Animal Models in Neuroscience

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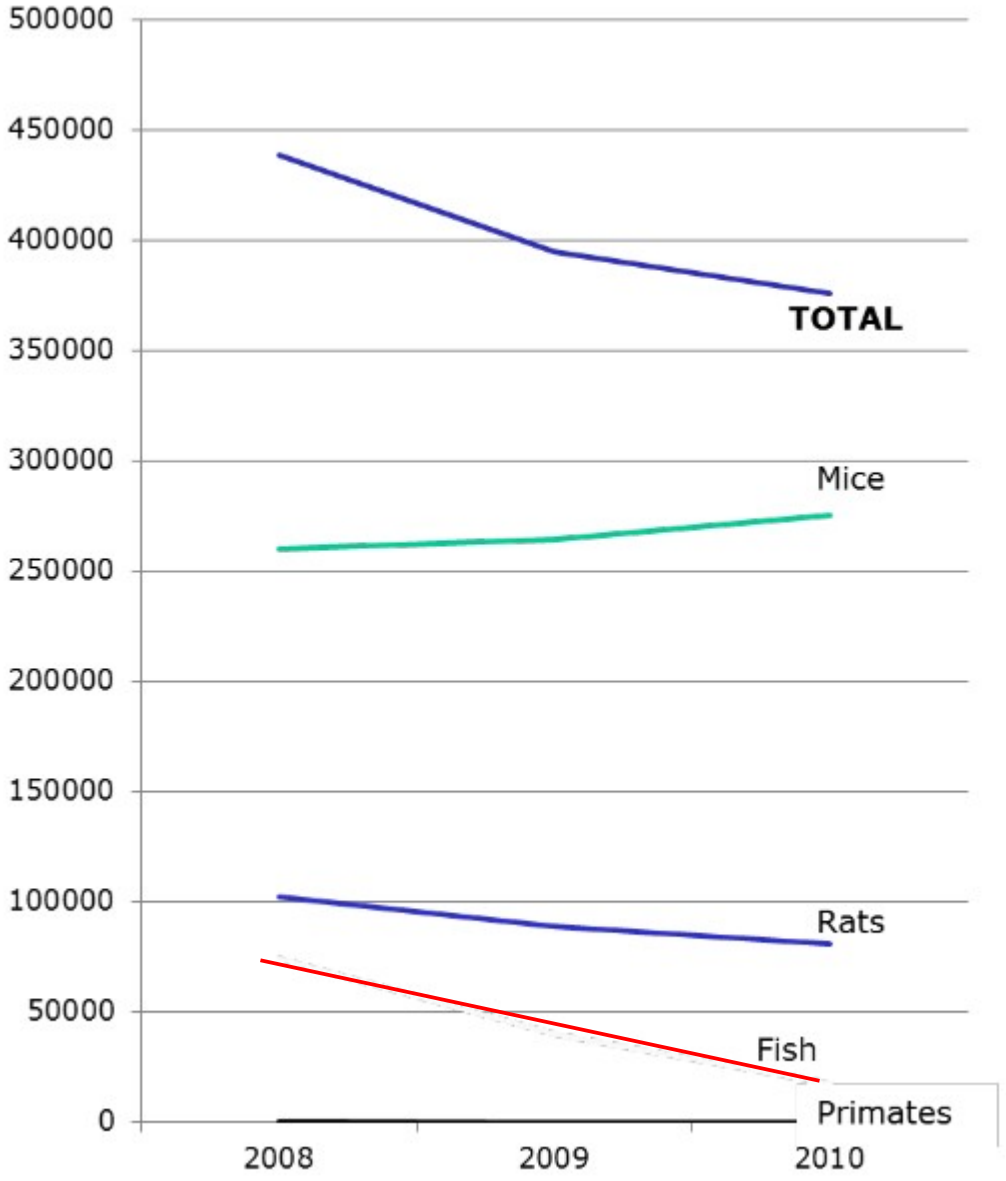
Todd Ahern/Emory University / Courtesy to The Chronicle

Number of animals (vertebrates) used in Neuroscience in the UK

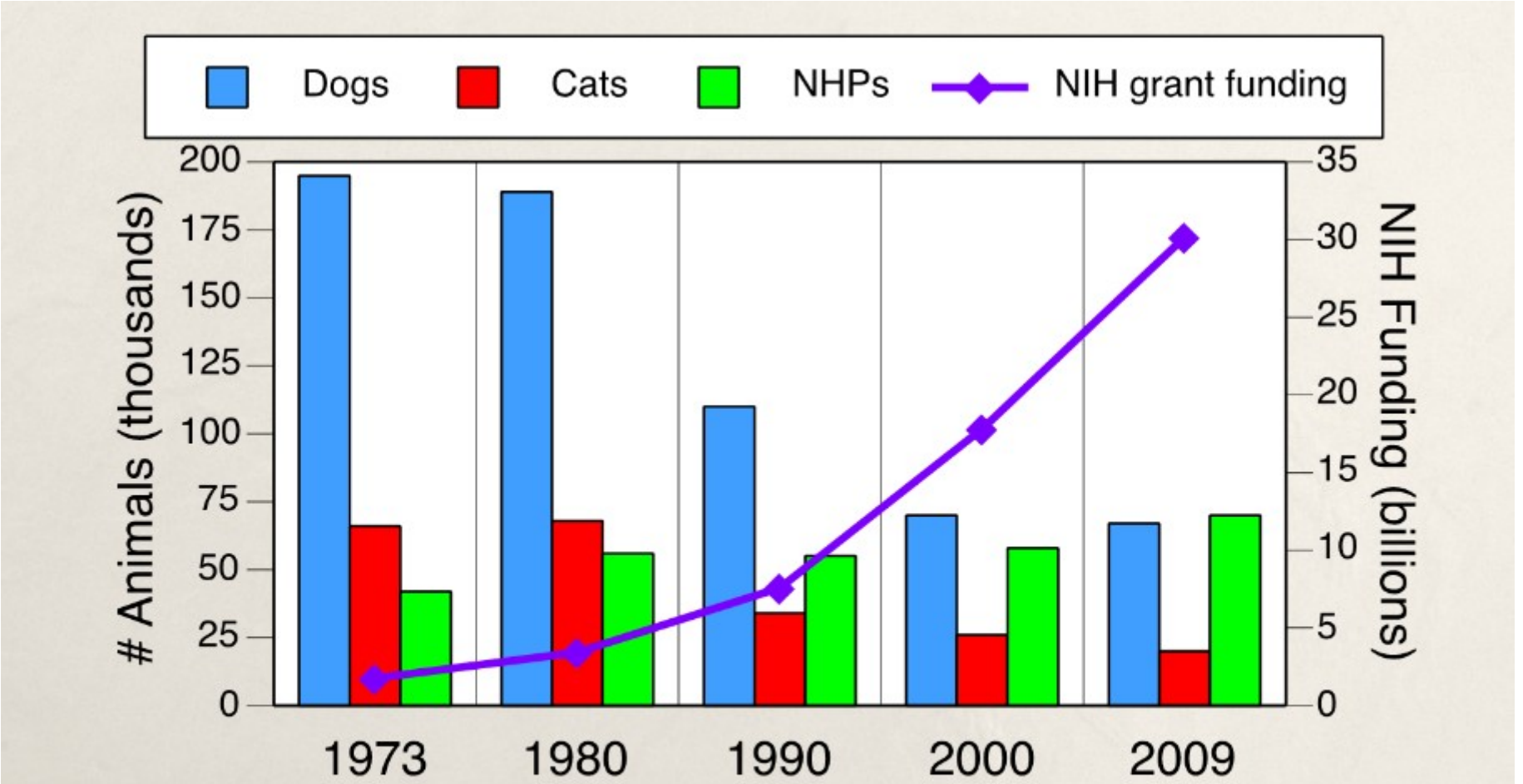


The types of institutions conducting animal research in the UK in 2004 were:

- universities (42.1%)
- commercial organizations (33.3%)
- non-profit organizations (4.9%)
- government departments (2.4%)
- NHS hospitals (0.9%)
- public health laboratories (0.6%)
- other public bodies (15.8%)

Invertebrates are not considered animals by the legislator!

Number of animals in the USA



Data for the United States. Data for rats, mice, birds, and cold-blooded vertebrates are not tracked.

Source: United States Department of Agriculture (USDA) Annual Reports and National Institutes of Health (NIH) website.

The fall of the feline

Although rodents have traditionally been the major species utilized for many types of neuroscience research (e.g., behavioral studies), felines were a major species used for neurophysiological studies prior to the mid-1980s.[1]

Examples are studies of visual processing by the brain (Nobel prize for Hubel and Wiesel in 1981) and processing of somatosensory inputs by the spinal cord (Nobel prize for Eccles in 1963).

Cats were popular research animals for classical neurophysiological studies because they:

- Could withstand the extensive surgeries required
- Were large enough to accommodate bulky instrumentation
- Were inexpensive models (obtained from pounds or animal shelters; limited paperwork requirements)

The fall of the feline

- In the mid-1980s, new regulations substantially increased the cost of the feline model.
- Miniaturization of instrumentation allowed rodents to serve as replacements for felines in some studies.
- Public opinion became negatively biased against the use of companion animals in research.
- Chronic recording techniques allowed a single animal to be studied over a prolonged time, such that fewer animals were needed for a study.
- The use of nonhuman primates became economically feasible.
- Nonhuman primates can be trained for more elaborate tasks than cats, allowing for sophisticated studies of neural function.

Choice of the model – the law

In deciding whether to grant a license, **the Home Office** refers to the Act's cost-benefit analysis, which is defined as

"the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the programme to be specified in the license" (Section 5(4)). A license should not be granted if there is a "reasonably practicable method not entailing the use of protected animals" (Section 5(5) (a)). The experiments must use "the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress, or lasting harm, and [be the] most likely to produce satisfactory results" (Section 5(5) (b)).[11]

What kind of model we need?

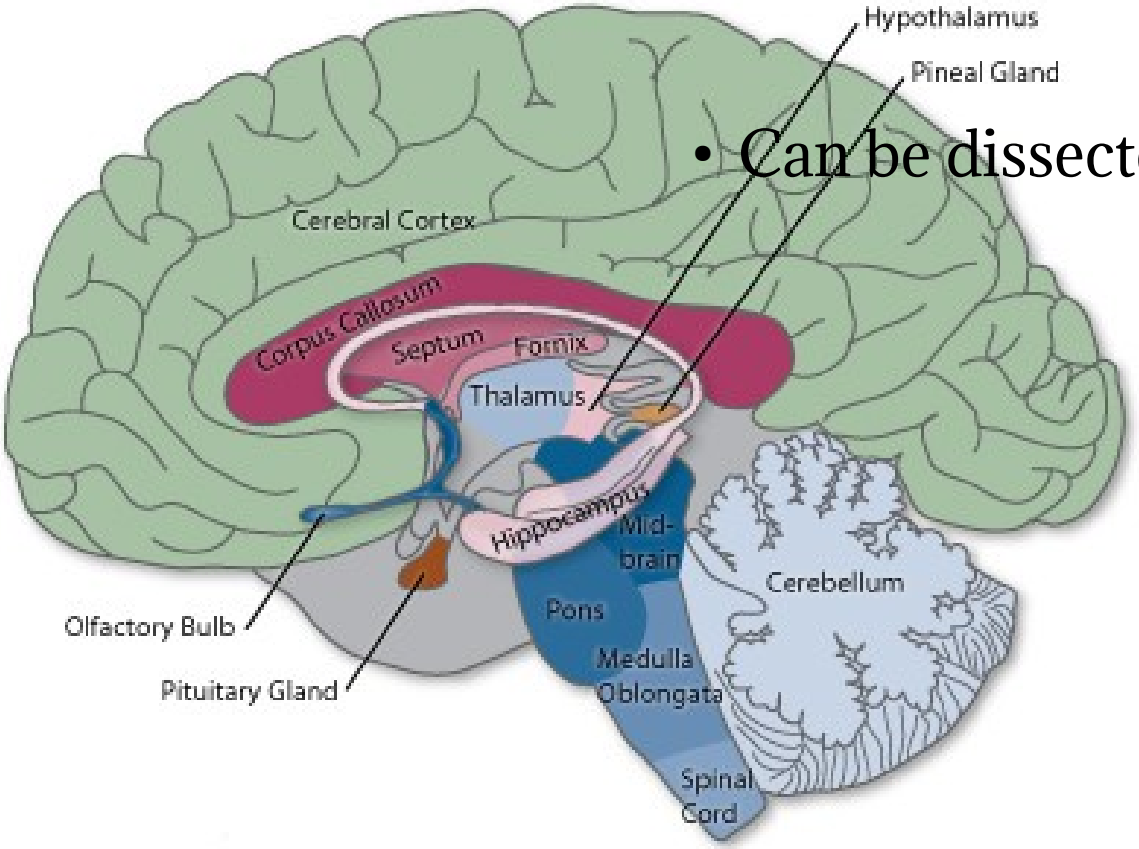
- Genetic model (e.g. rodents, flies, worms)
- Pharmacological model (e.g. primates)

- Model for anatomy or development (e.g. cats, rodents, flies)
- Behavioural model (e.g. rodents, apes, honeybees, ants)
- Electrophysiological model (e.g. rodents, cats, slugs)
- Models for cellular neuroscience (e.g. aplysia)

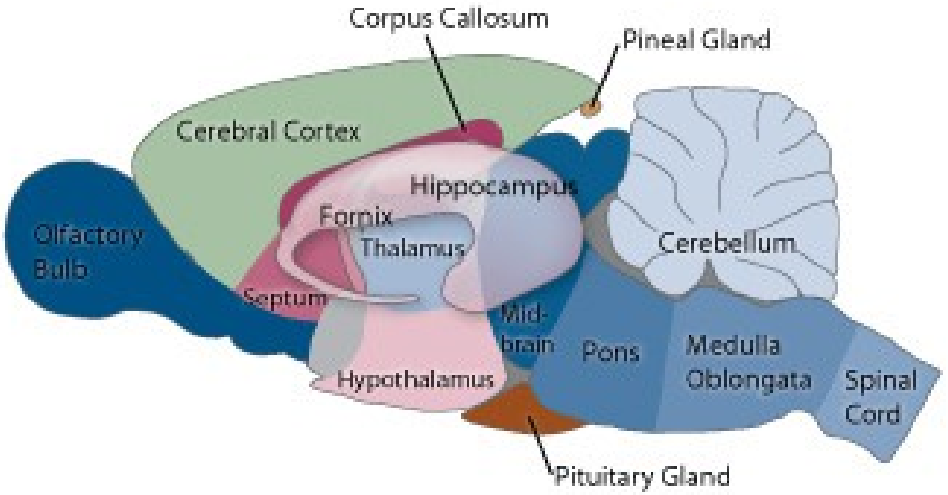
Choice of the model – the scientific rationale

- A genetic model (e.g. rodents, flies, worms)
- A pharmacological model (e.g. primates)
- An anatomical model (e.g. cats)
- A behavioural model (e.g. rodents, apes, honeybees, ants)
- An electrophysiological model (e.g. rodents, cats, slugs)
- Similarities to human brain (exp. anatomical)
- Similarities to human genes
- Specific biological problem
- Highlighted feature (e.g . special memory, big axons...)
- Availability of genetic tools
- Personal /ethical preferences

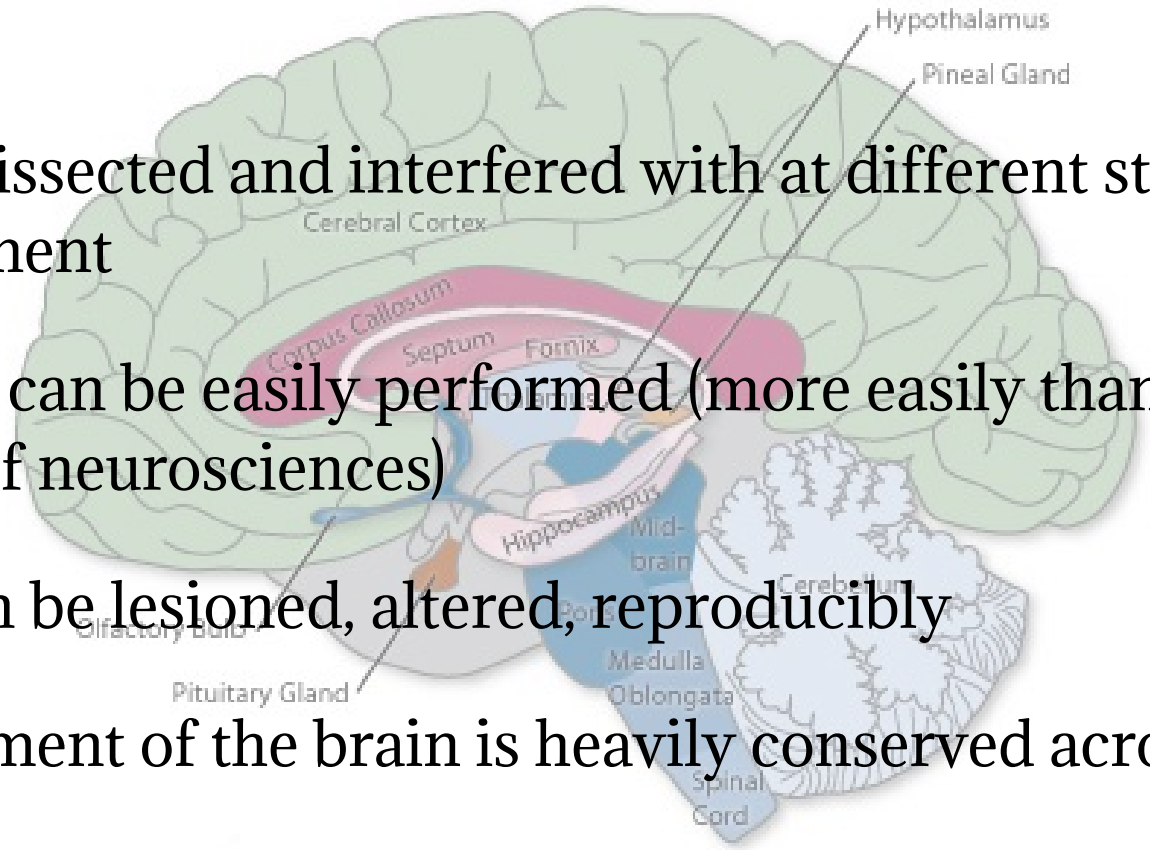
Anatomical models



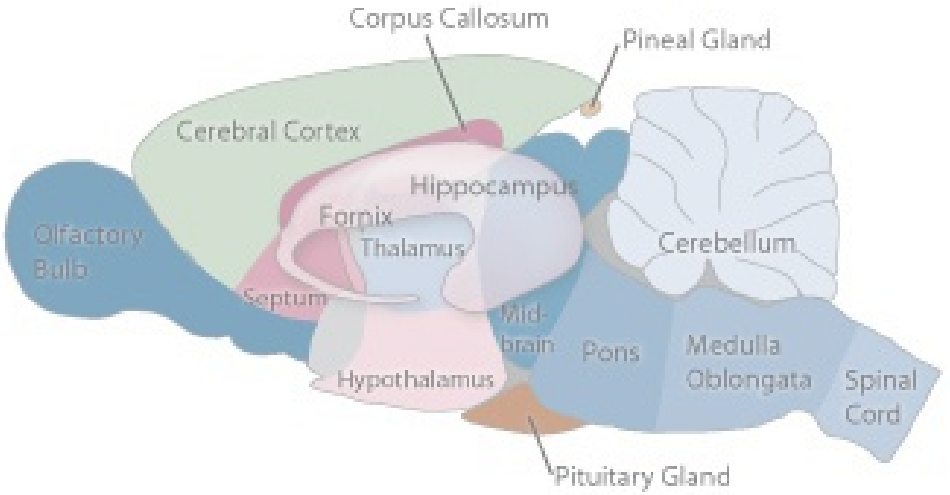
- Can be dissected and interfered



Anatomical models



- Can be dissected and interfered with at different stages of development
- Genetics can be easily performed (more easily than for other aspects of neurosciences)
- Brain can be lesioned, altered, reproducibly
- Development of the brain is heavily conserved across species



Eight Phases in Embryonic and Fetal Development at a Cellular Level

Mitosis/Proliferation

Migration

Differentiation

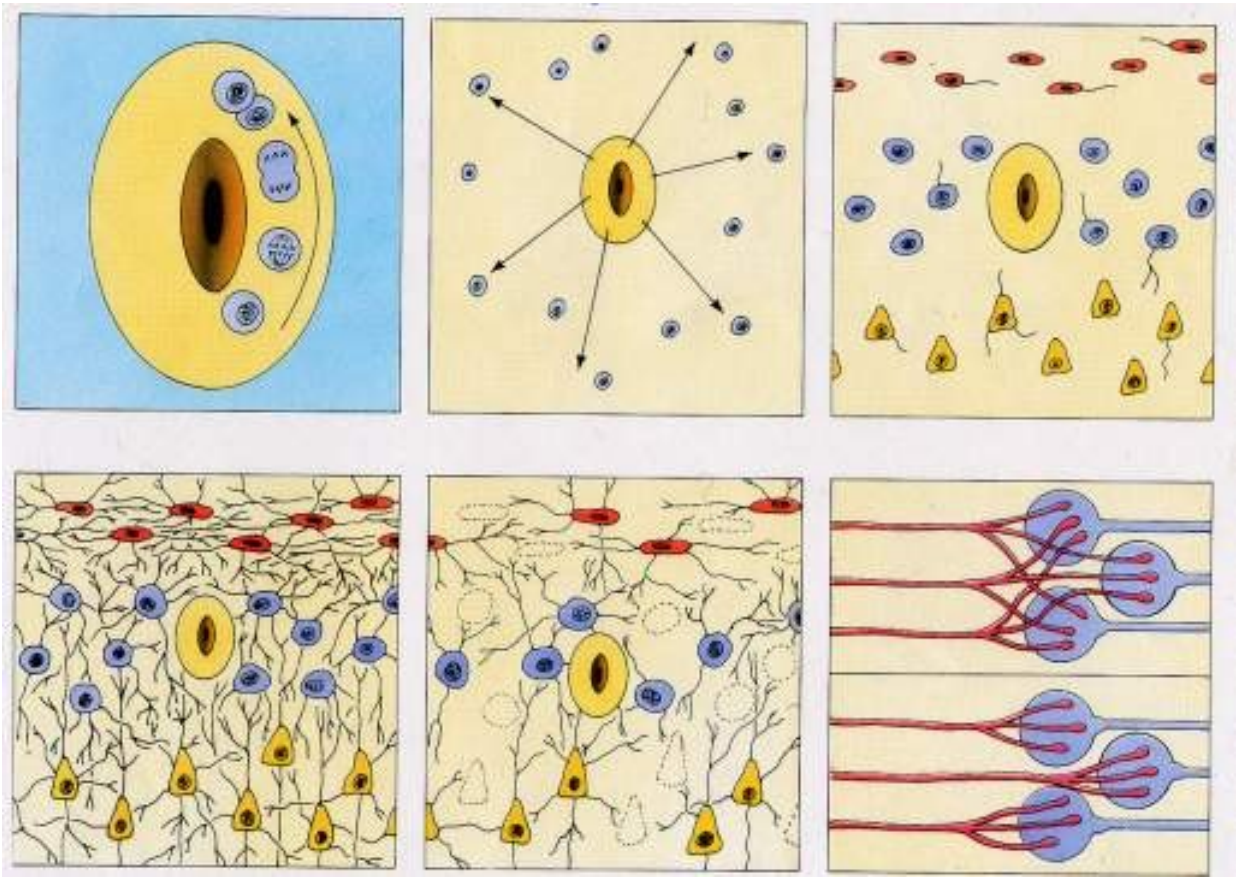
Aggregation

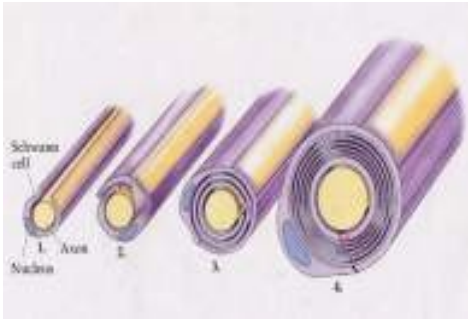
Synaptogenesis

Neuron Death

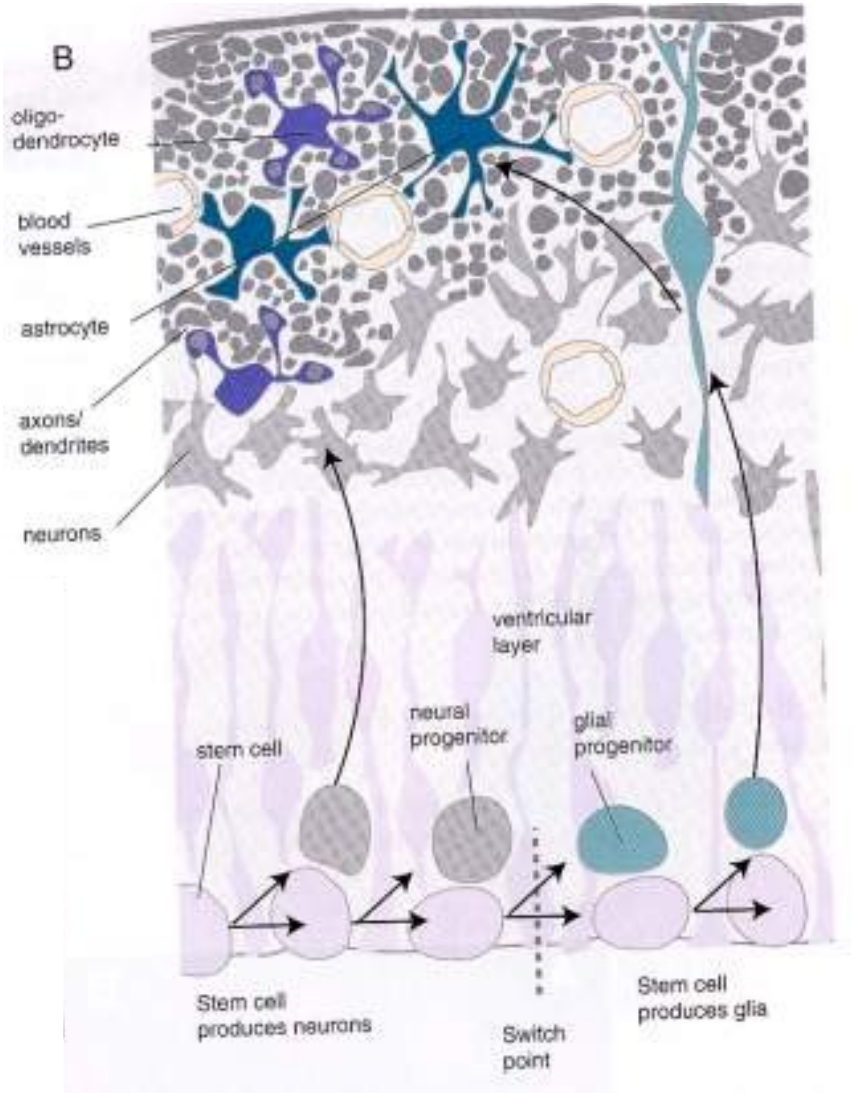
Synapse Rearrangement

Myelination





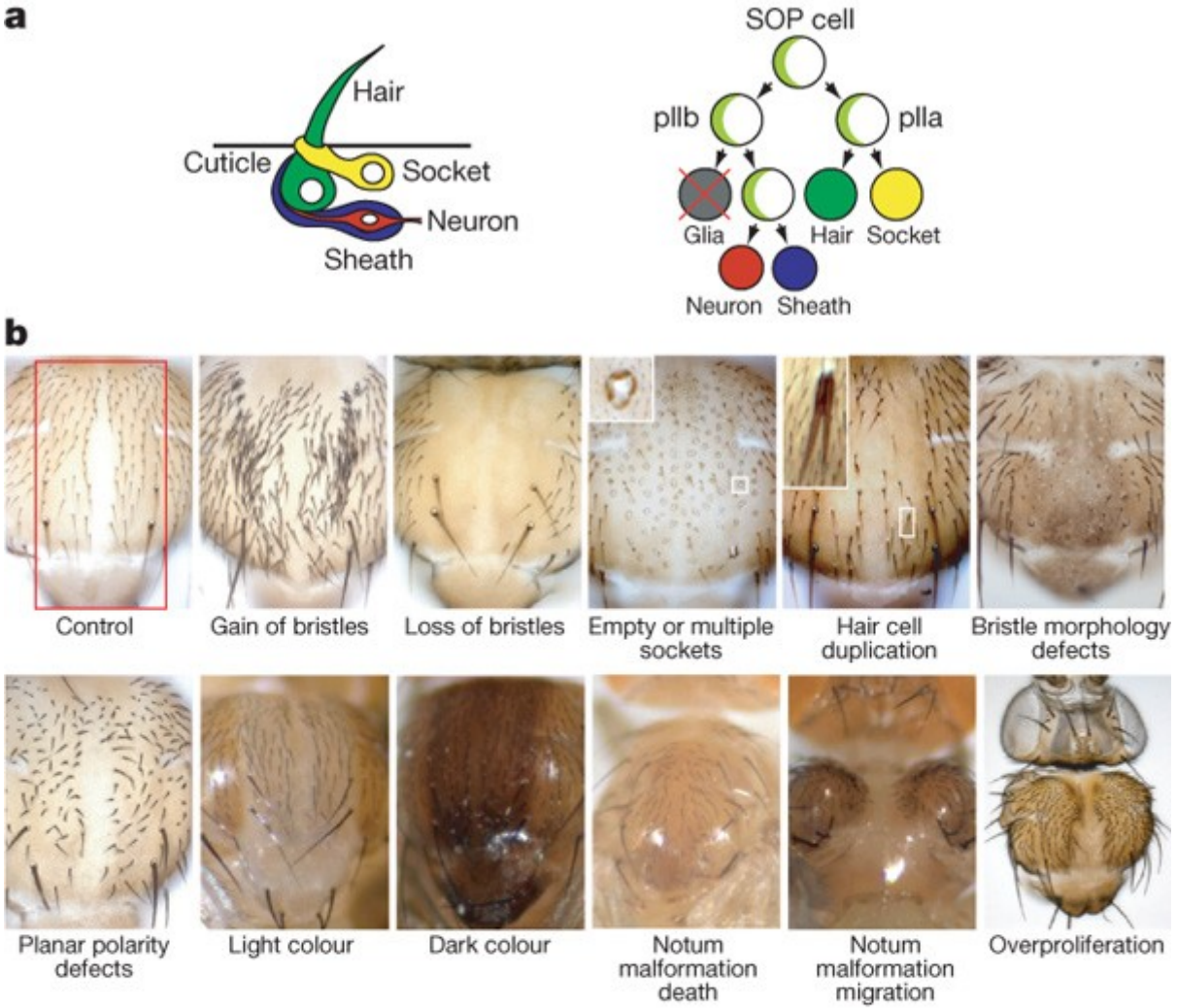
1. Mitosis / Profileration : neurons and glia

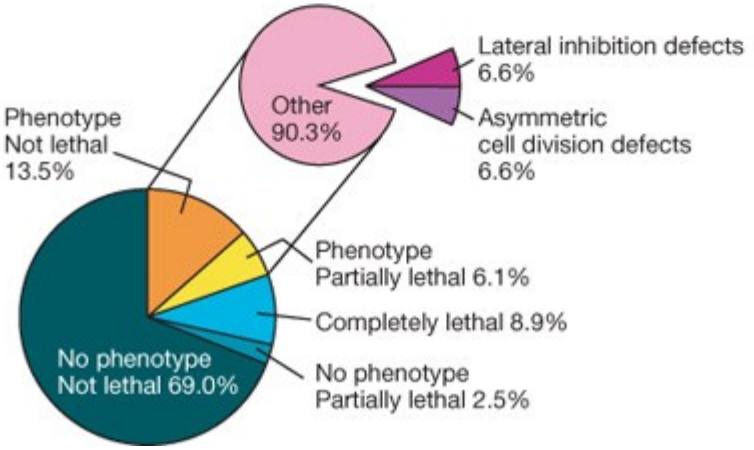


At early stages, a stem cell generates neuroblasts.

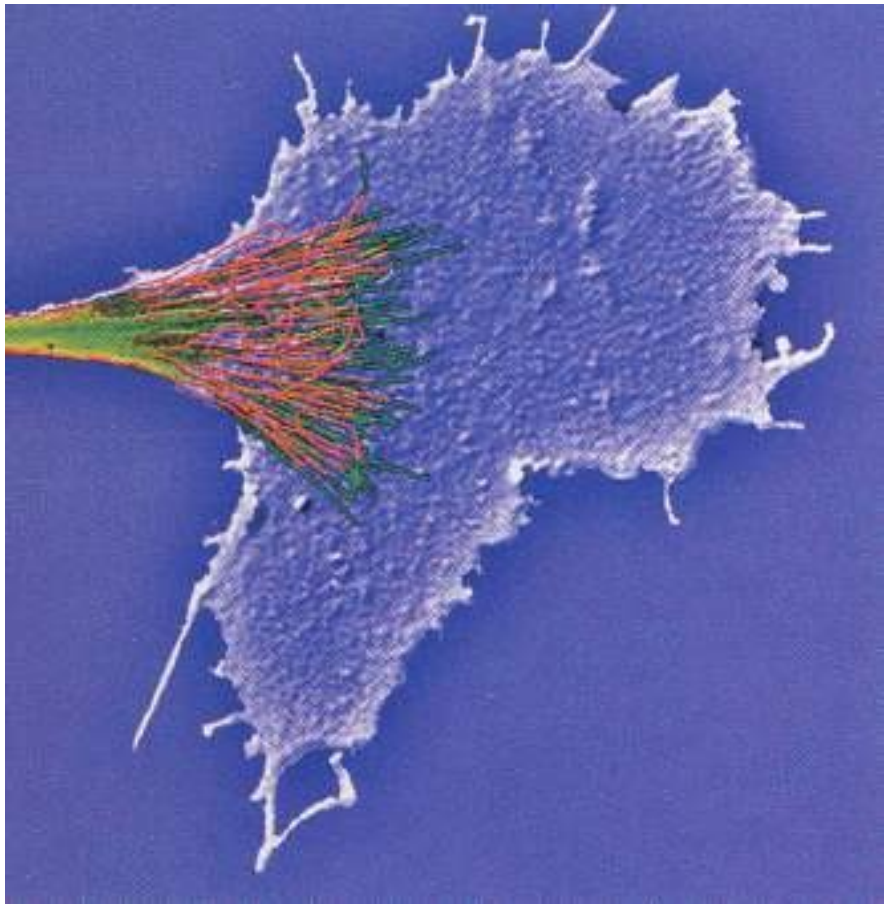
Later, it undergoes a specific asymmetric division (the “switch point”) at which it changes from making neurons to making glia

1. Mitosis / Profileration : Asymettric cell Division





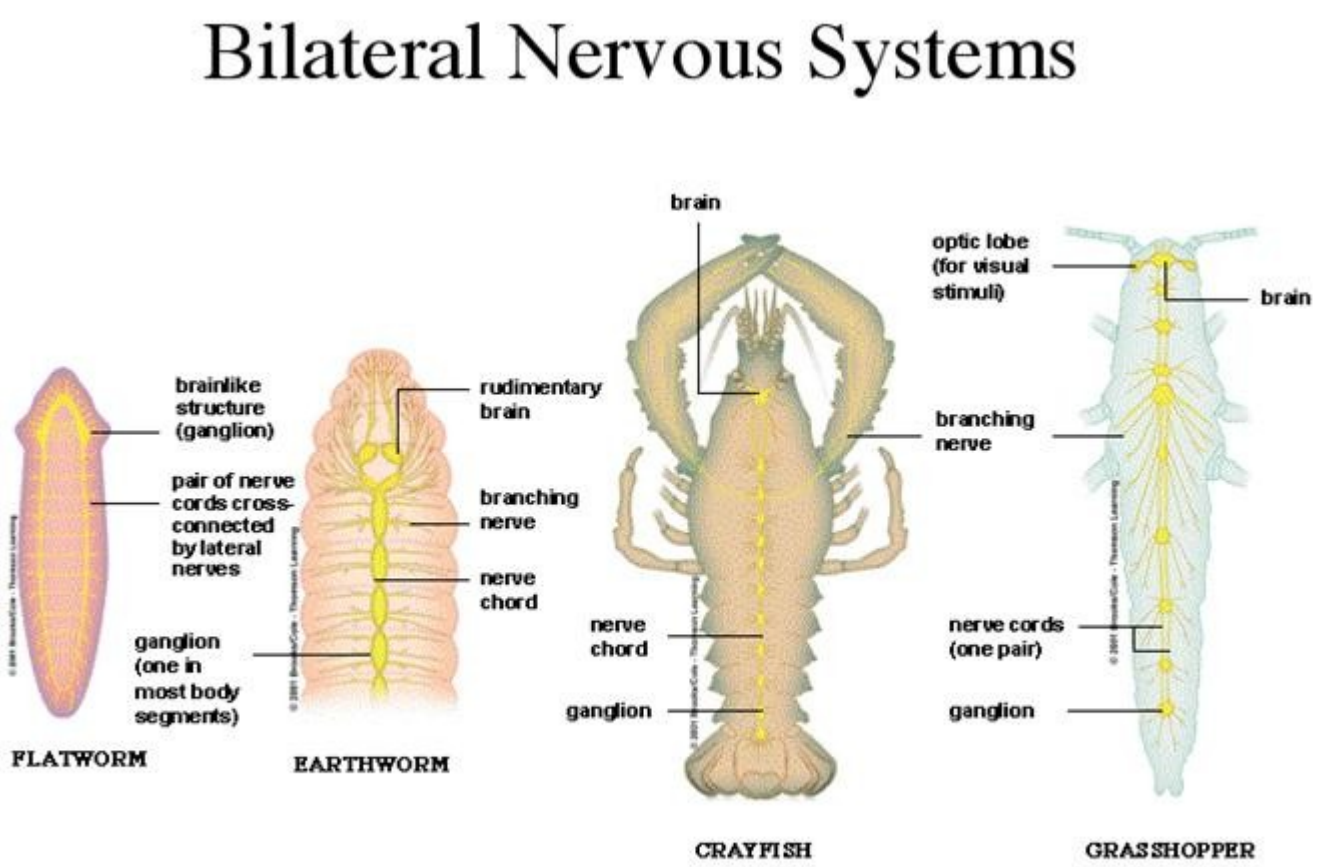
2. Migration



Growth cones crawl forward as they elaborate the axons training behind them. Their extension is controlled by cues in their outside environment that ultimately direct them toward their appropriate targets.

The fine threadlike extensions shown in red and green are filopodia, which find adhesive surfaces and pull the growth cone and therefore the growing axon to the right.

2. Migration: how do neurons know where to go. The case of midline crossing



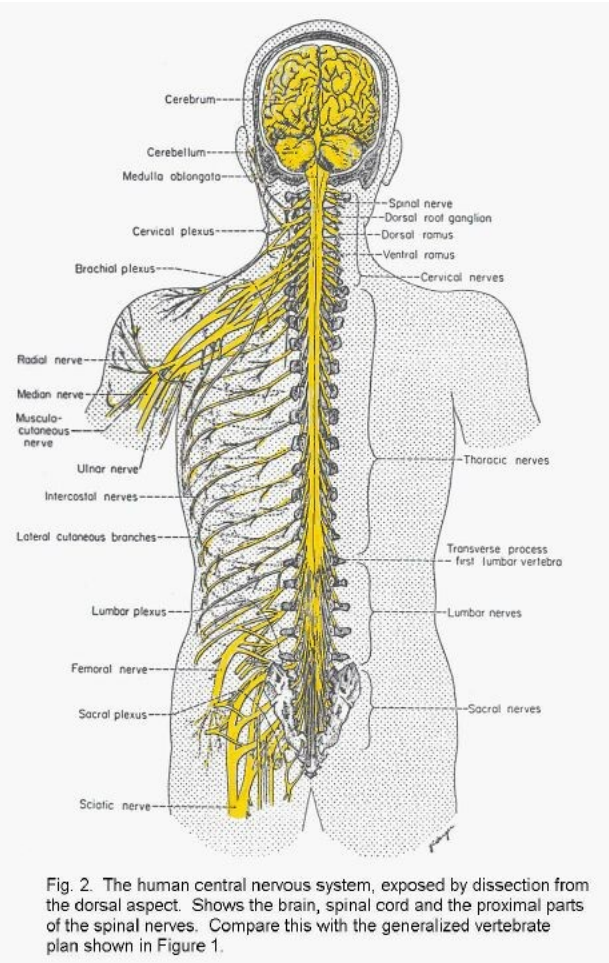
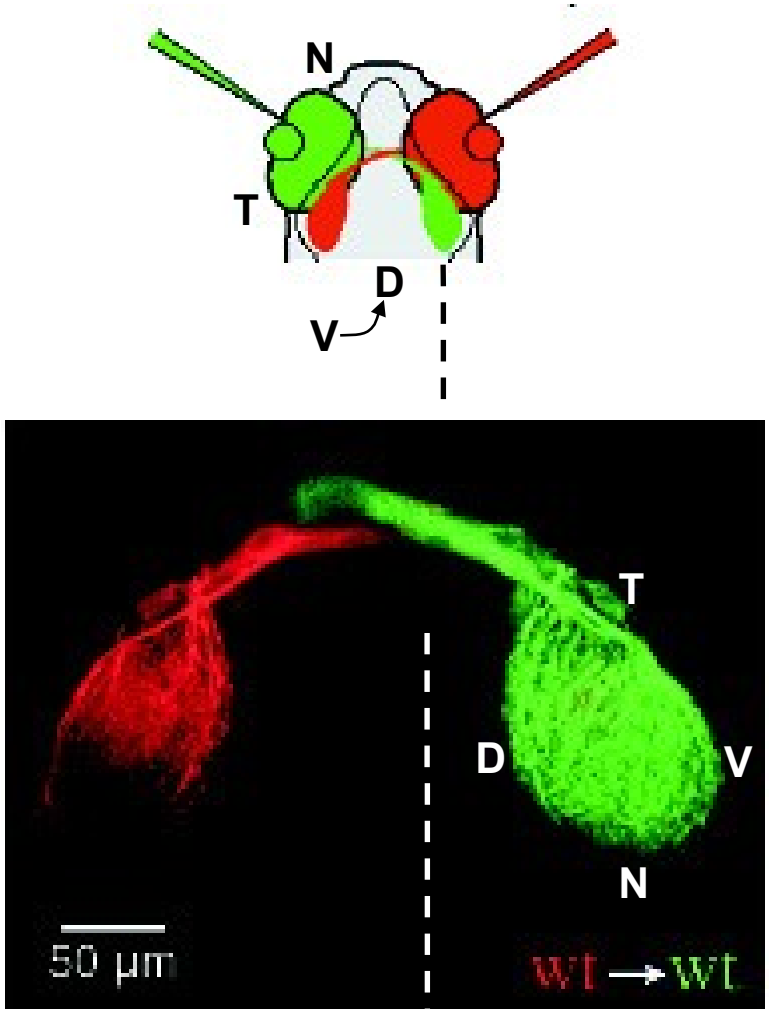


Fig. 2. The human central nervous system, exposed by dissection from the dorsal aspect. Shows the brain, spinal cord and the proximal parts of the spinal nerves. Compare this with the generalized vertebrate plan shown in Figure 1.

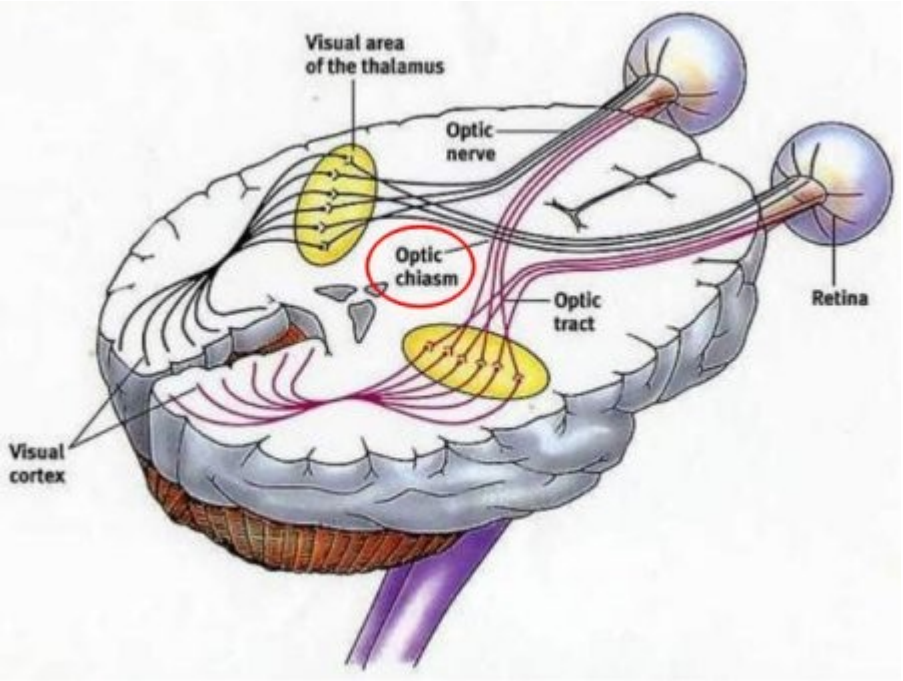
In bilaterally symmetrical organisms, about 90% of neurons are contralateral and only 10% ipsilateral

2. Migration: how do neurons know where to go. The case of midline crossing



(Friche, *et al.* 2001)

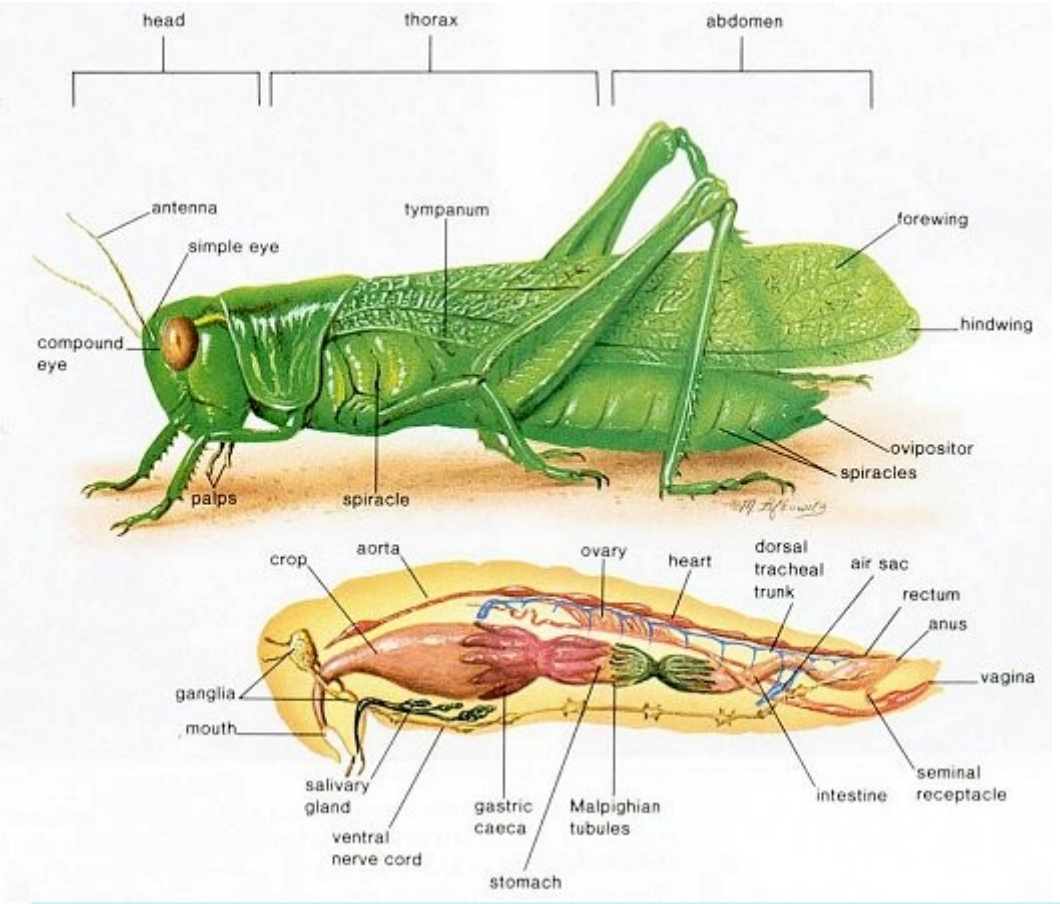
Retinotectal Mapping Visualized by Dye Injection in Zebrafish

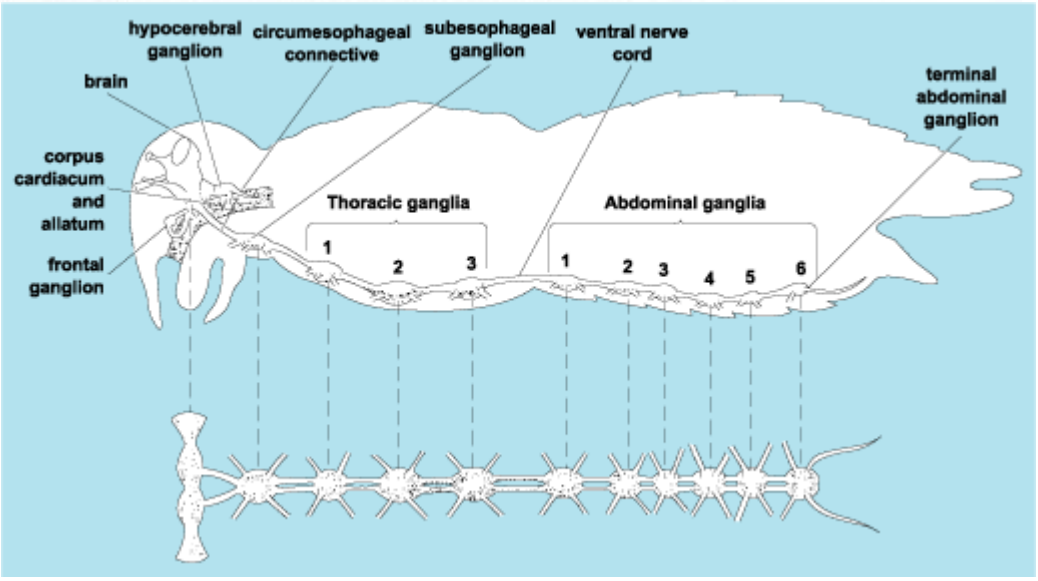


© Warren Photographic

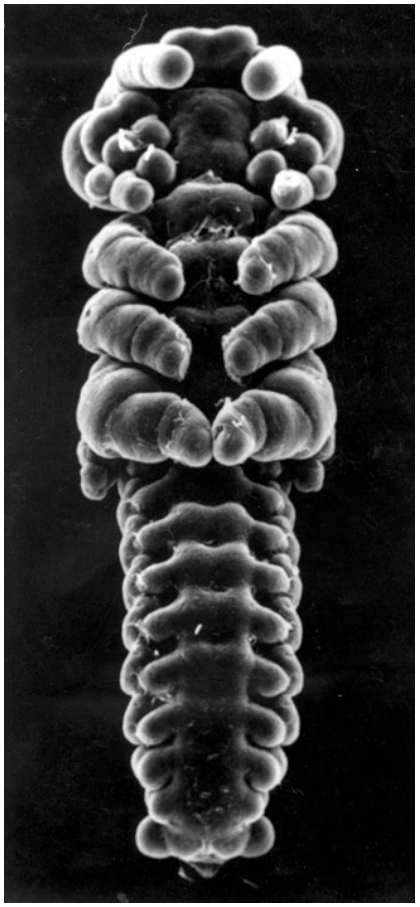
The grasshopper has a brain located between its eyes, just above the esophagus. The brain is connected to the 1st ventral ganglion by a pair of ventral nerves that surround the gut. The grasshopper can do many things, like walking and jumping, WITHOUT its brain. The brain is used to relay sensory information to other parts of the body and to help with movement. The first ventral ganglion is used primarily to control movement of the mouth. The segmental ganglia throughout the length of the grasshopper are connected to the first ventral ganglion by a double nerve cord and serve to coordinate local activities.

Insects have a compound eye containing many different units called "ommatidia". Each ommatidia is like an individual lens that samples a small part of the visual field. There can be thousands of ommatidia in a single insect eye. Science fiction/horror/monster movies that show an insect that sees thousands of identical images of the ENTIRE visual field are WRONG -- an insect sees only ONE picture at a time because each ommatidia sees only a small part of the entire field. Some insects are sensitive to ultraviolet light and others can detect infrared wavelengths of light.

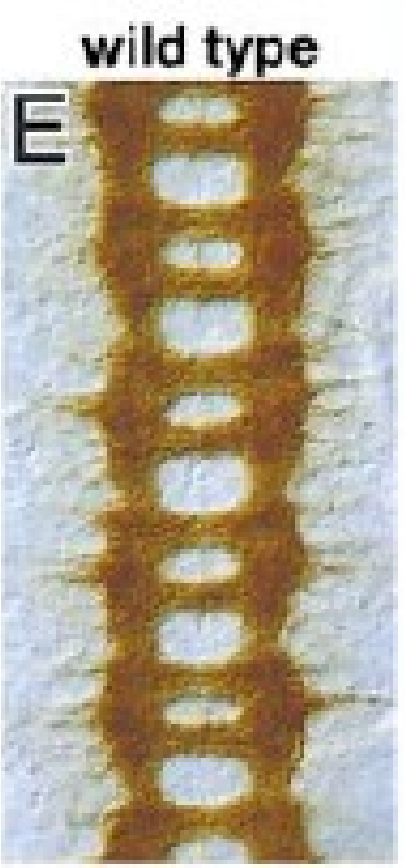


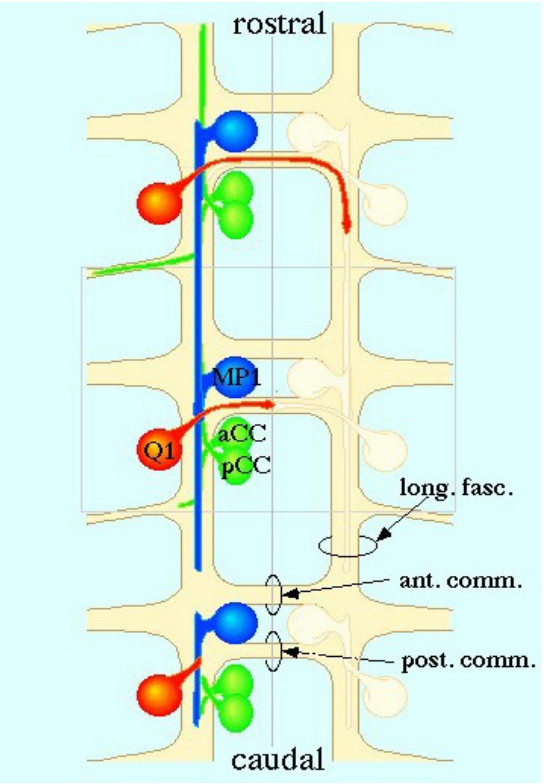


The segmented nervous system



Spider





D. melanogaster

2. Migration: how do neurons know where to go. Identification of molecules using biochemical *ex vivo* approaches (rat, mouse, chicken)

Observe Neuronal Specificity

⬇

Functional Assay

⬇

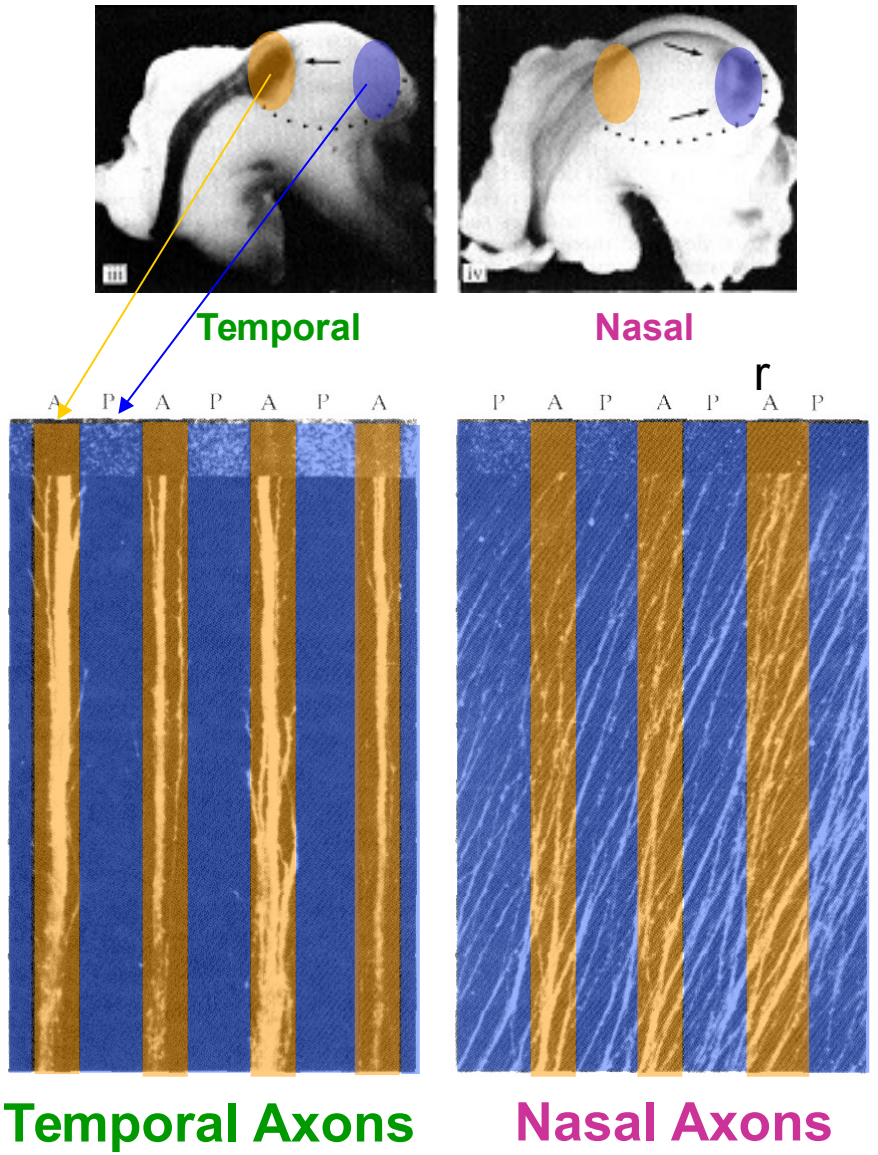
Fractionate Native Factors

⬇

Purify and Identify Factor

⬇

(Ephrins...)



2. Migration: how do neurons know where to go. Identification of molecules using genetics (c.elegans, Drosophila)

Observe WT Neuronal Specificity

⬇

Screen for Mutants of Neuronal Specificity

⬇

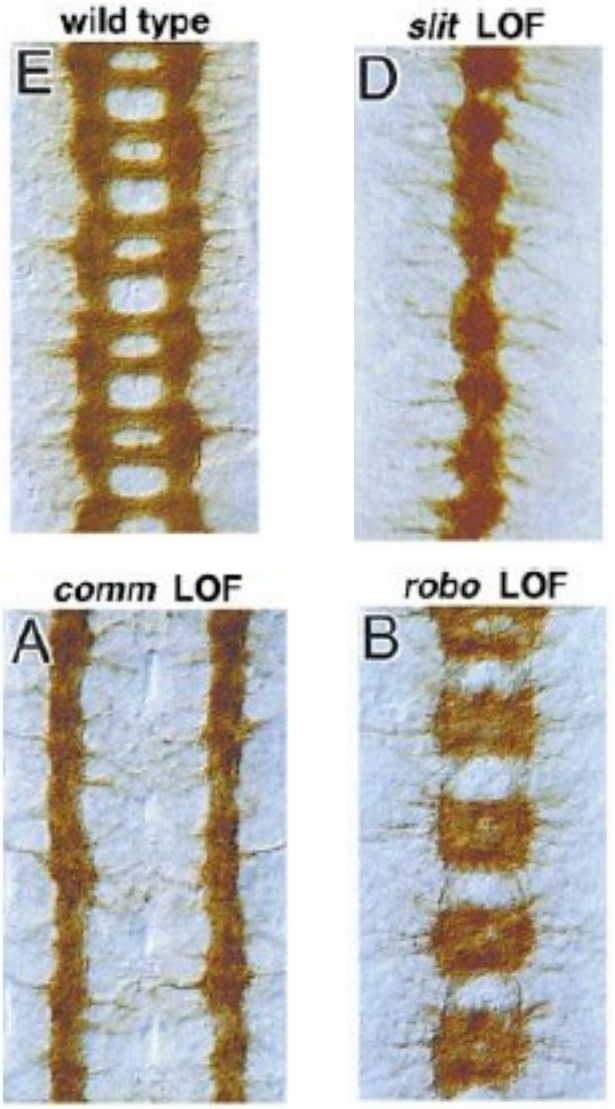
Clone Mutant Genes

⬇

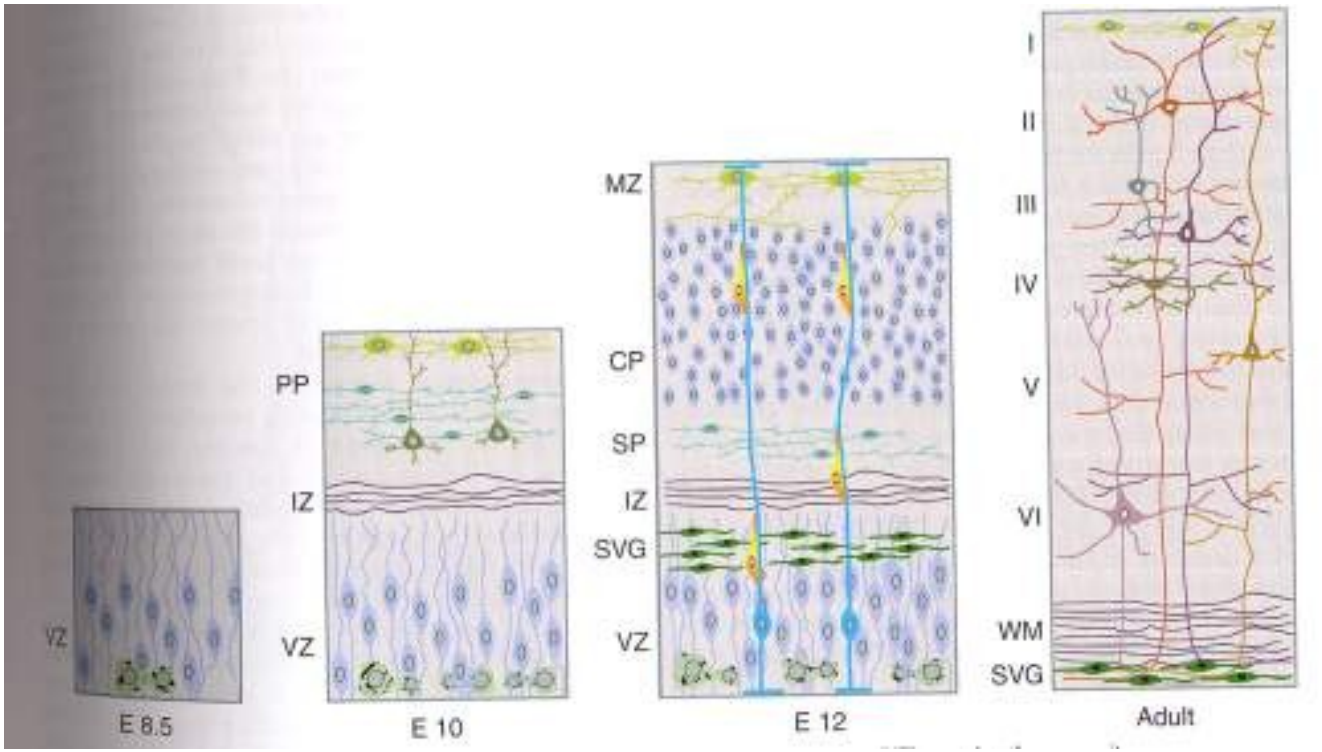
Identify Factors

⬇

(Semphorins, Slit, Robo, Commissureless...)



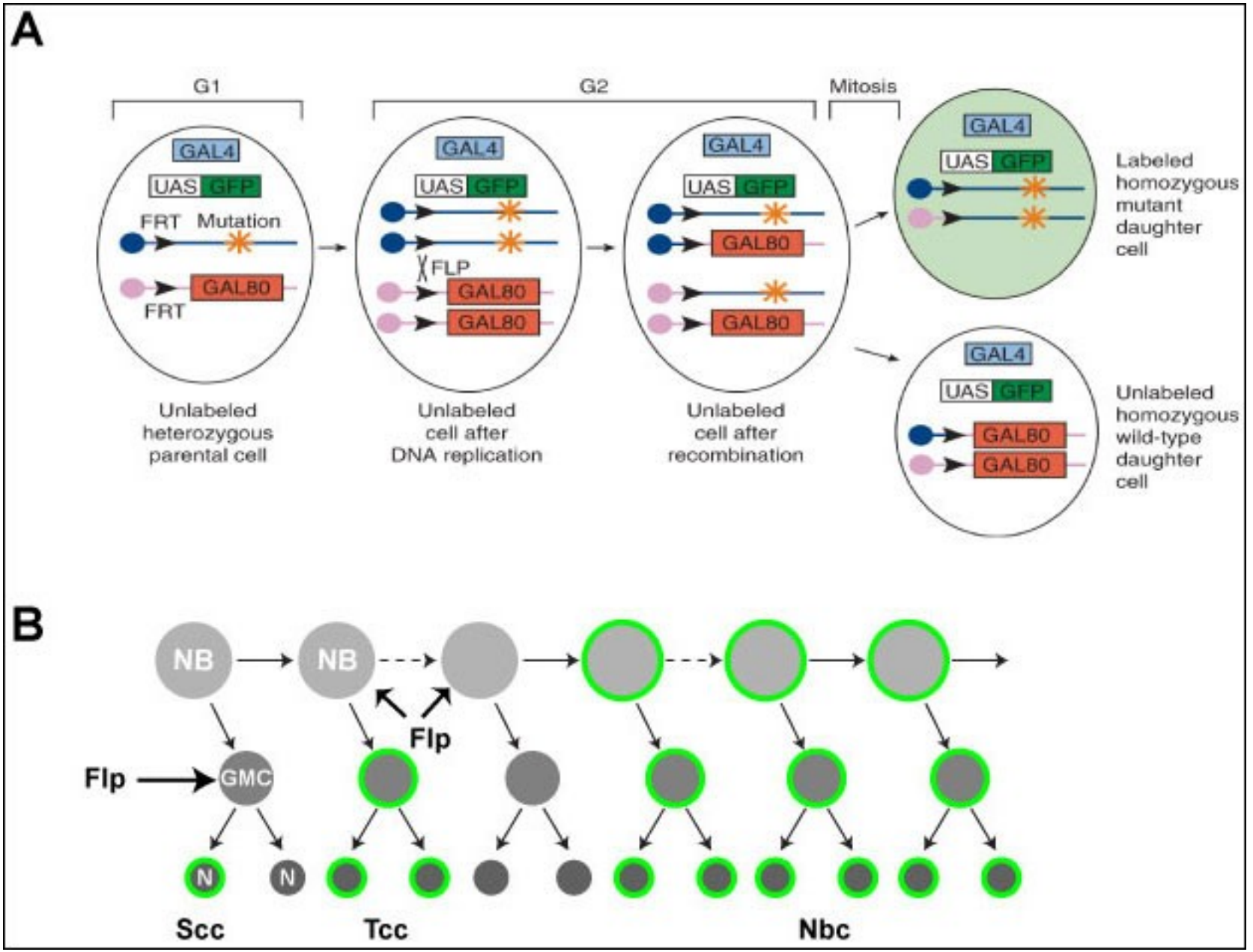
3. Differentiation



Development of the cerebral cortex

The ventricular zone (VZ) contains progenitors of neurons and glia. 1st neurons establish the preplate (PP); their axons an ingrowing axons from the thalamus establish the intermediate zone (IZ). Later generated neurons establish layers II-VI. After migration and differentiation there are 6 cortical layers.

3. Differentiation
MARCM in Drosophila



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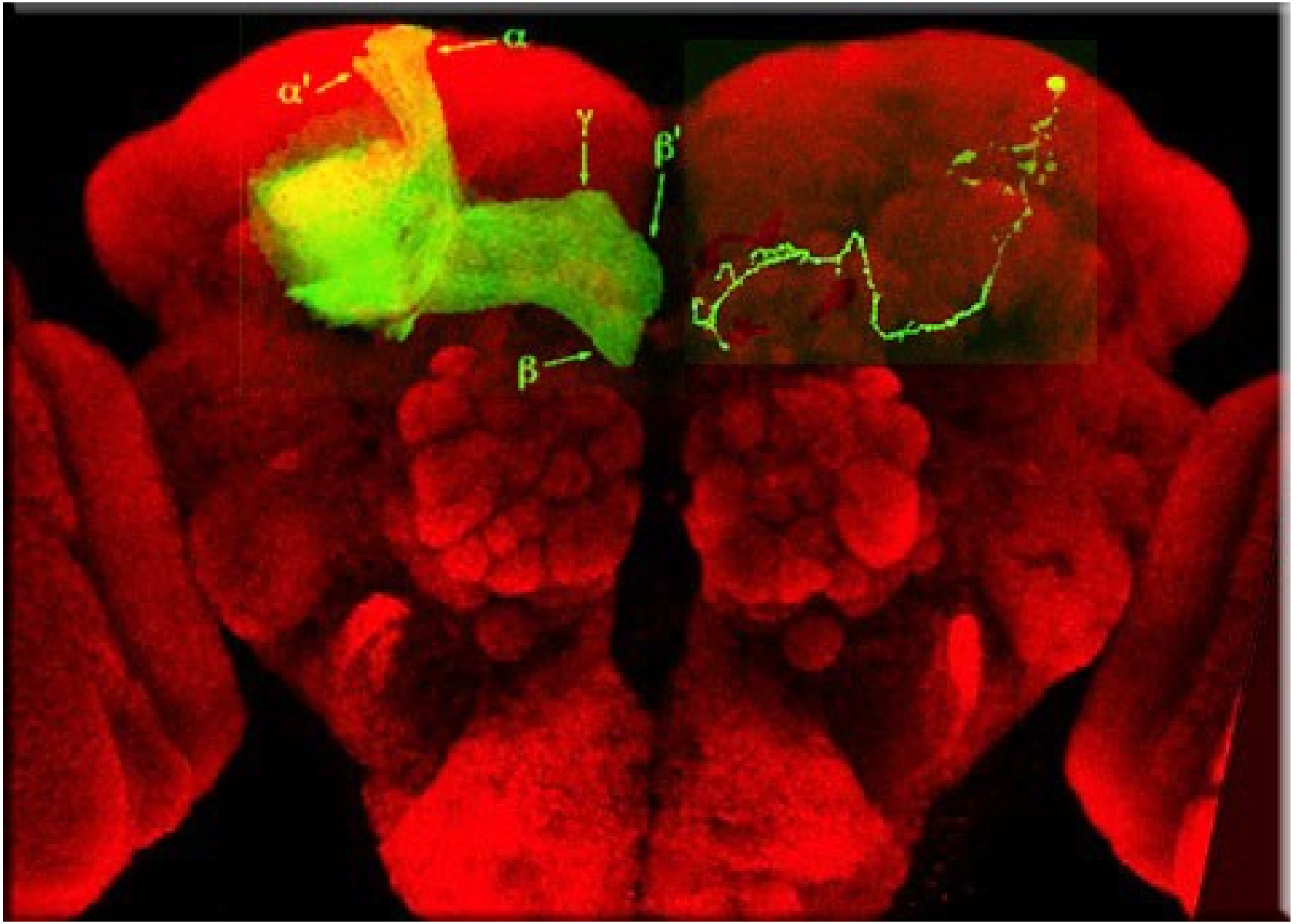
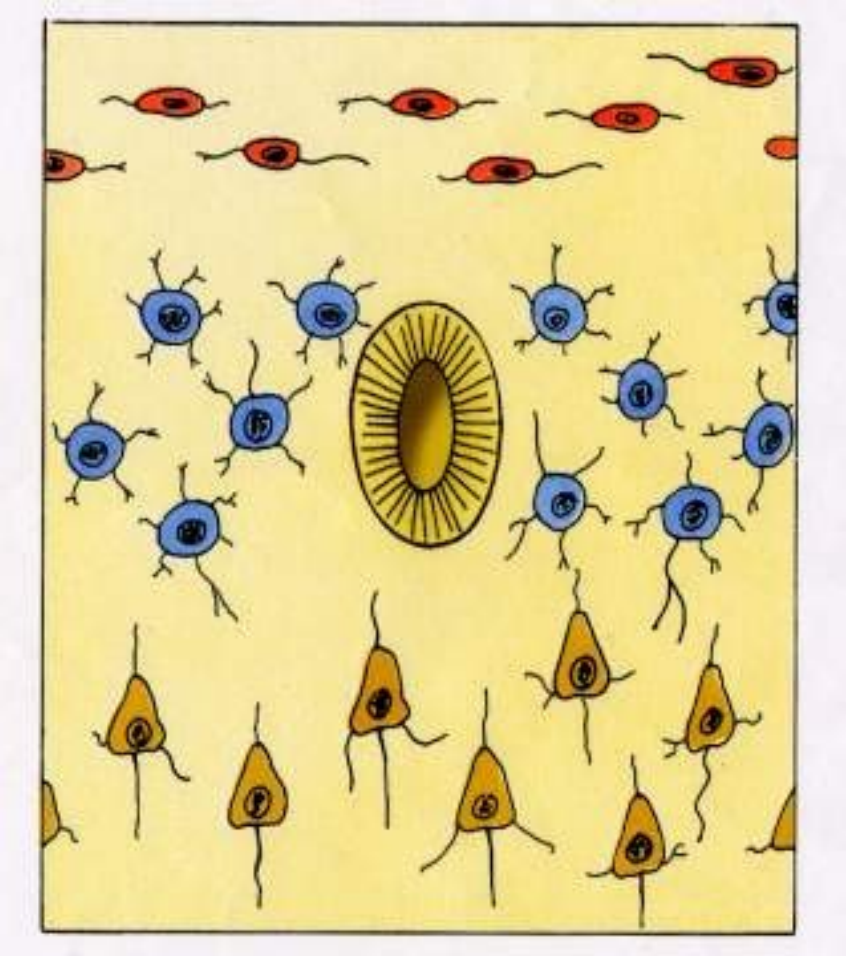
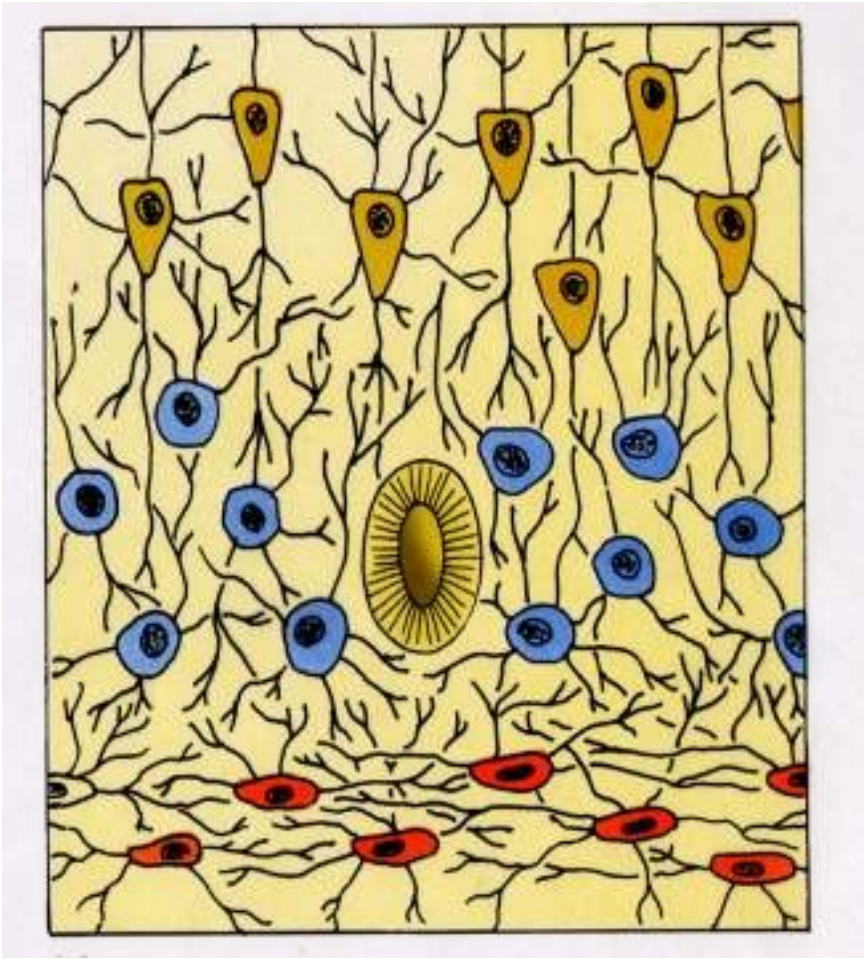


3. Aggregation



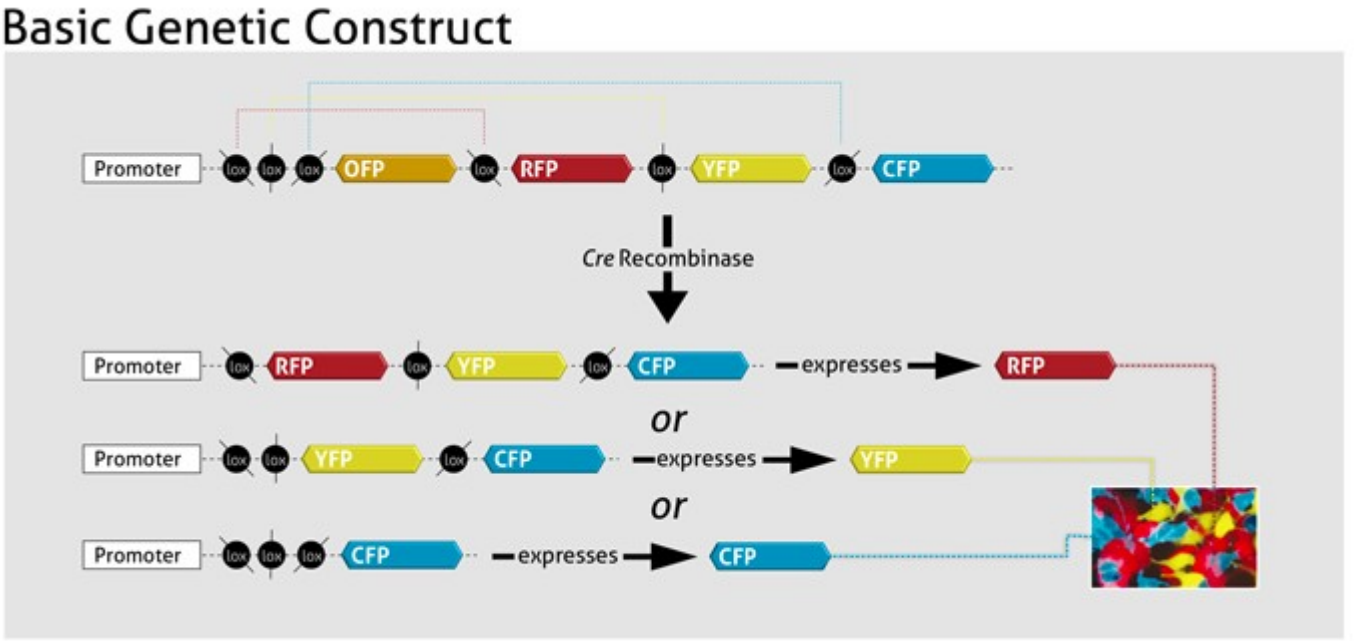
Like neurons move together and form layers

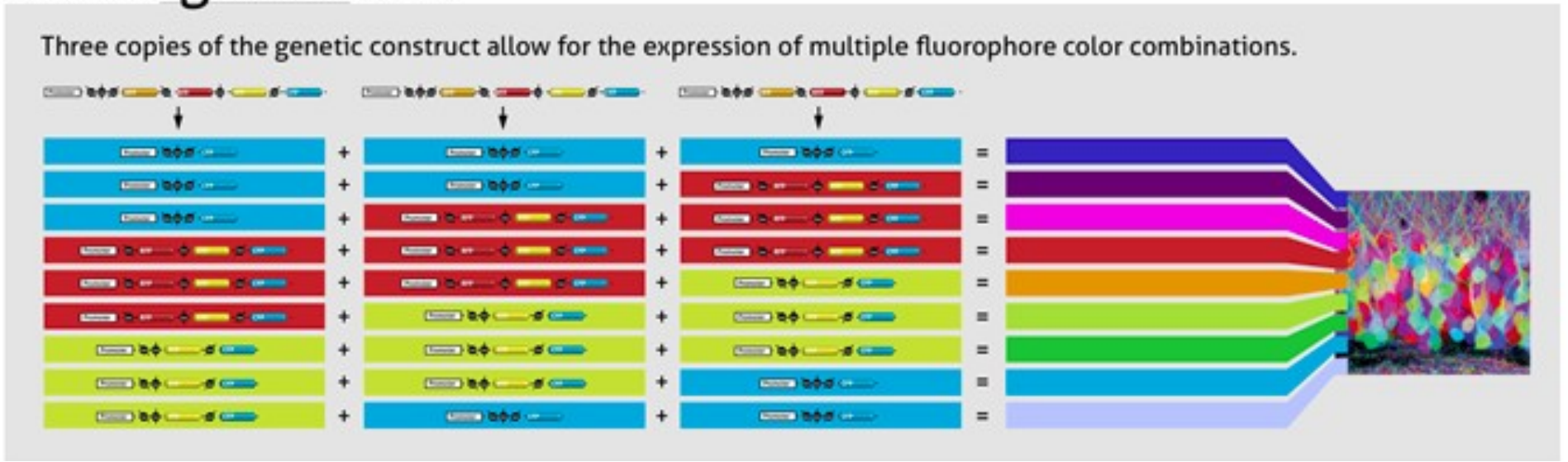
3. Synaptogenesys



Axons (with growth cones on end) form a synapse with other neurons or tissue (e.g. muscle)

3. Synaptogenesys and Connectivity
Brainbow in mice





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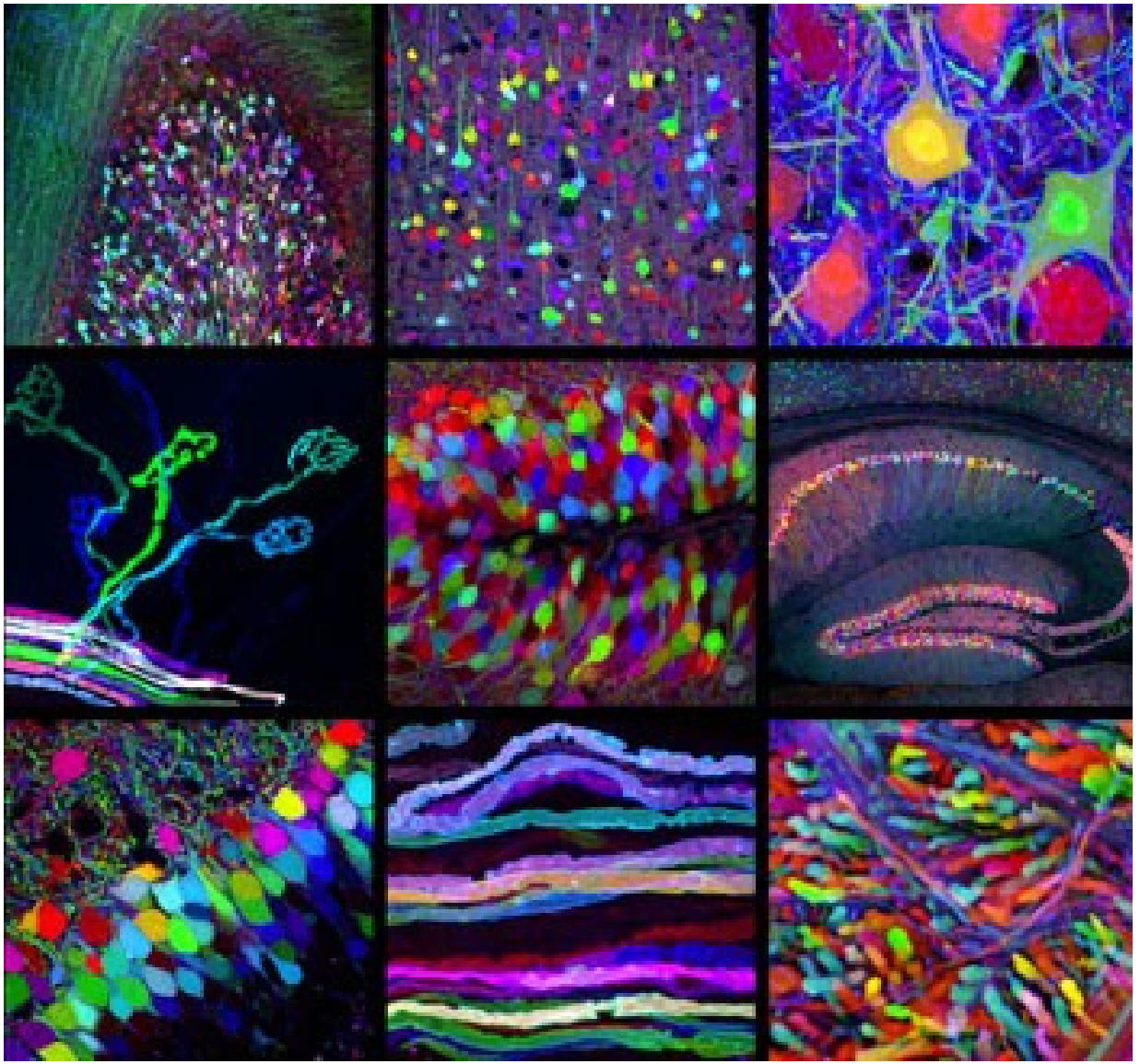
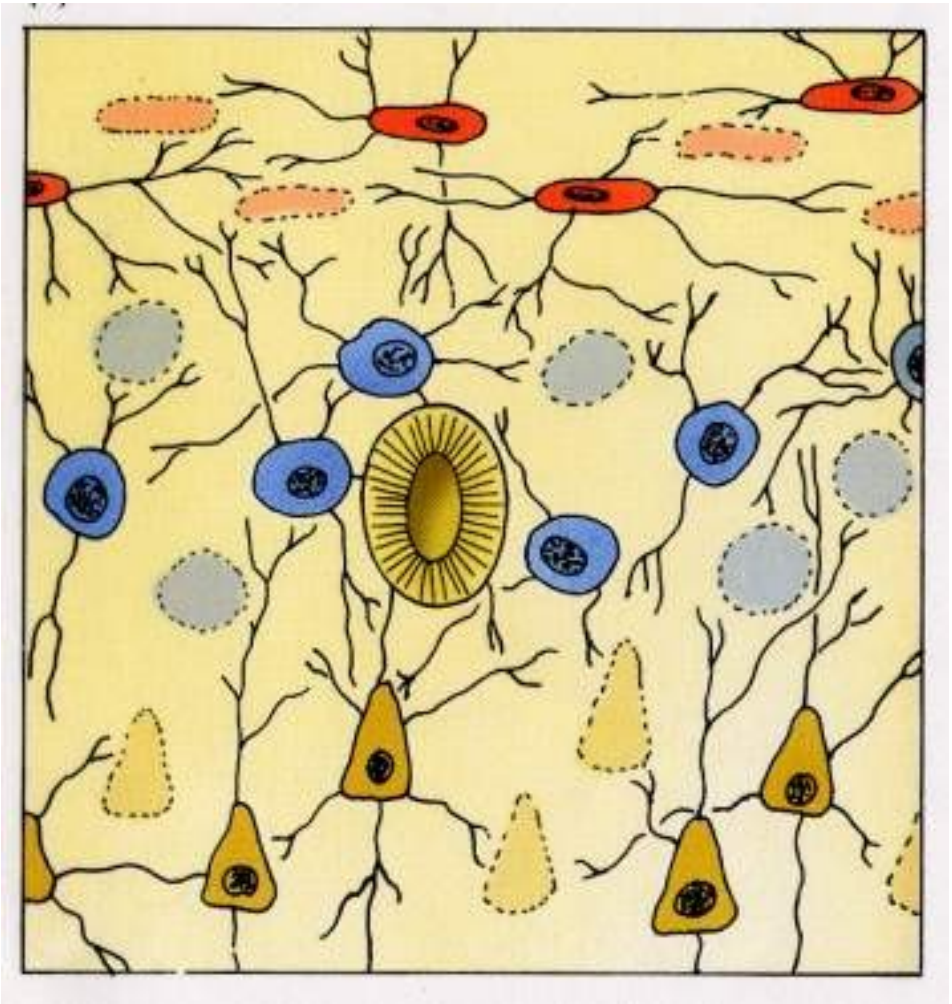
Brainbow in mice

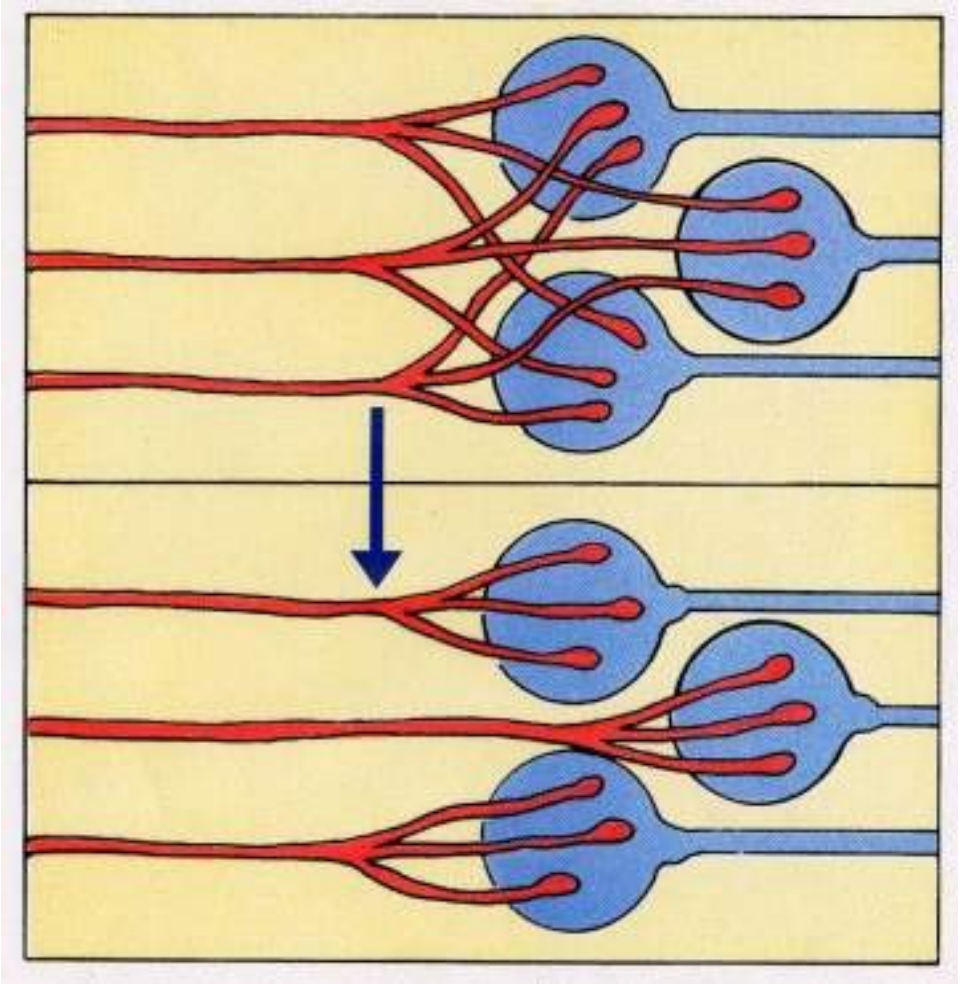


Neuronal Death



- Between 40 and 75 percent of all neurons born in embryonic and fetal development do not survive.
- They fail to make optimal synapses.

Synapse Rearrangment

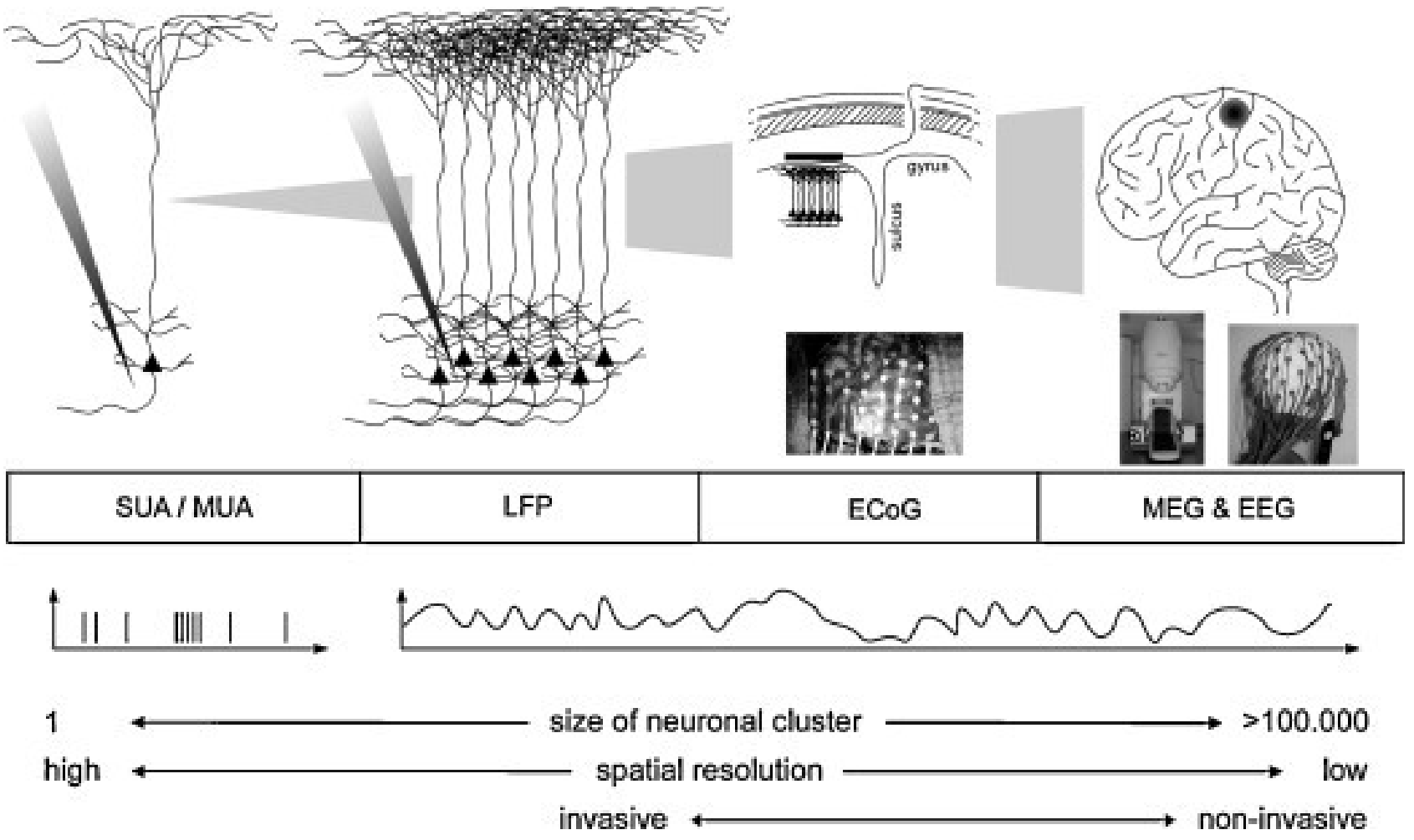


- Active synapses likely take up neurotrophic factor that maintains the synapse
- Inactive synapses get too little trophic factor to remain stable

Electrophysiological models

- Physiology can be studied in humans but only in a subset of patients
- Studied can be performed in vivo and ex vivo
- Electrophysiological properties of a neuron are extremely conserved

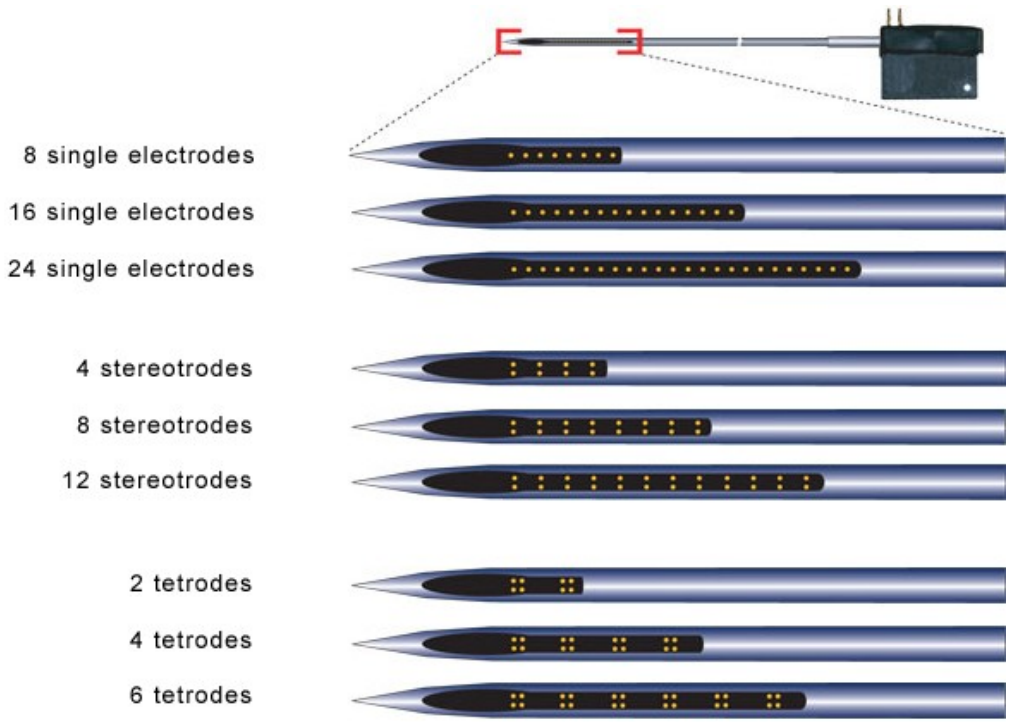
Electrophysiological models

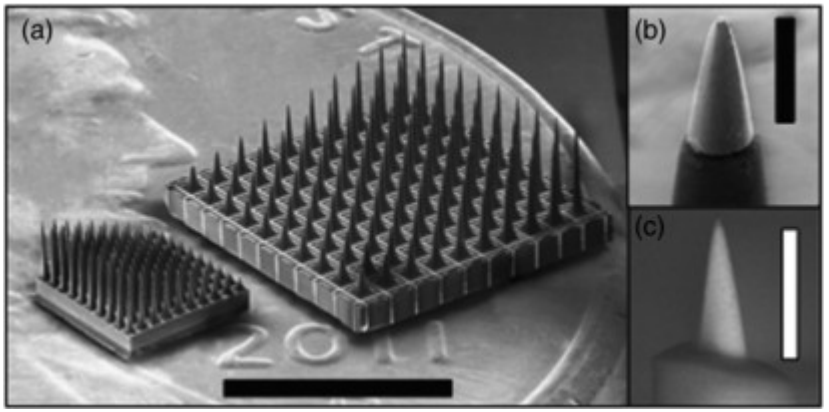


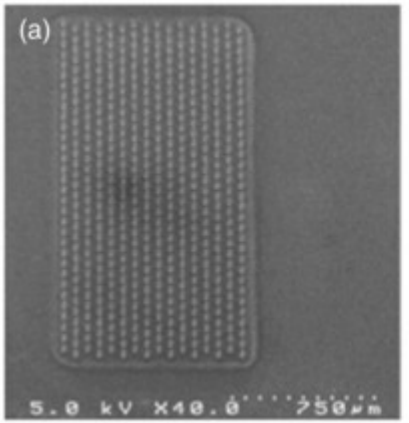
Single Unit Recordings

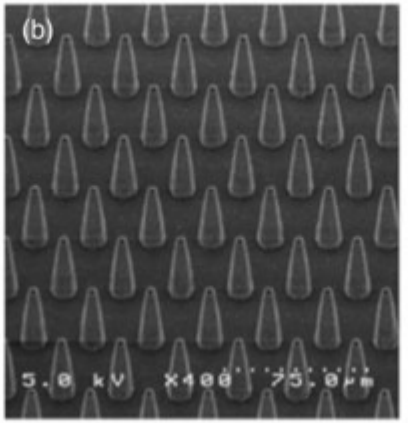
- Microelectrode
  - Glass micropipette
  - Conductive wire (platinum or tungsten)
  - Neutral solution
- Place in or near neuron and record the electrical activity
- Precise enough to isolate individual neurons

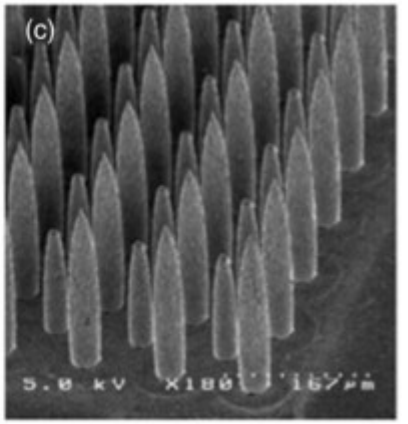
Single Unit Recordings



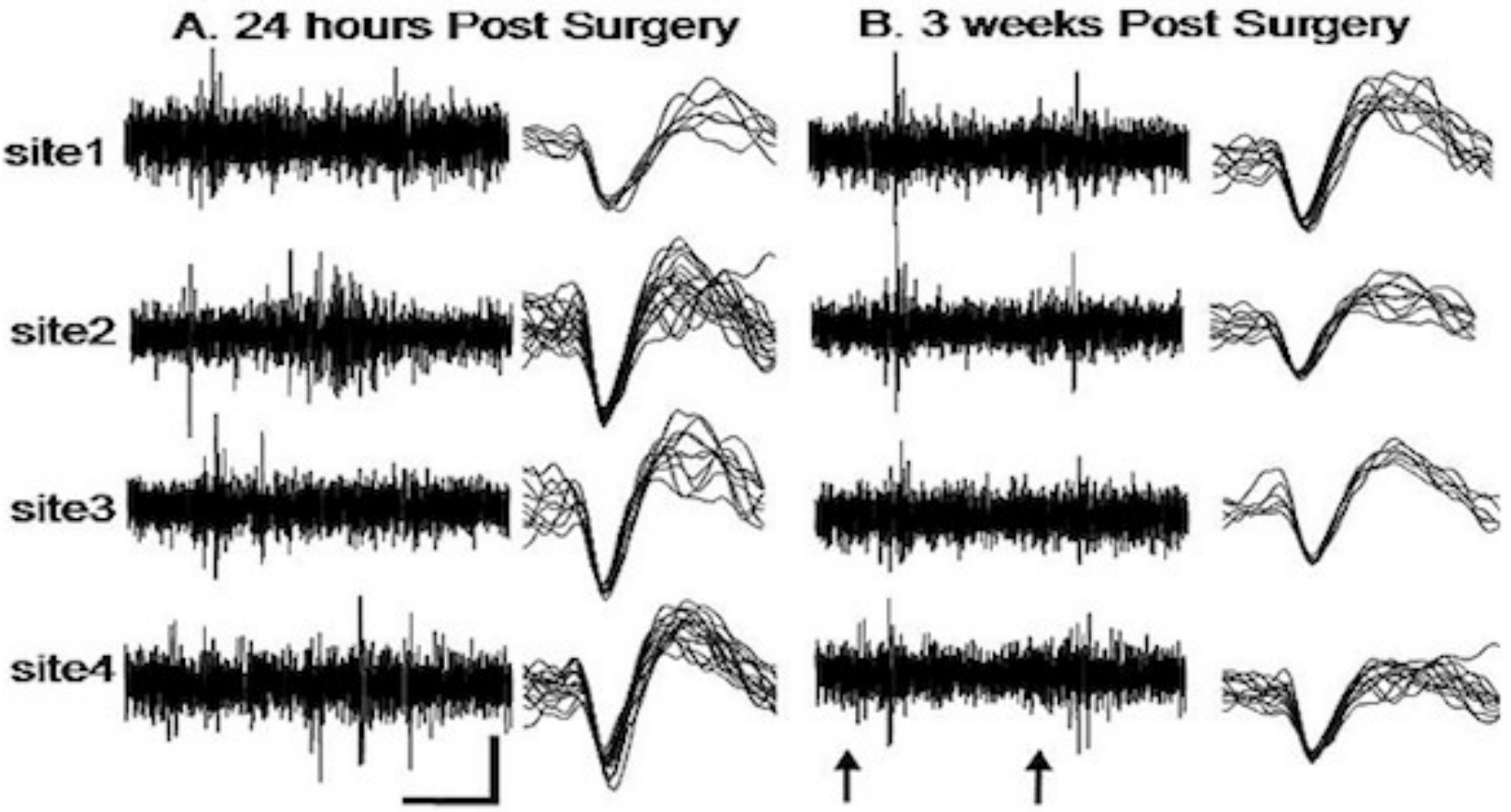








Single Unit Recordings



Extracellular Recording (LFP)

- Microelectrode
  - Very small tip (3-10 micron ~ 1/1,000,000 m)
  - Small enough to isolate a single neuron

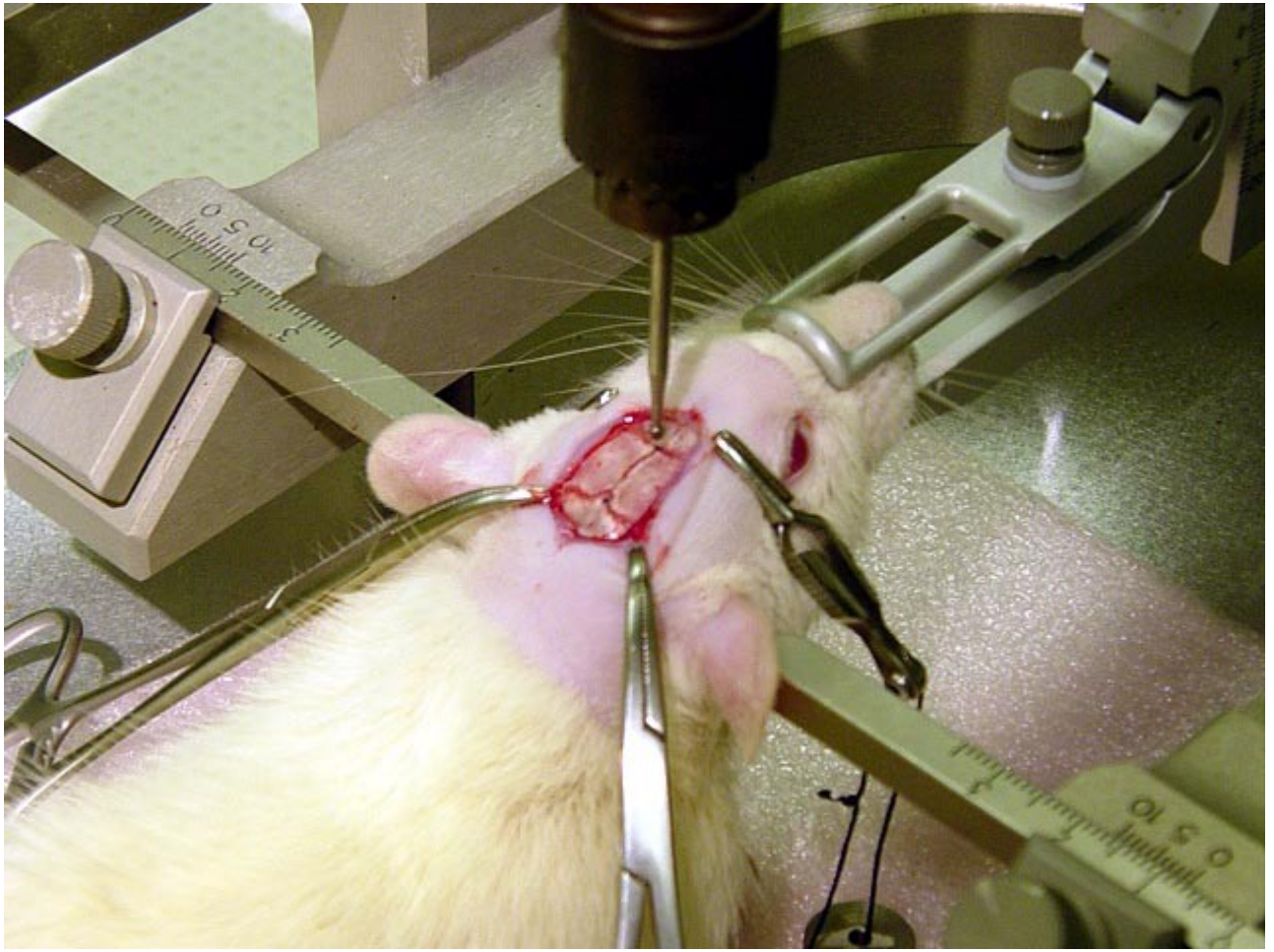
- Place near cell and record changes in electrical activity
  - Dendrites
    - EPSP
    - IPSP

  - Place near a node of Ranvier
    - Action potential

Relationship between MUA and LFP

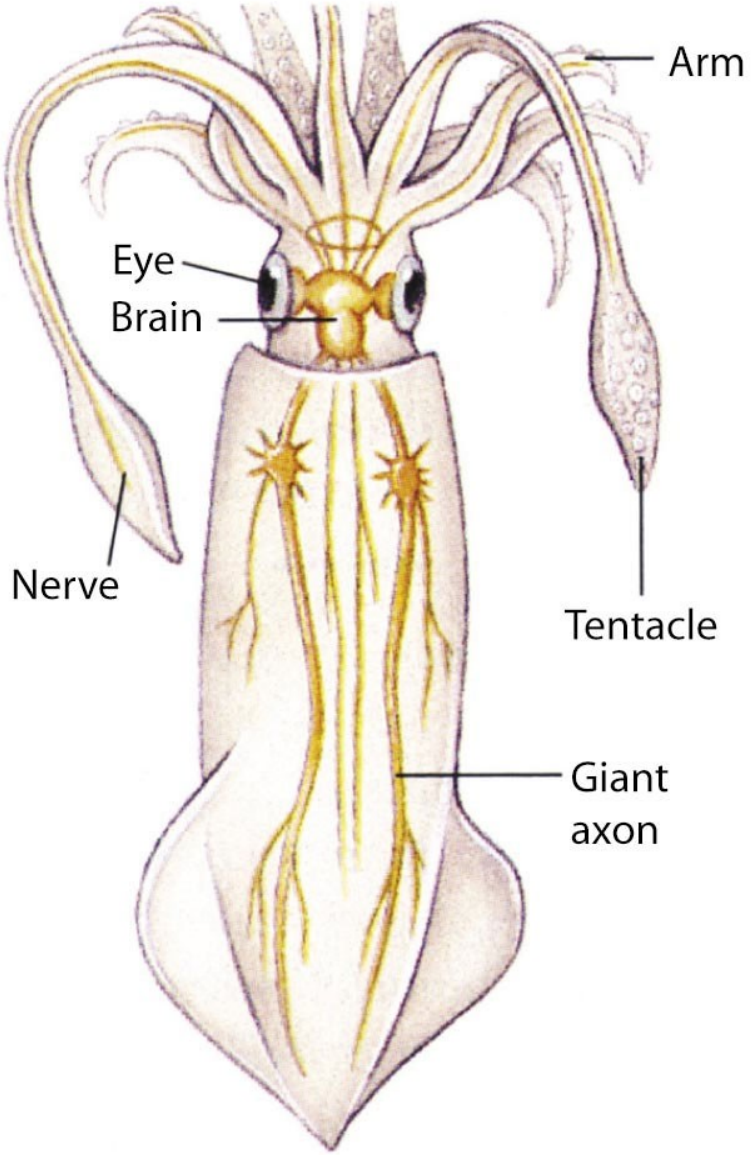
Electrophysiological models

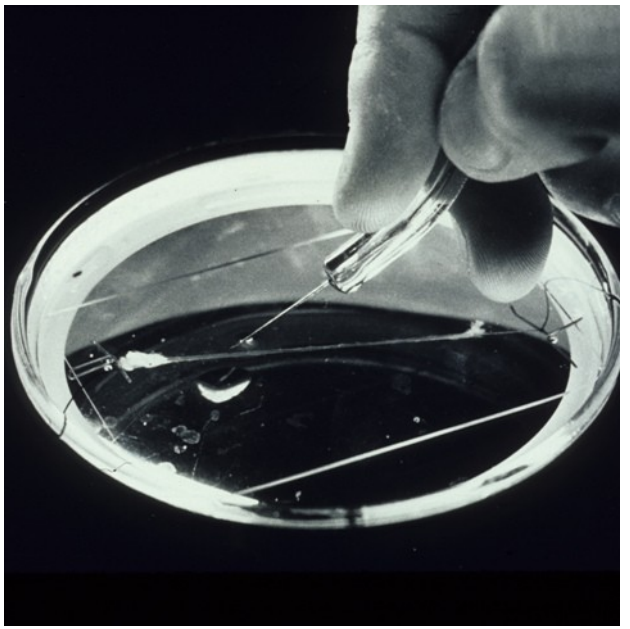
Stereotactic surgery

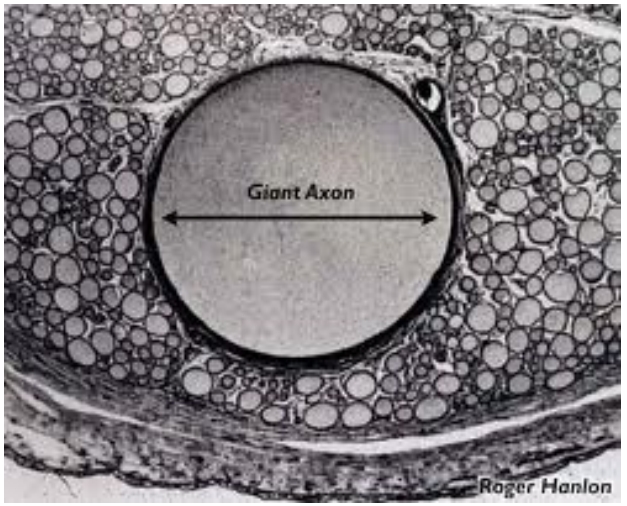


Electrophysiology in invertebrates

Squid giant axon

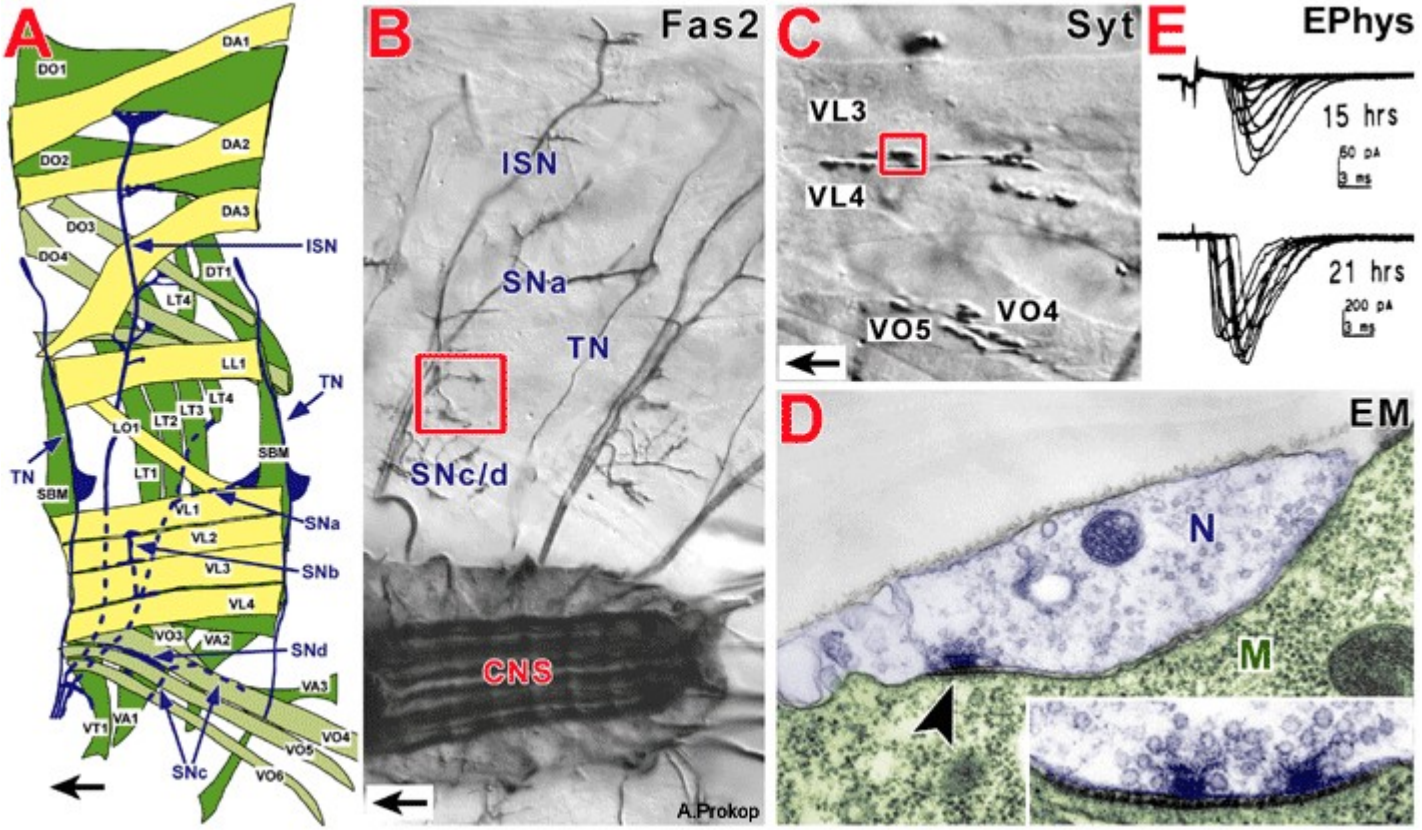






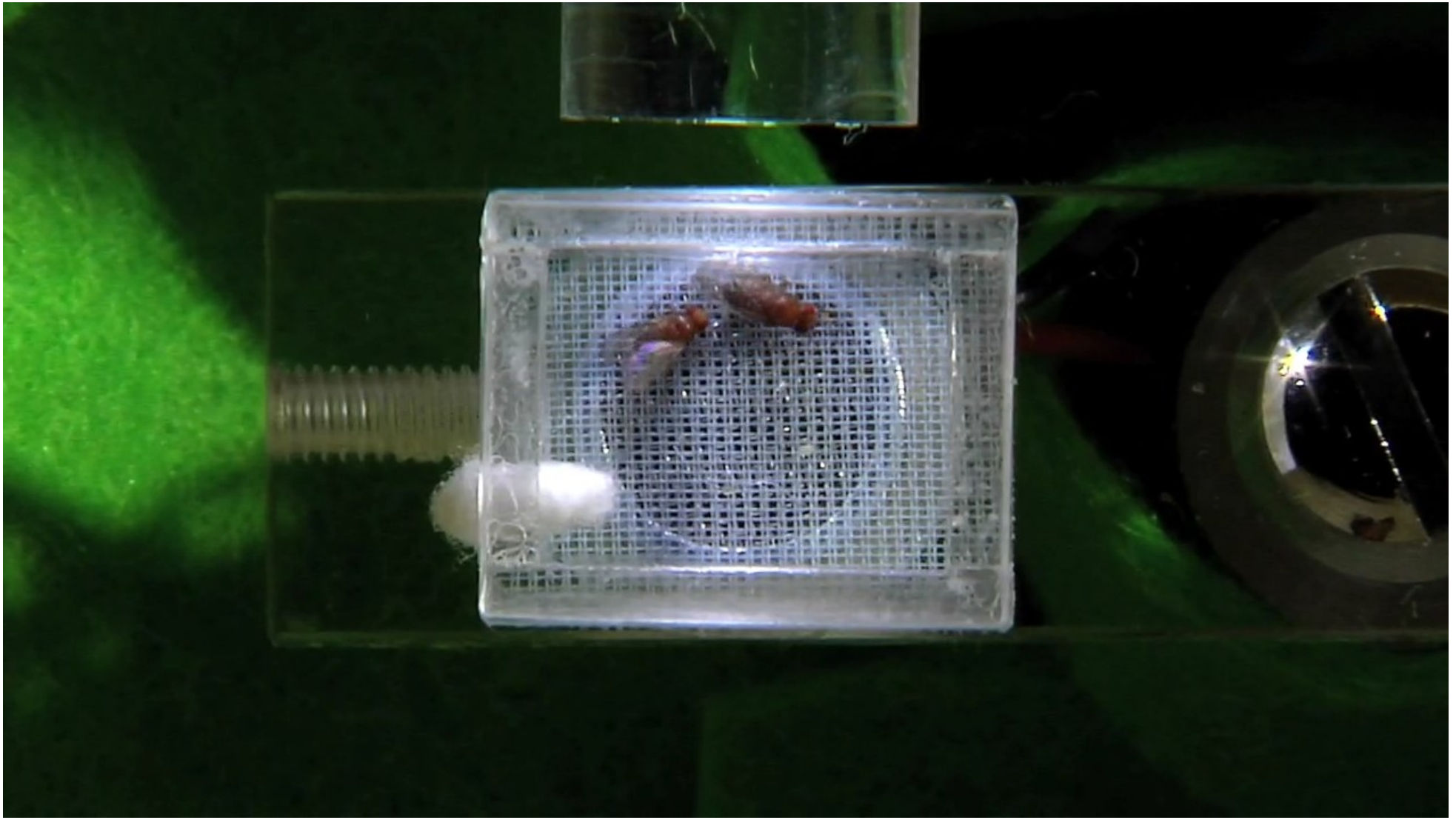
Electrophysiology in invertebrates

Drosophila neuromuscolar junction



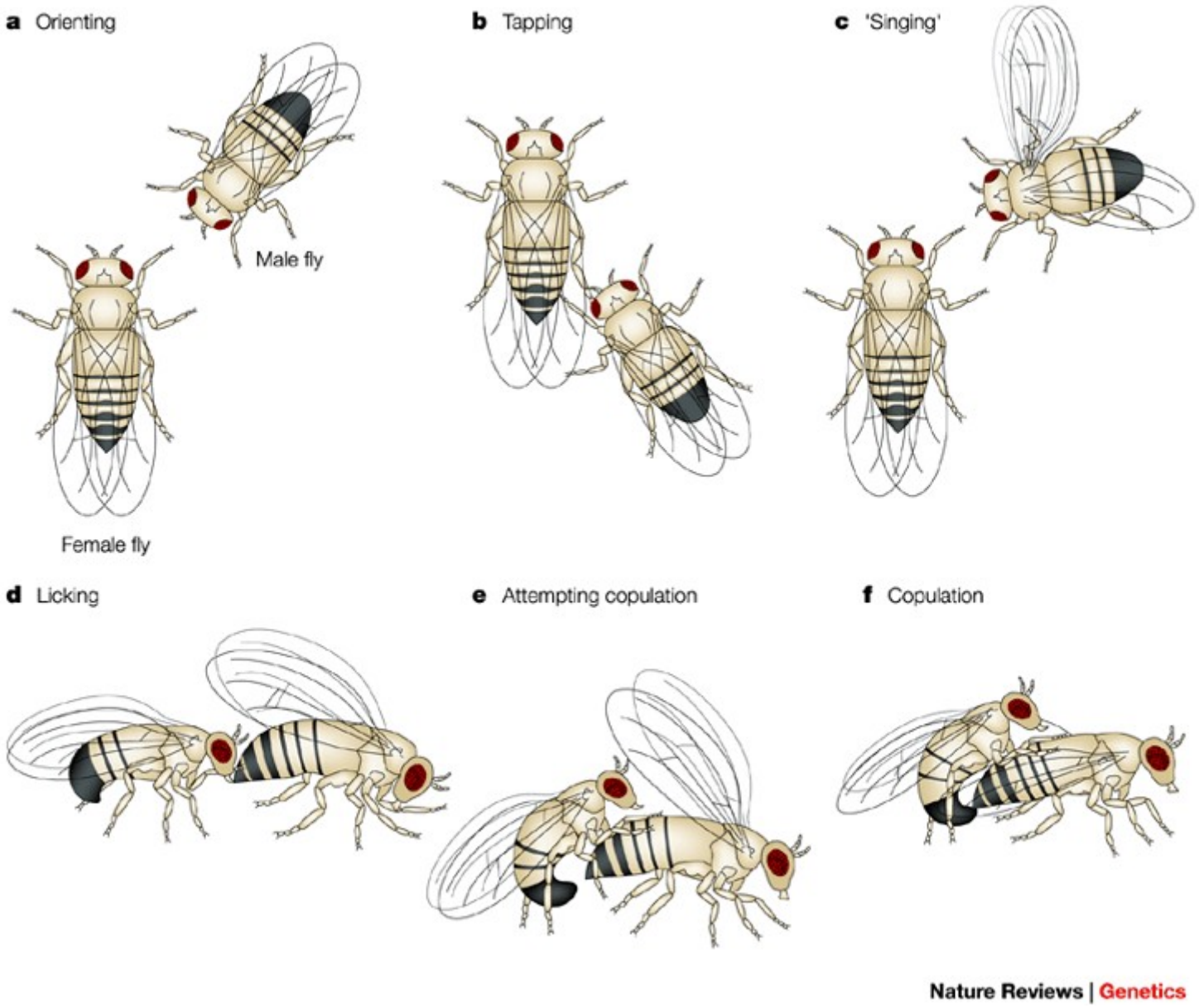
Behavioural models

Dissecting genes and circuits
Drosophila courtship



Drosophila courtship song (link on youtube)

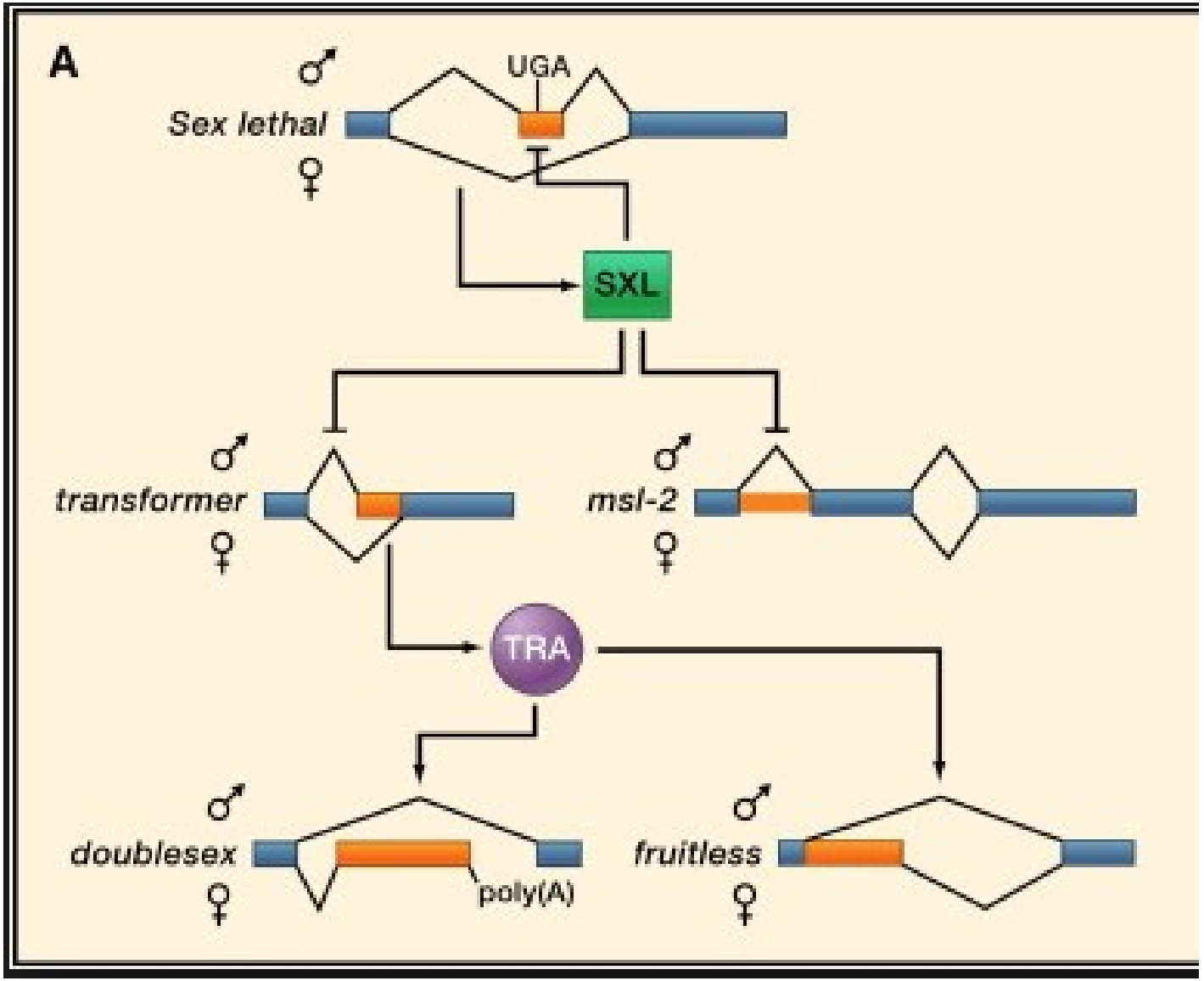
Neuronal control of Drosophila courtship



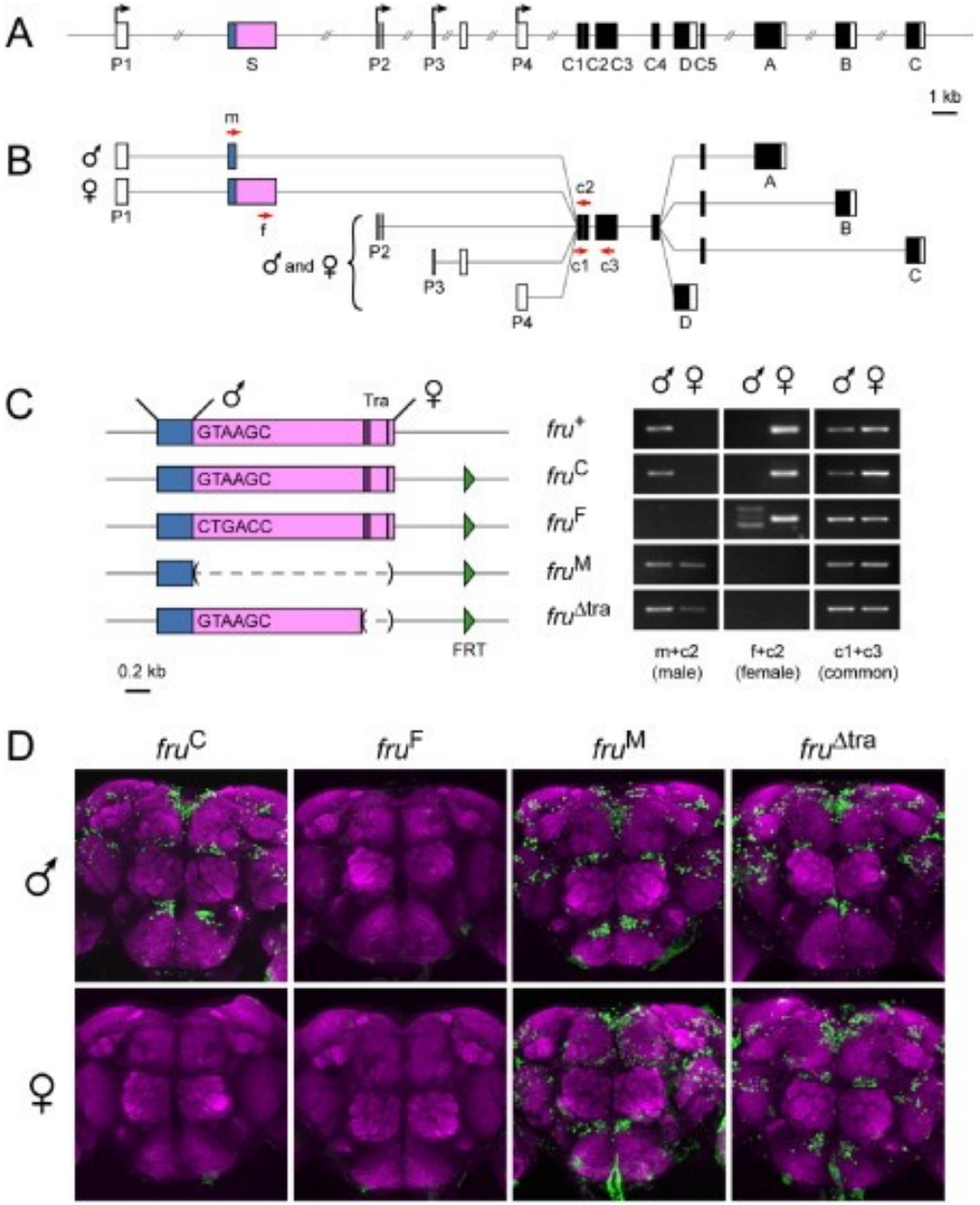
Dissecting genes and circuits
Drosophila courtship

Drosophila courtship ritual (link on youtube)

Dissecting genes and circuits
Drosophila courtship: the *fruitless* gene



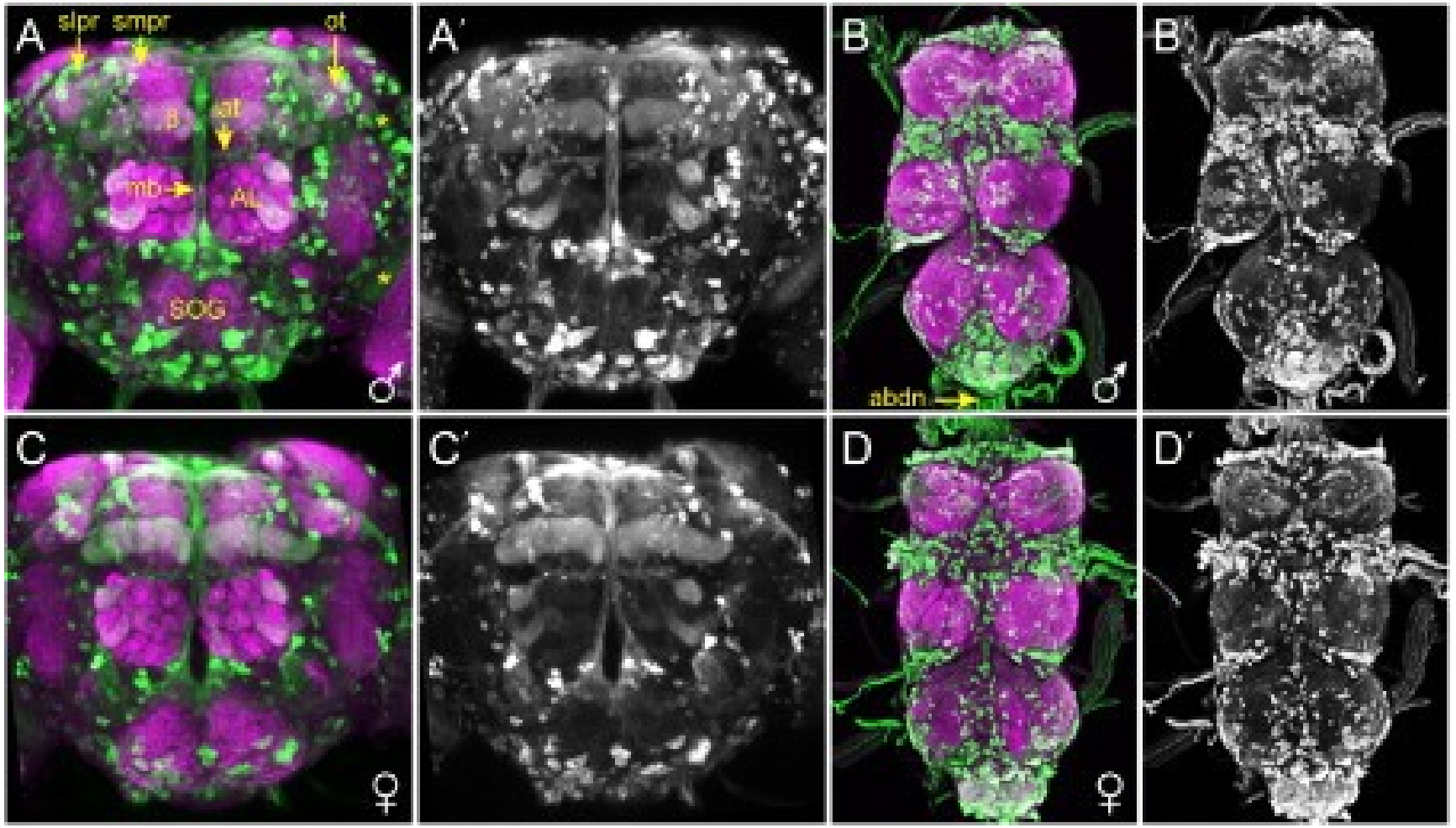
Dissecting genes and circuits
Drosophila courtship: the *fruitless* gene



Dissecting genes and circuits
Drosophila courtship: the *fruitless* gene

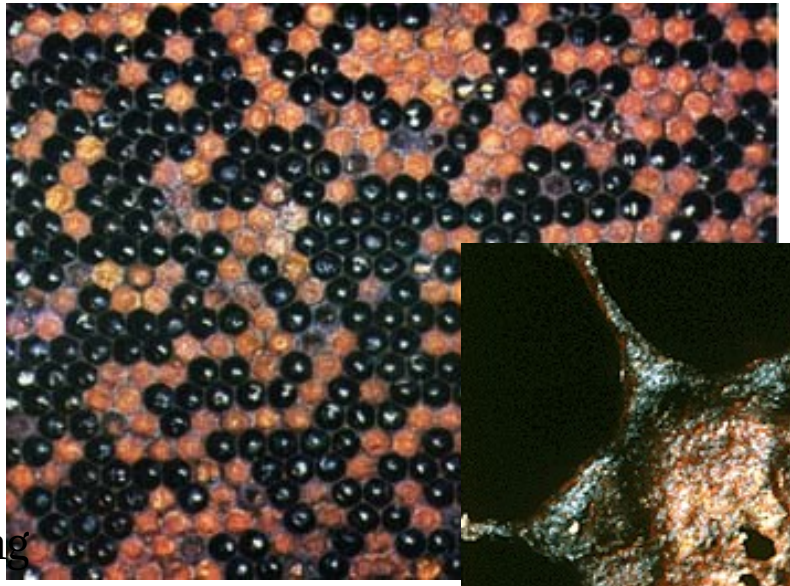
Fruitless mutant – see Supplementary Videos in Demir and Dickson 2005

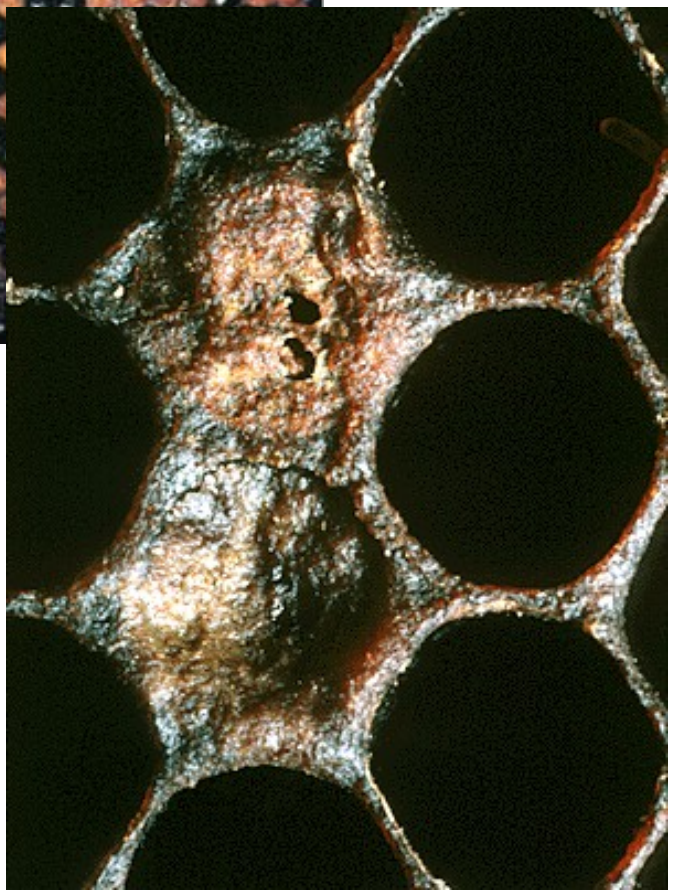
Dissecting genes and circuits
Drosophila courtship: the *fruitless* gene



Dissecting genes and beheaviours in Honey Bees

American foulbrood disease in honeybees





- Caused by a spore-forming bacteria
- Highly resistant to treatment
- Spores last for years in hive products
- Can result in the death of the hive

In the 1940's it was discovered that some hives could effectively manage AFB by uncapping infected larval cells and dragging the infected larvae out of the colony before it became infectious

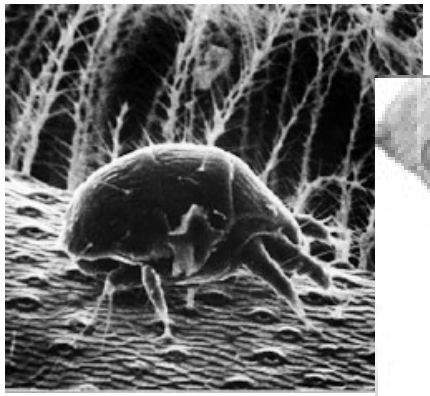
1960's Rothenbhuler demonstrates that two separate and recessive genes control uncapping and removal behaviors

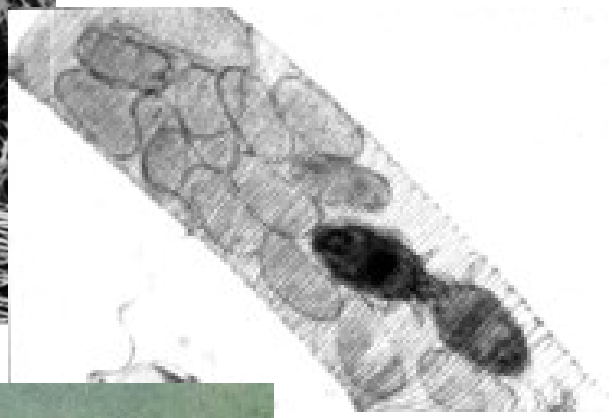
This behavior effectively controls this and a variety of other foulbrood diseases.

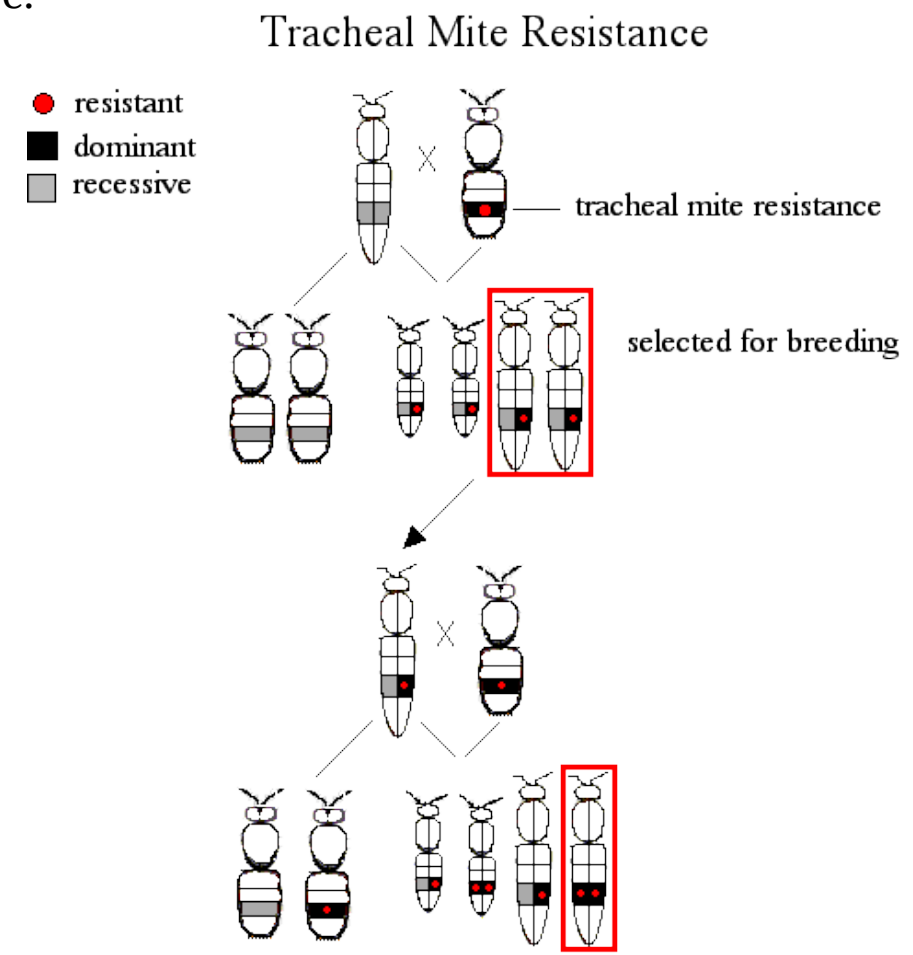
Dissecting genes and beheaviours in Honey Bees

Tracheal mite control behaviors

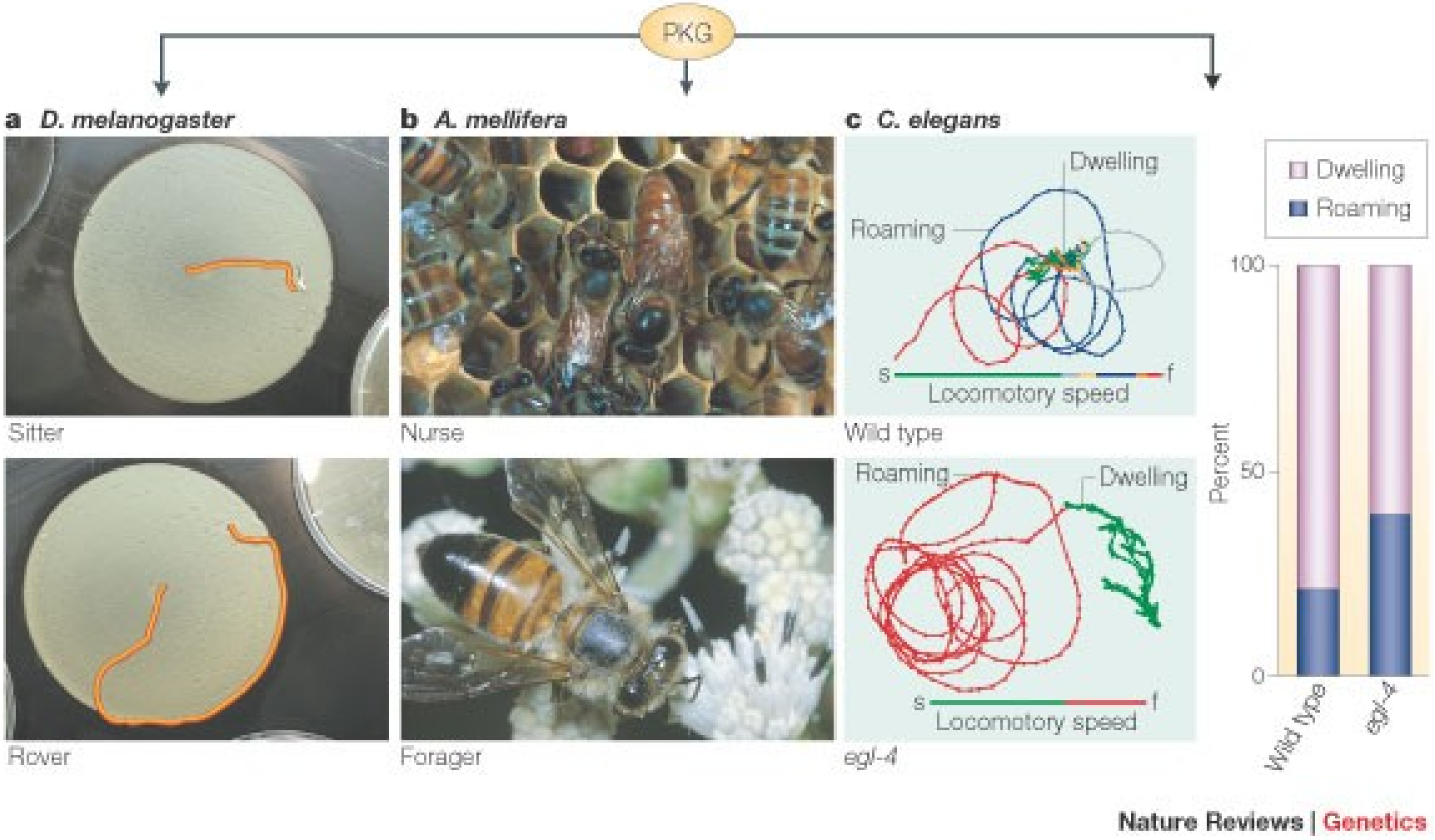
- Bob Danka demonstrated that some bees could effectively resist the mite
- These bees used their middle leg to groom the mites away from the tracheae preventing infestation.
- He also found that this grooming behavior was controlled by a single dominant gene.
- Tracheal mites get into the trachea of honeybees lay eggs which clog the trachea, eventually suffocating the bee.





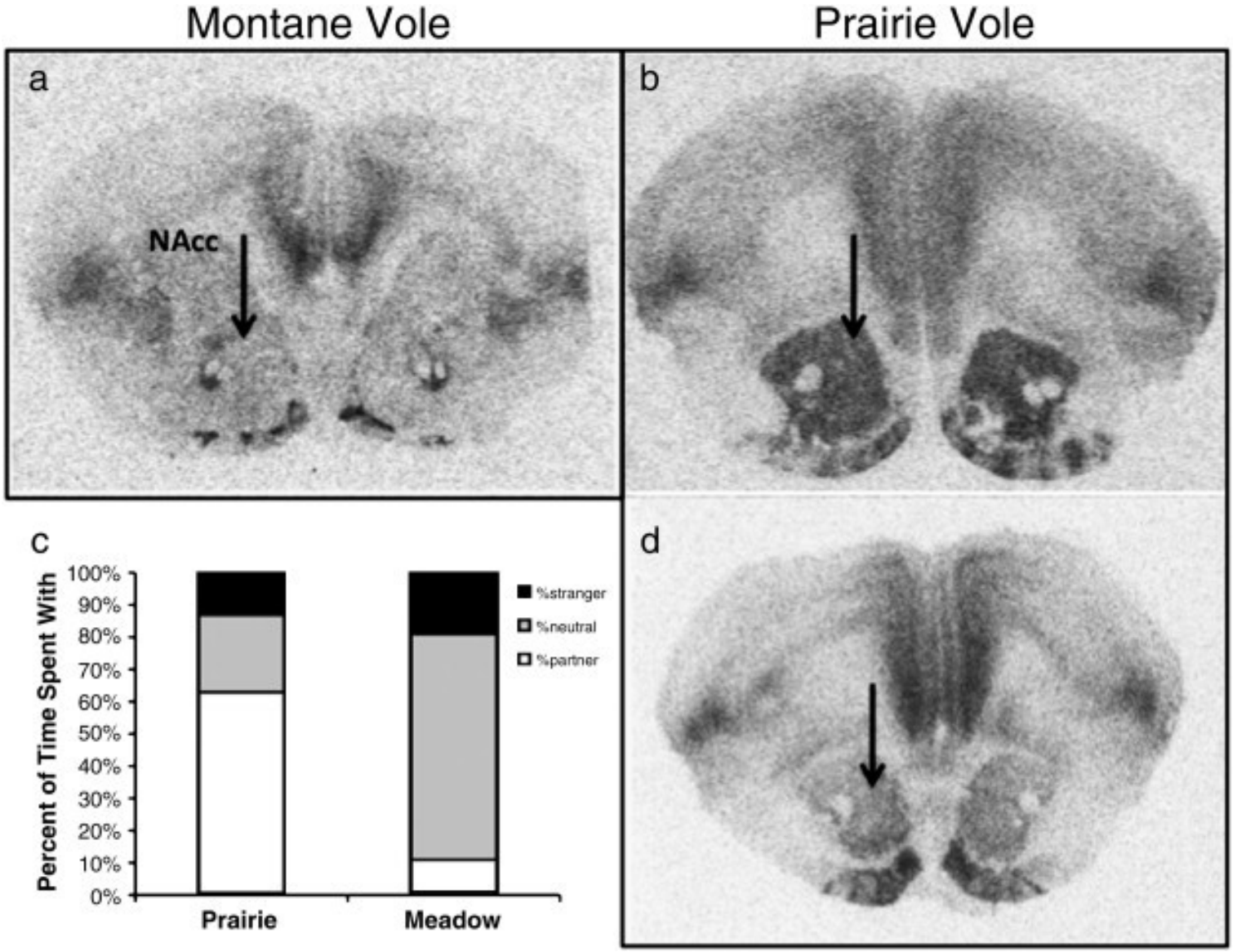


Dissecting genes and beheaviours in Honey Bees

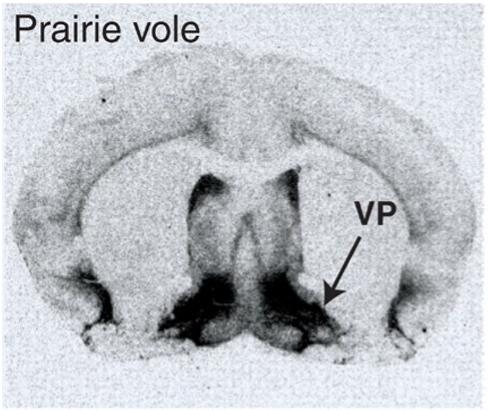


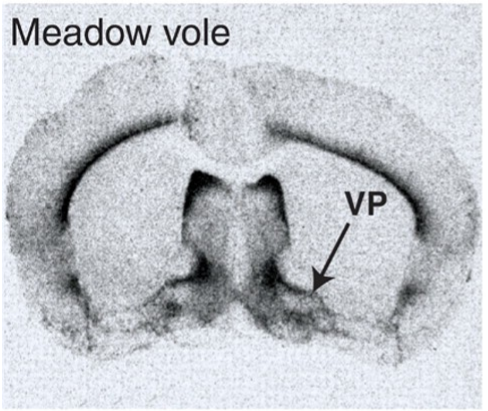
Nature Reviews | Genetics

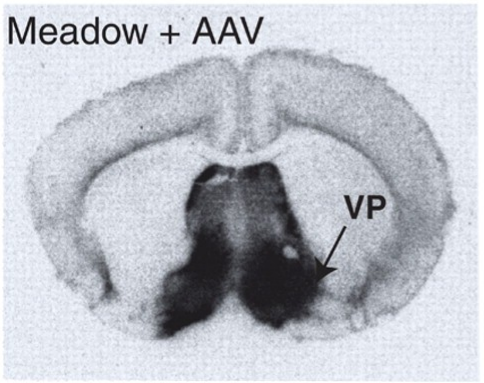
Dissecting genes
Oxytocin in Voles

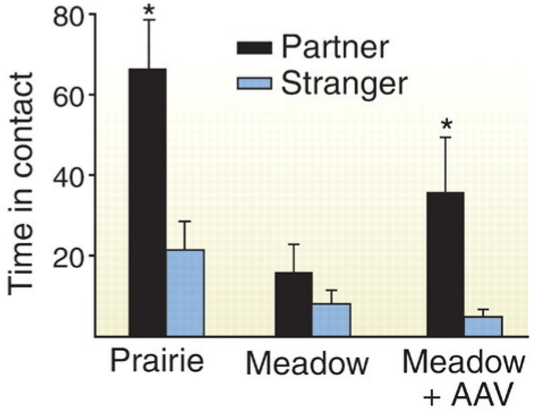


Dissecting genes
Oxytocin in Voles







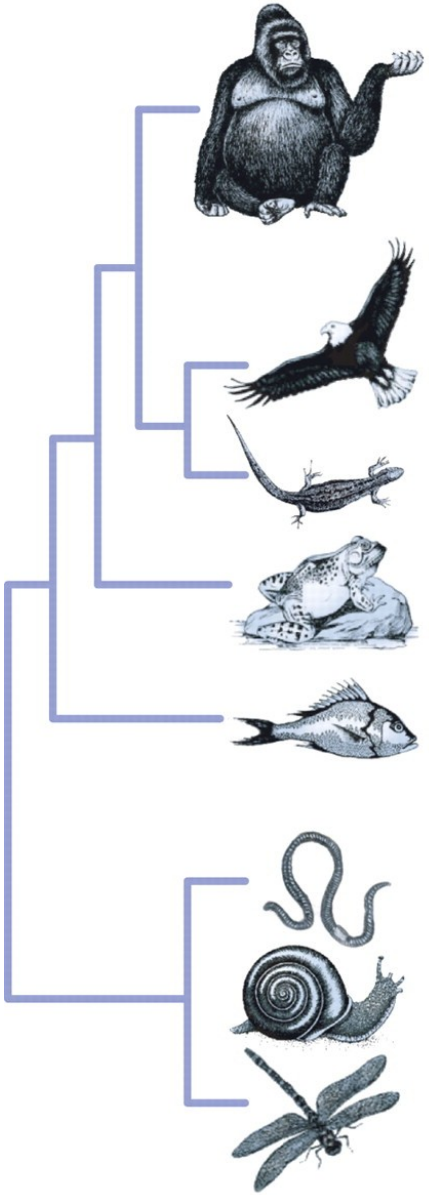


Dissecting genes
Oxytocin in Voles

- In Praire Voles (monogamous), the OXTR is express in the nucleus Accumbens a brain center involved with addiction and pleasure
- Montane Voles (polygamous) do not have OXTR in theNAcc
- Viral infection in Montane Voles can radically transform their behaviour
- Ox is a conserved molecule and acts in regulating pair bonding, pleasure and social interaction in many species including humans
- Oxytocin and Vasopressin are products of the same gene

oxytocin--milk ejection, uterine contractions, maternal behavior, sex behavior, stress, grooming, and development of social bonds (sheep)
vasopressin--water balance, aggressive behavior, scent marking, stress, grooming

Dissecting genes
Oxytocin in Voles



Vasopressin (mammals)
Cys·Tyr·Phe·Gln·**Asn**·**Cys**·**Pro**·Arg·**Gly**·NH_2

Lysipressin (pigs, marsupials)
Cys·Tyr·Phe·Gln·**Asn**·**Cys**·**Pro**·Lys·**Gly**·NH_2

Phenypressin (marsupials)
Cys·Phe·Phe·Gln·**Asn**·**Cys**·**Pro**·Arg·**Gly**·NH_2

Oxytocin
Cys·Tyr·Ile·Gln·**Asn**·**Cys**·**Pro**·Leu·**Gly**·NH_2

Vasotocin
Cys·Tyr·Ile·Gln·**Asn**·**Cys**·**Pro**·Arg·**Gly**·NH_2

Mesotocin
Cys·Tyr·Ile·Gln·**Asn**·**Cys**·**Pro**·Ile·**Gly**·NH_2

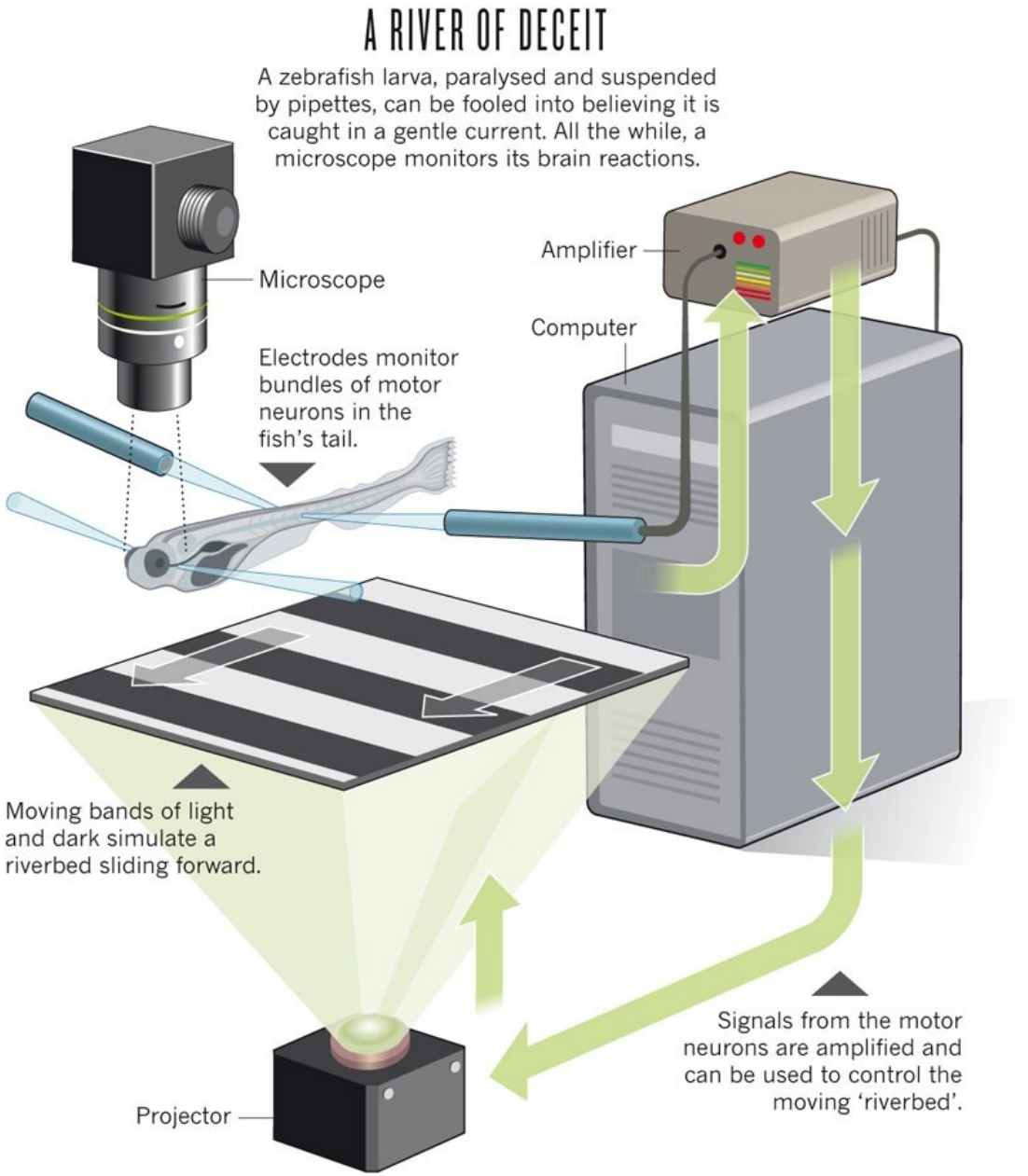
Isotocin
Cys·Tyr·Ile·Ser·**Asn**·**Cys**·**Pro**·Leu·**Gly**·NH_2

Annepressin (annelid worms)
Cys·Phe·Val·Arg·**Asn**·**Cys**·**Pro**·Thr·**Gly**·NH_2

Conopressin (snails, cones, sea hare, leeches)
Cys·Phe/Ile·Ile·Arg·**Asn**·**Cys**·**Pro**·Lys/Arg·**Gly**·NH_2

Inotocin (some insects)
Cys·Leu·Ile·Thr·**Asn**·**Cys**·**Pro**·Arg·**Gly**·NH_2

Dissecting circuits
In vivo analysis and perturbation of behaviour



Models for cellular neuroscience

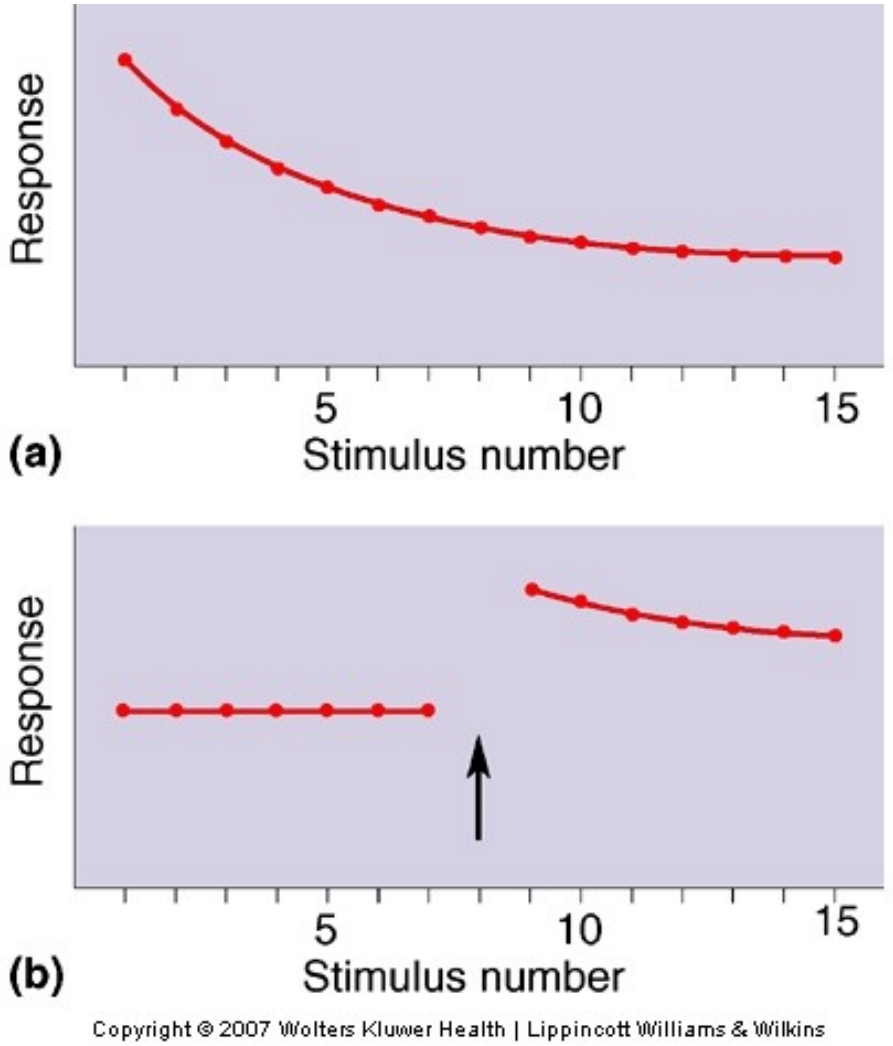
Models for cellular neuroscience

Cellular and Molecular basis of learning

- Neurobiology of memory
  - Identifying where and how different types of information are stored
- Hebb
  - Memory results from synaptic modification
- Study of simple invertebrates
  - Synaptic alterations underlie memories (procedural)
- Electrical stimulation of brain
  - Experimentally produce measurable synaptic alterations - dissect mechanisms

Cellular and Molecular basis of learning
Procedural Learning

- Procedural memories amenable to investigation
- Nonassociative Learning
  - Habituation
    - Learning to ignore stimulus that lacks meaning
  - Sensitization
    - Learning to intensify response to stimuli



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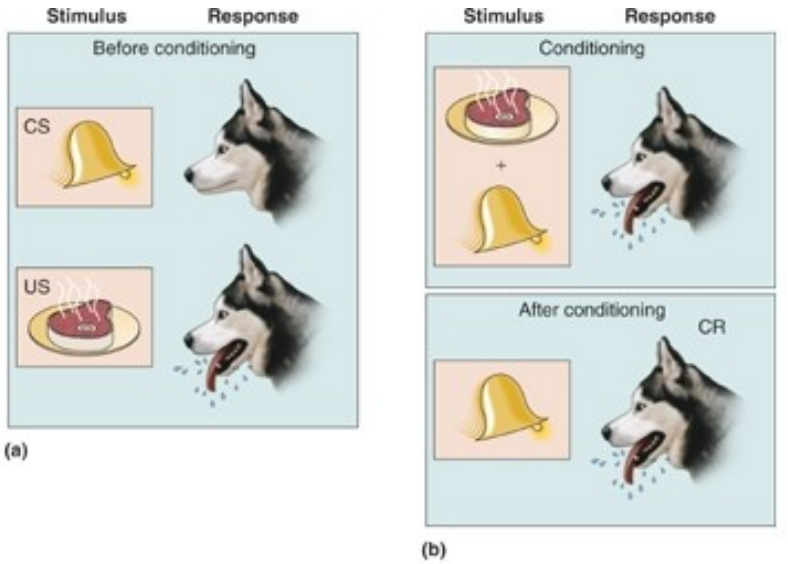
Cellular and Molecular basis of learning
Procedural Learning

- Associative Learning
  - Classical Conditioning: Pair an unconditional stimulus (UC) with a conditional stimulus (CS) to get a conditioned response (CR)
  - Instrumental Conditioning: Learn to associate a response with a meaningful stimulus, e.g., reward lever pressing for food. Complex neural circuits related to role played by motivation



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Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in *Aplysia*
  - Gill-withdrawal reflex
  - Habituation

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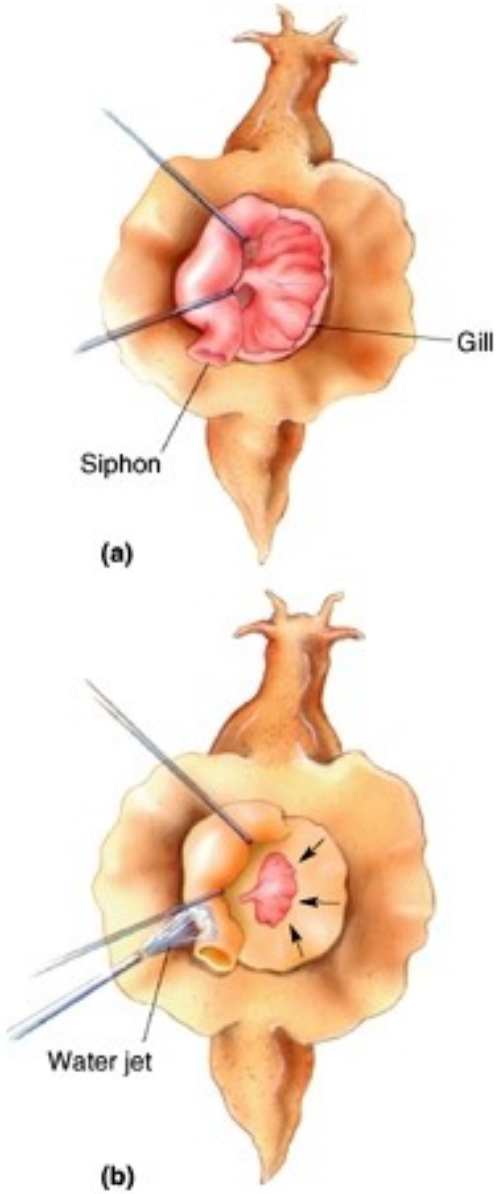

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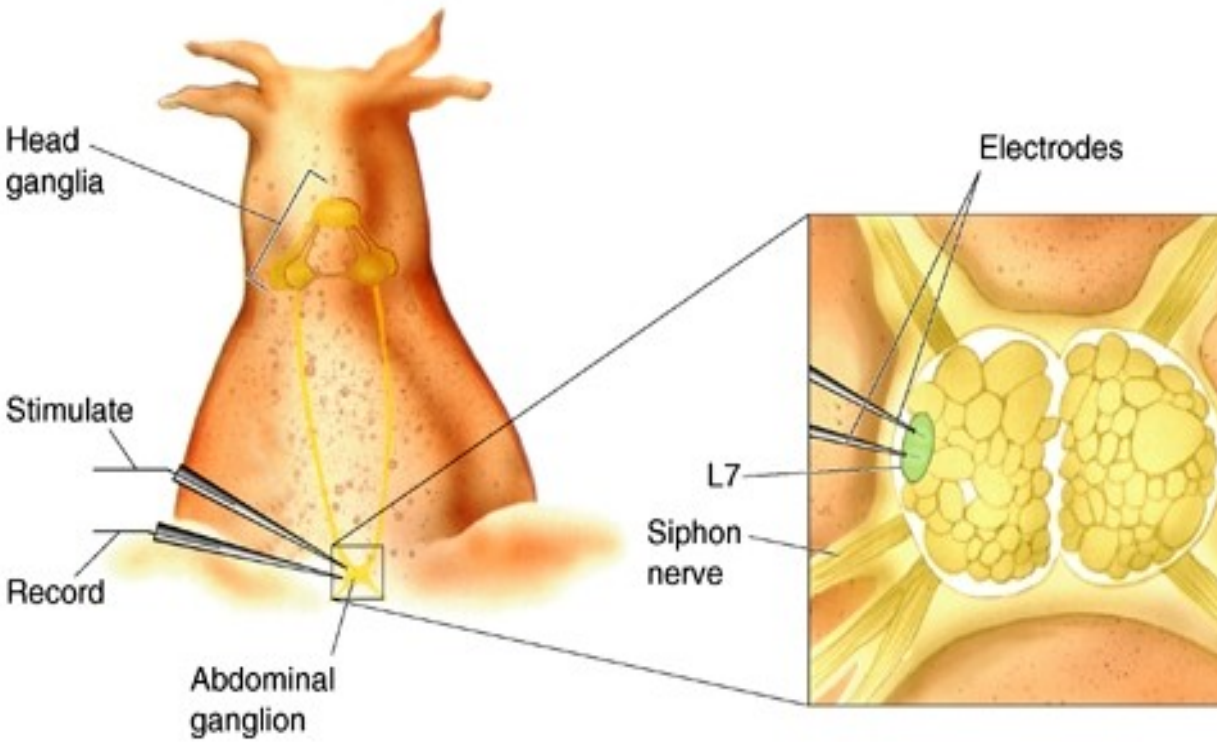
Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in *Aplysia*
  - Habituation results from presynaptic modification at L7



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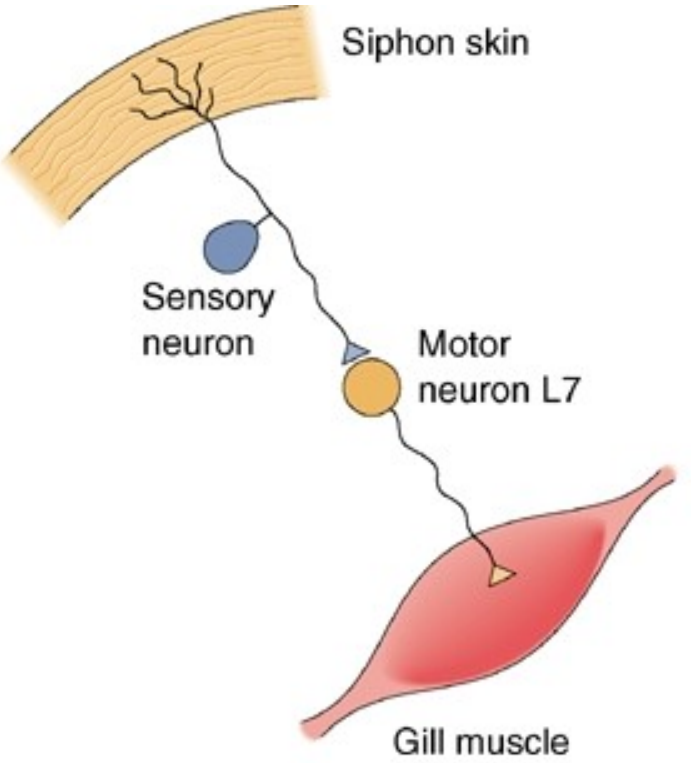

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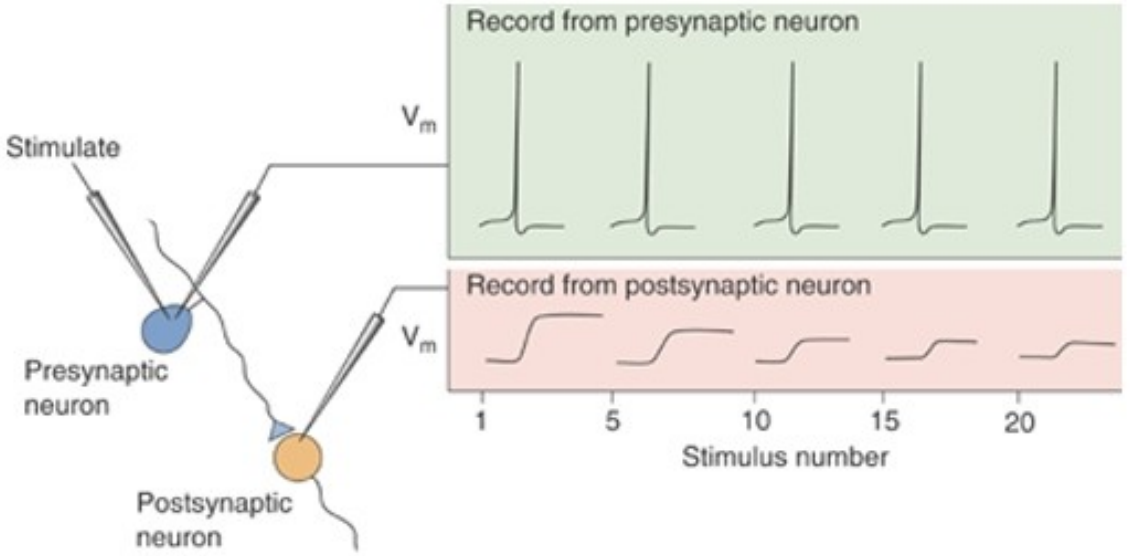
Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in *Aplysia*
  - Repeated electrical stimulation of a sensory neuron leads to a progressively smaller EPSP in the postsynaptic motor neuron



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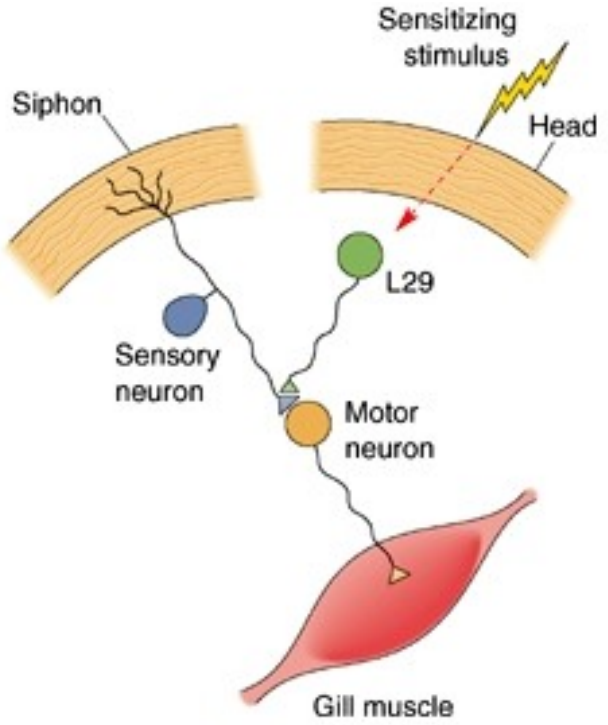
Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in Aplysia
  - Sensitization of the Gill-Withdrawal Reflex involves L29 axoaxonic synapse



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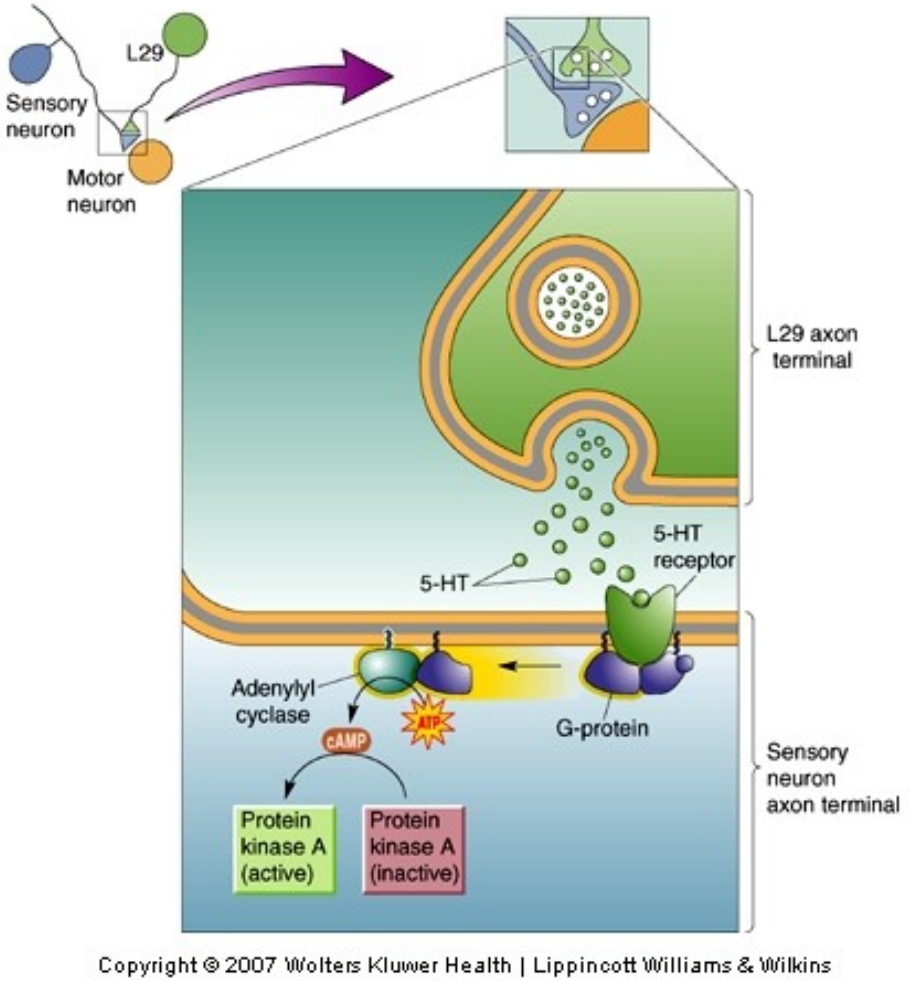
Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in Aplysia
  - 5-HT released by L29 in response to head shock leads to G-protein coupled activation of adenylyl cyclase in sensory axon terminal.
  - Cyclic AMP production activates protein kinase A.
  - Phosphate groups attach to a potassium channel, causing it to close



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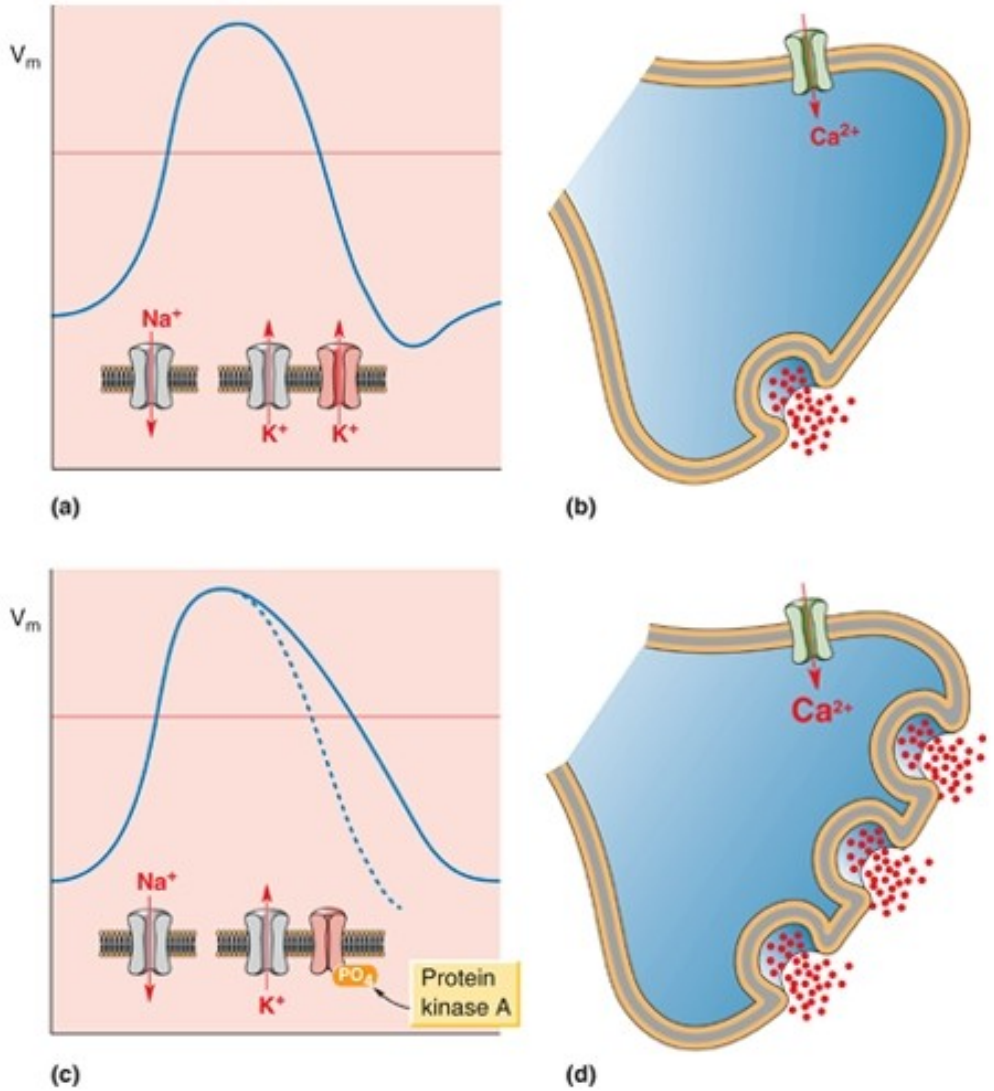
Simple systems: Invertebrate Models of Learning

- Nonassociative Learning in Aplysia
  - Effect of decreased potassium conductance in sensory axon terminal
  - More calcium ions admitted into terminal and more transmitter release



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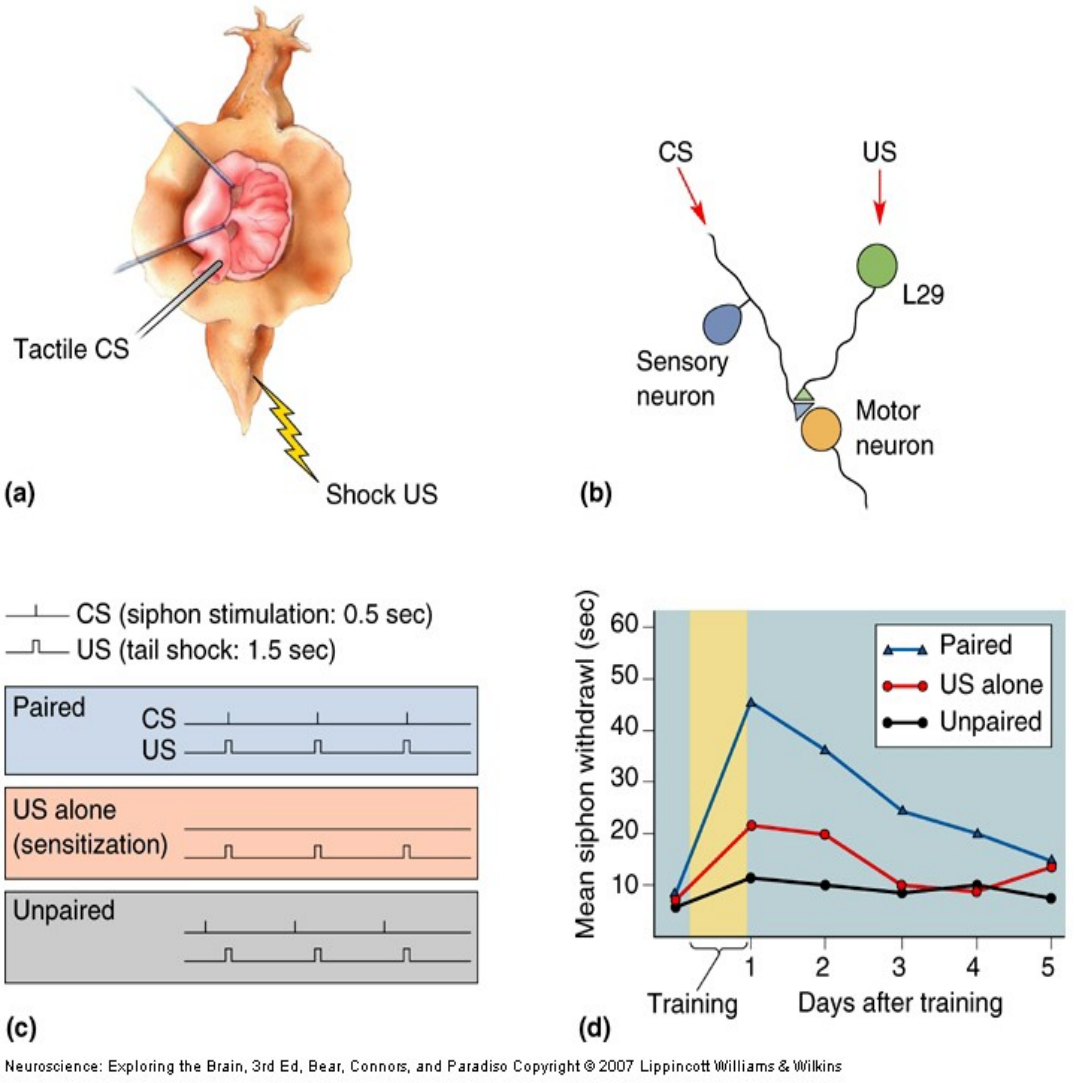
Simple systems: Invertebrate Models of Learning

- Associative Learning in *Aplysia*
  - Classical conditioning: CS initially produces no response but after pairing with US, causes withdrawal



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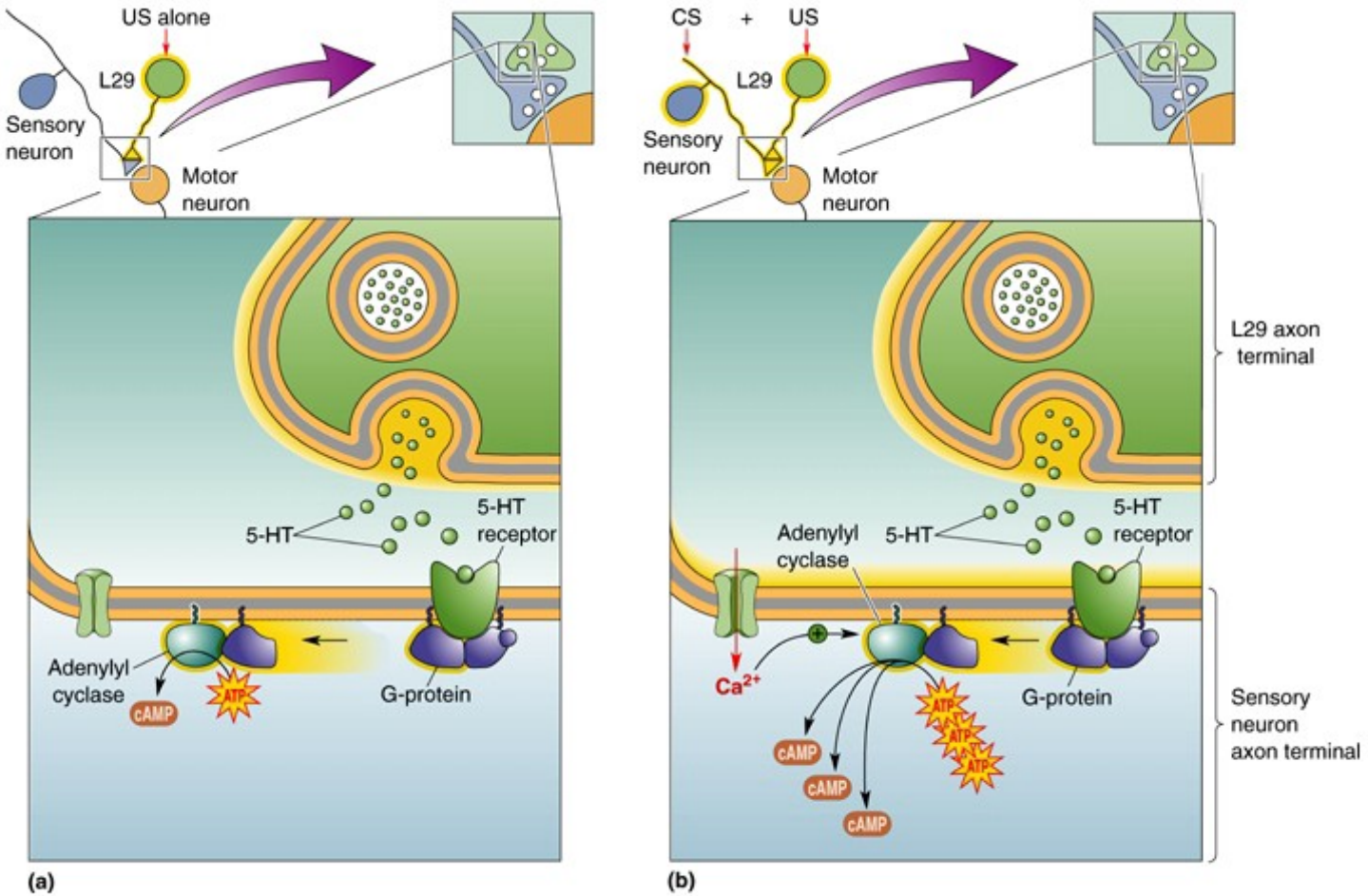
Simple systems: Invertebrate Models of Learning

- The molecular basis for classical conditioning in *Aplysia*
  - Pairing CS and US causes greater activation of adenylyl cyclase because CS admits Ca^{2+} into the presynaptic terminal



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Simple systems: Invertebrate Models of Learning

- Neural basis of memory: principles learned from invertebrate studies
  - Learning and memory can result from modifications of synaptic transmission
  - Synaptic modifications can be triggered by conversion of neural activity into intracellular second messengers
  - Memories can result from alterations in existing synaptic proteins