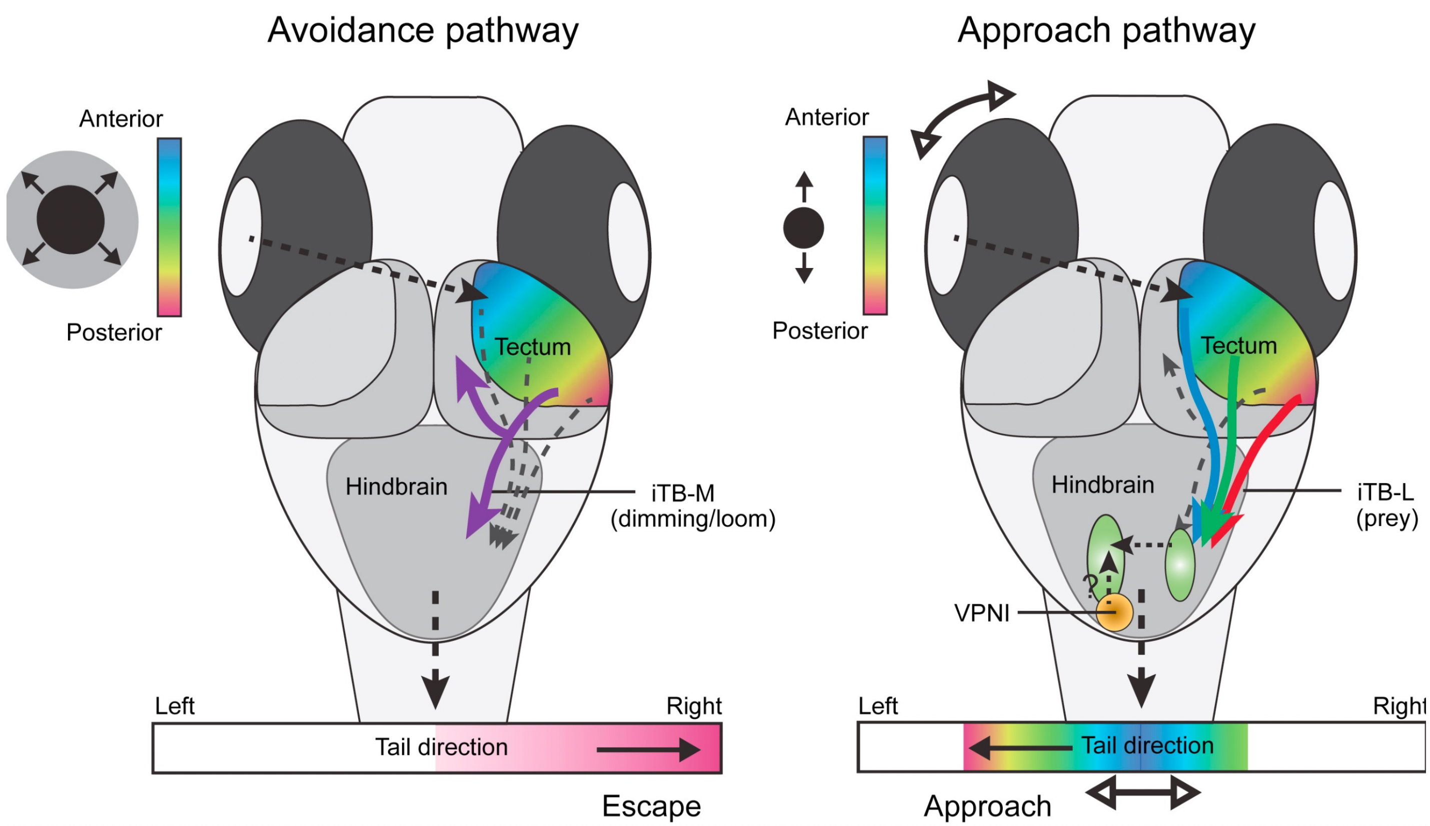
Vs

Avoid



Direct behavior either toward prey or away from predators in egocentric space

Separate circuits for prey capture and predator avoidance



(Helmbrecht 2023)

Summary

- Center-surround model and it’s ethological relevance (motion)
- Anatomy of the visual pathway, and in particular, cortical layers
- The retina is an evolutionary old structure, and adapted to the niche of the animal
- Zebrafish are a great model systems neuroscience due to their small size and optical transparency
- Zebrafish display a wide diversity of visually driven behaviour such as the OMR, prey capture and predator avoidance
- The organisation of the zebrafish retina – 4 cones with UV cone being integral for detecting prey
- Retinal ganglion cells act as feature detectors, providing parallel processing streams to the brain
- The tectum has a highly organised structure and acts as a local motion detector classifying prey and predators
- The tectum uses this information to trigger approach and avoidance behavior

Section tomorrow:

1. Journal club:

Neural circuits underlying visually evoked escapes – Dunn et al., 2016

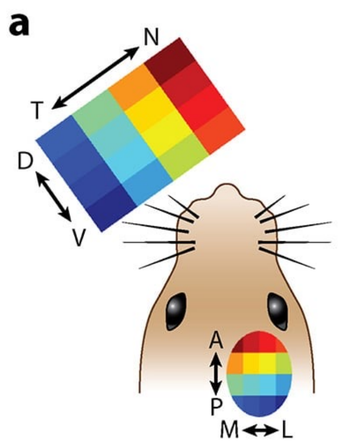
2. Zebrafish experiment:

Psychopy – https://www.psychopy.org/

We can then test these stimuli on some actual zebrafish!

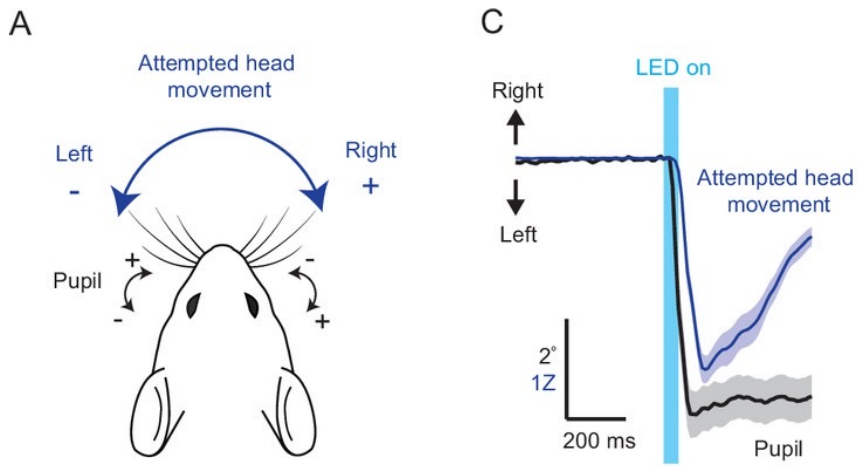
Superior colliculus in mammals also contains a retinotopic maps and motor maps

Retinotopic map of visual space



Cang J, et al. 2018.
Annu. Rev. Vis. Sci. 4:239–62

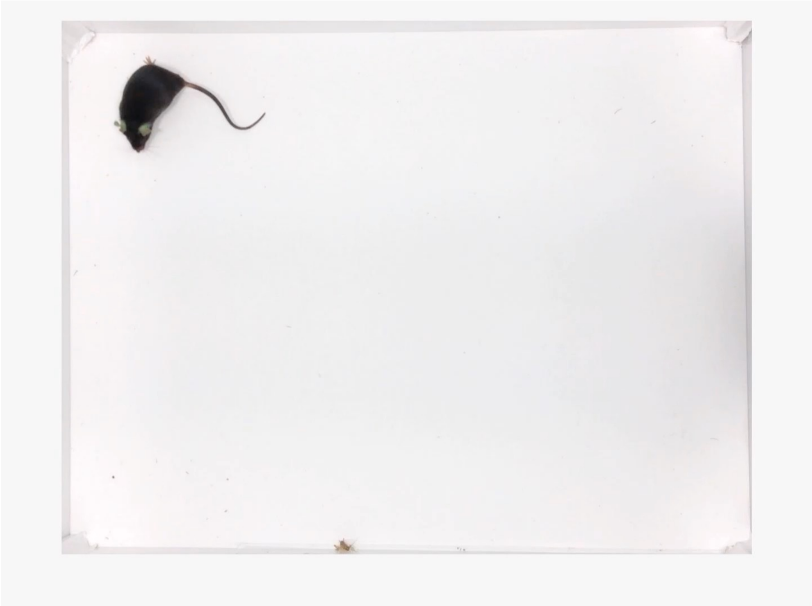
Optogenetic stimulation of different regions of the superior colliculus produces head movements



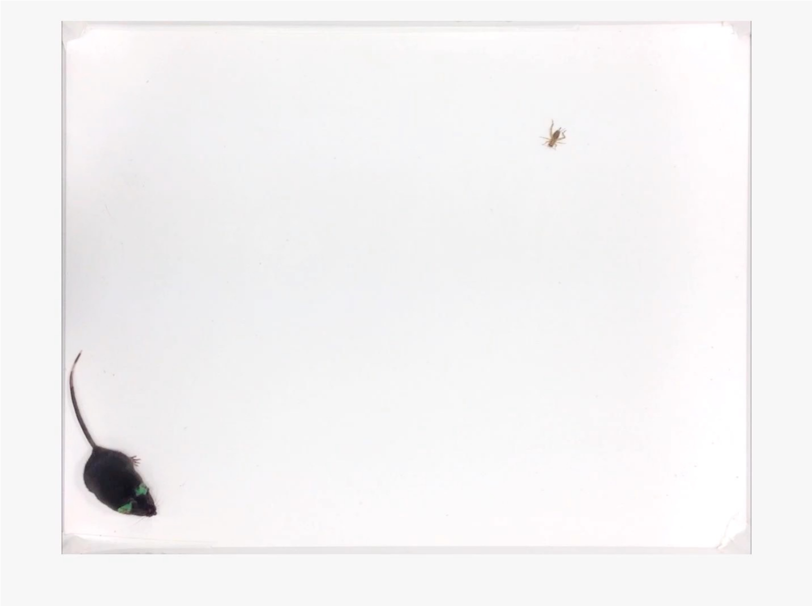
Does the SC also control similar behaviors?

Prey capture in mice requires specific cell types in the SC

WT



Silencing cells in the superior colliculus



(Hoy et al., 2020)