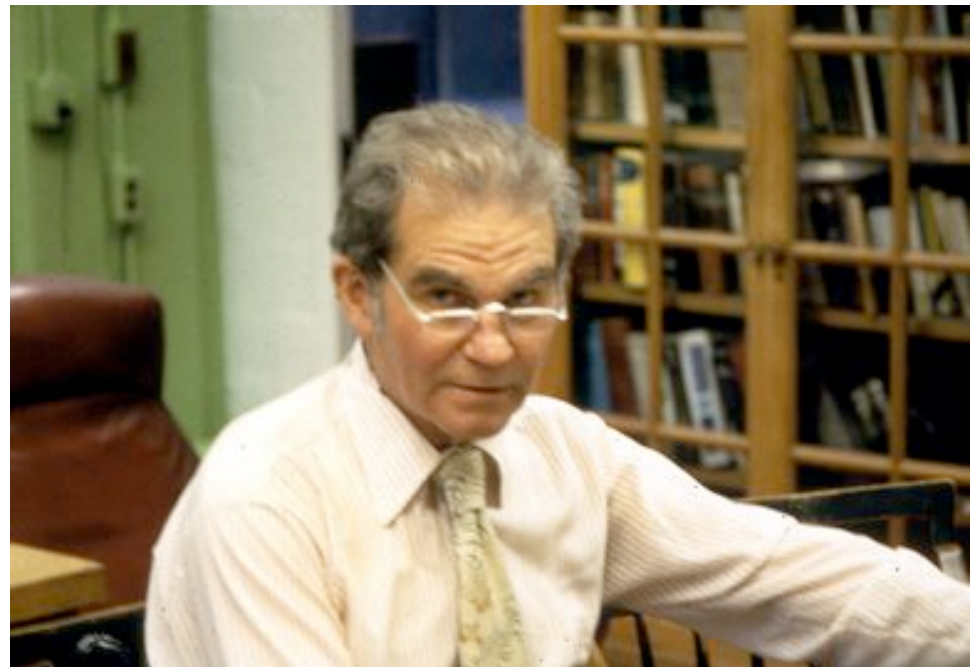


# ENDOCRINE SYSTEM OR NEUROENDOCRINE SYSTEM

Sir V.B. Wigglesworth



Carroll Williams



# **FUNCTIONS OF THE NEUROENDOCRINE SYSTEM**

Along with the nervous system, hormones provide the necessary communication between all the cells that constitute a multicellular animal

**Nervous system**-Is involved in rapid transfer of short-term events and coordination of short-term events. Electrochemical information involving neurons.

**Neurosecretory cells**-Neurons have electrical activity but involved in the production and release of neurosecretion that produces their effect as chemicals.

**Endocrine system**-Is involved in the integration and coordination of long-term events through chemicals called hormones.

# TECHNIQUES FOR STUDYING THE INSECT ENDOCRINE SYSTEM

**First generation insect endocrinologists (1920-1950's) used the following techniques:**

**Mainly identified the major endocrine glands and some of their functions**

- Ligation
- Parabiosis
- Extirpation
- Transplantation of gland
- Reimplantation of gland
- Light microscopy

**Second generation endocrinologists (1950-1960's) used the following techniques:**

**Hormones were chemically identified as to their structure and biosynthetic pathways**

- SEM and TEM of glands
- Antibodies to the hormone
- Gas and liquid chromatography
- Mass spectrometry
- Nuclear magnetic resonance (NMR)
- High-performance liquid chromatography

**Third generation endocrinologists (1970-1980's) used the following techniques:**

**Determined hormone titer**

- Enzyme-linked immunoassay (ELISA) and RIA-radioimmunoassay)

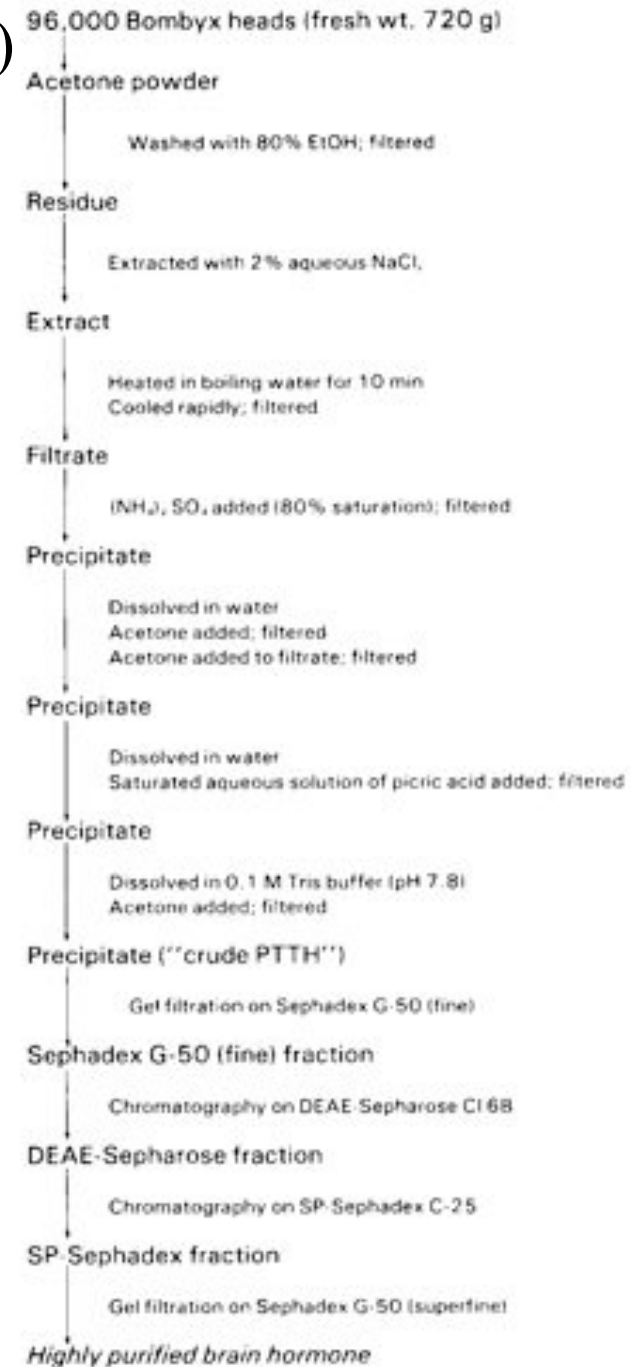
**Fourth generation endocrinologists (1980s until now) are using the following techniques:**

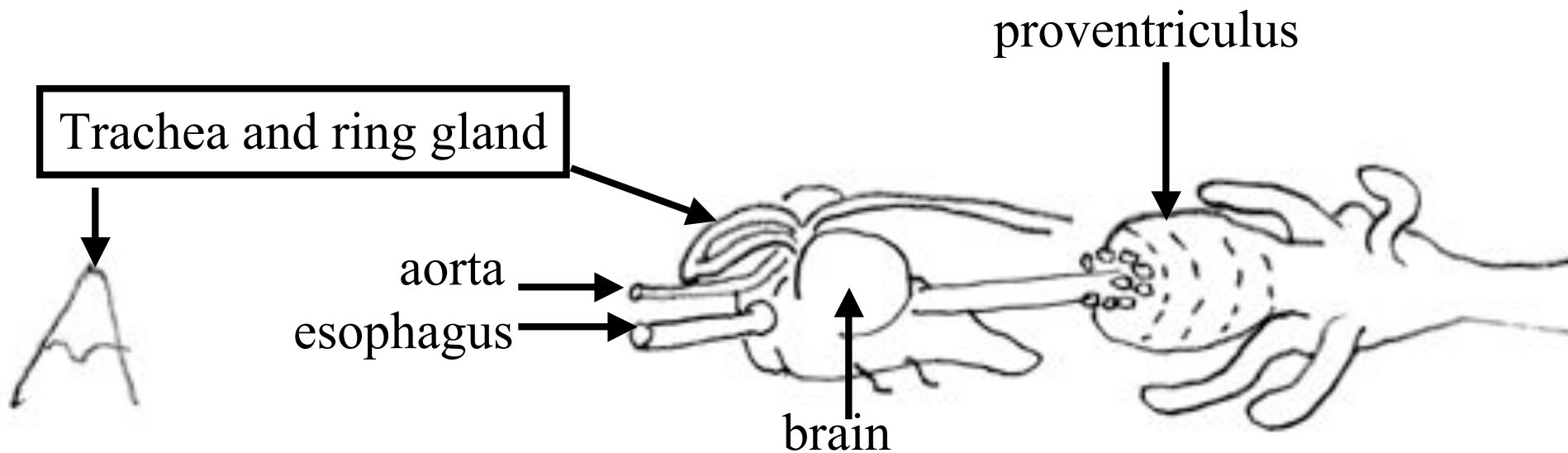
**Locate and determine the genes involved in hormone production, determining the molecular structure and action. Identifying various receptors.**

- PCR
- Other molecular and genetic techniques

Kopec's ligation experiment in 1917 with gypsy moth larva was the first to show that hormones were present in insects.

# Partial purification of brain hormone or PTTH) from the head of *Bombyx mori*





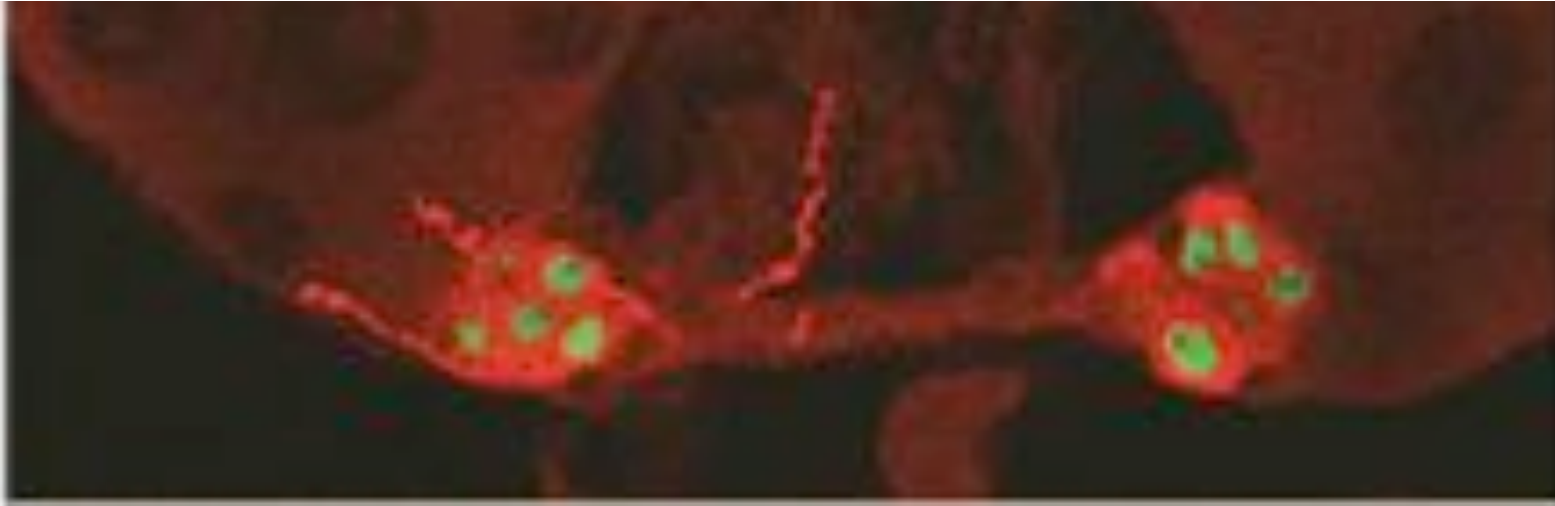
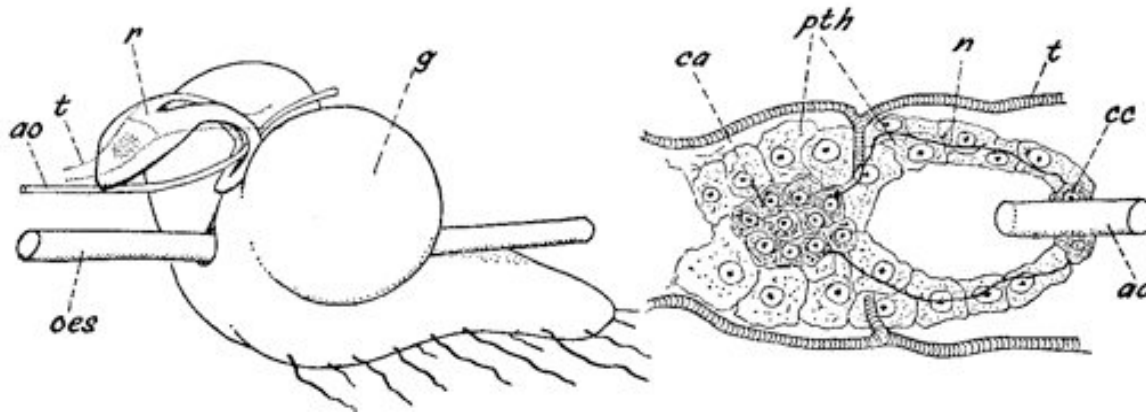
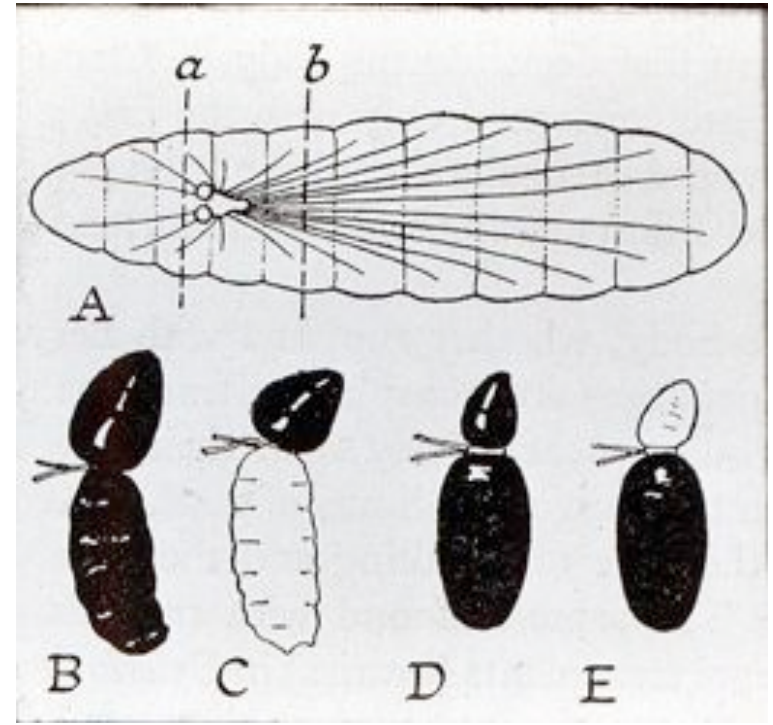


Figure 2: Aminergic cells (green) with serotonin (red) in the *Drosophila* larval ring gland.  
<http://www.ncbs.res.in/gaiti/research.html>

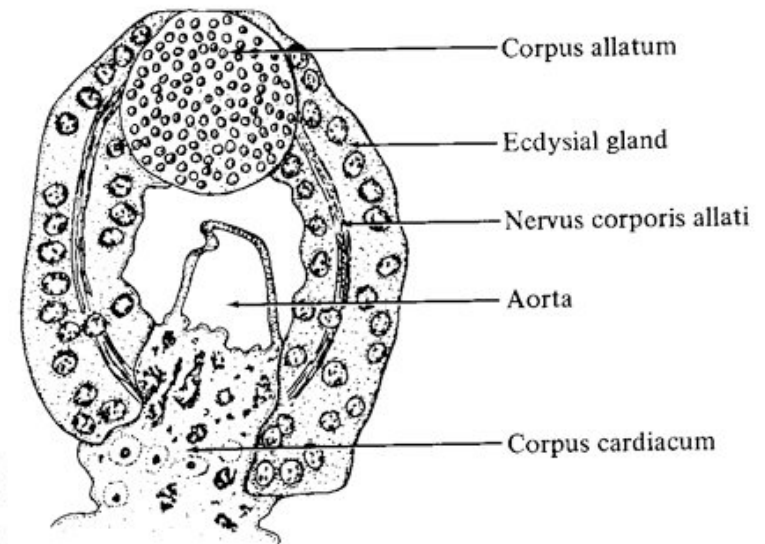
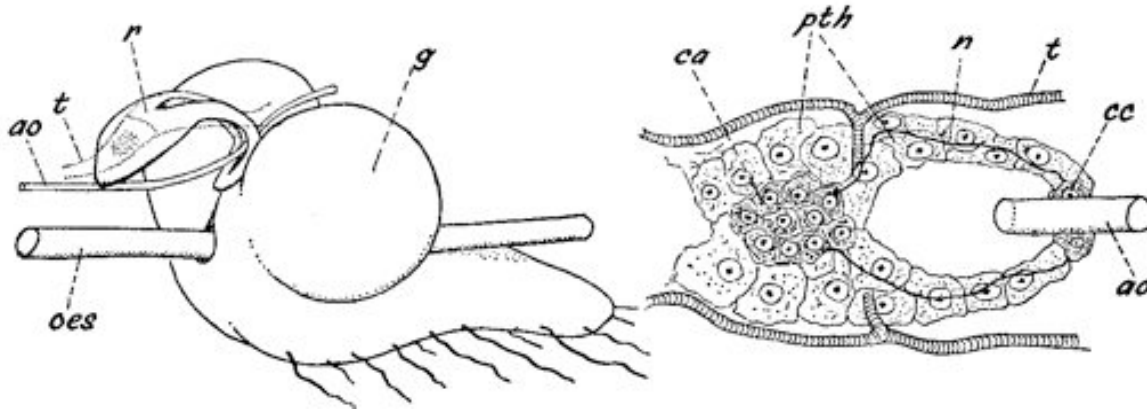




**Ligation**-Separating parts of the body by using human hair, silk, or fine string to tie off and separate the blood supply of one area from the other. Note in photo to the right that the ring gland (Weismann's ring) is found within the section between a and b.

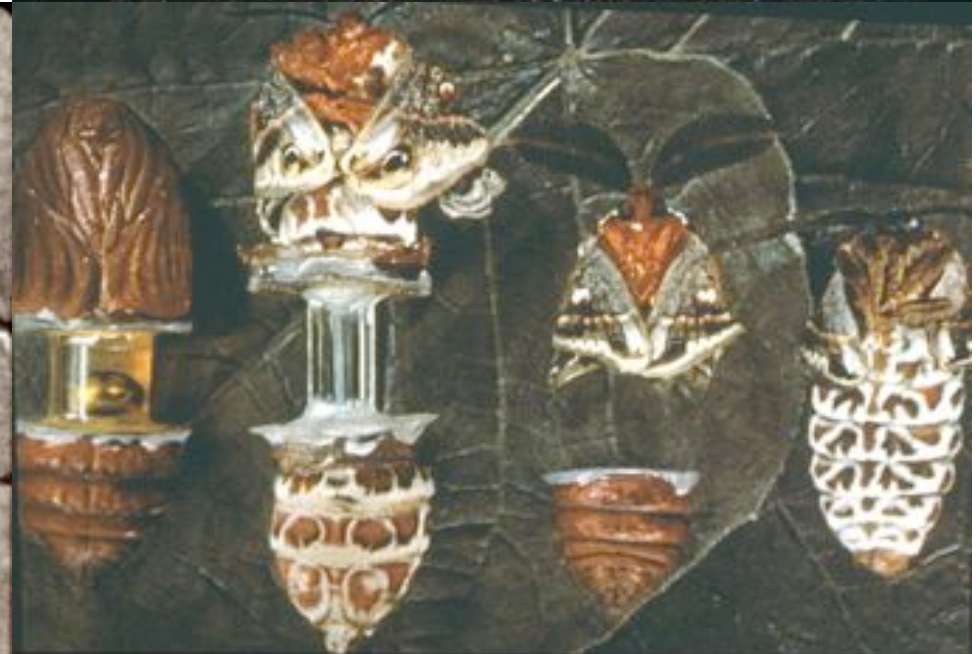
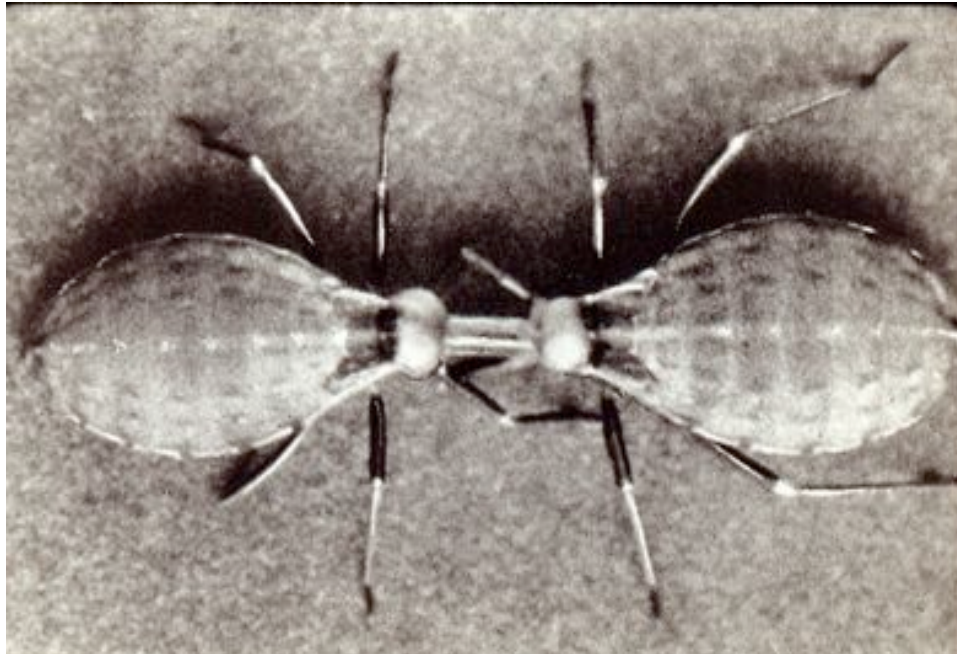
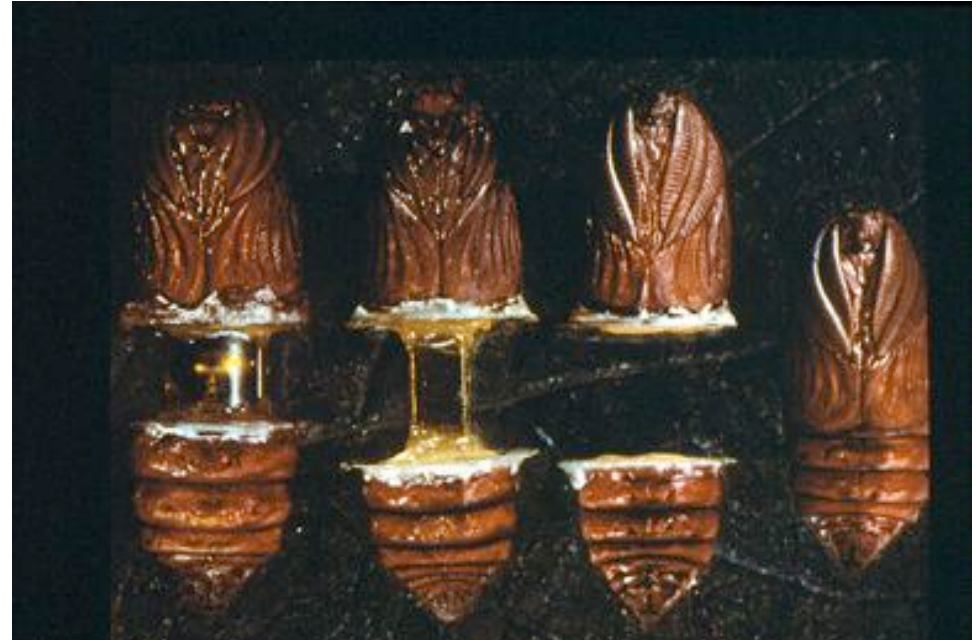


### Weismann's ring or rind gland

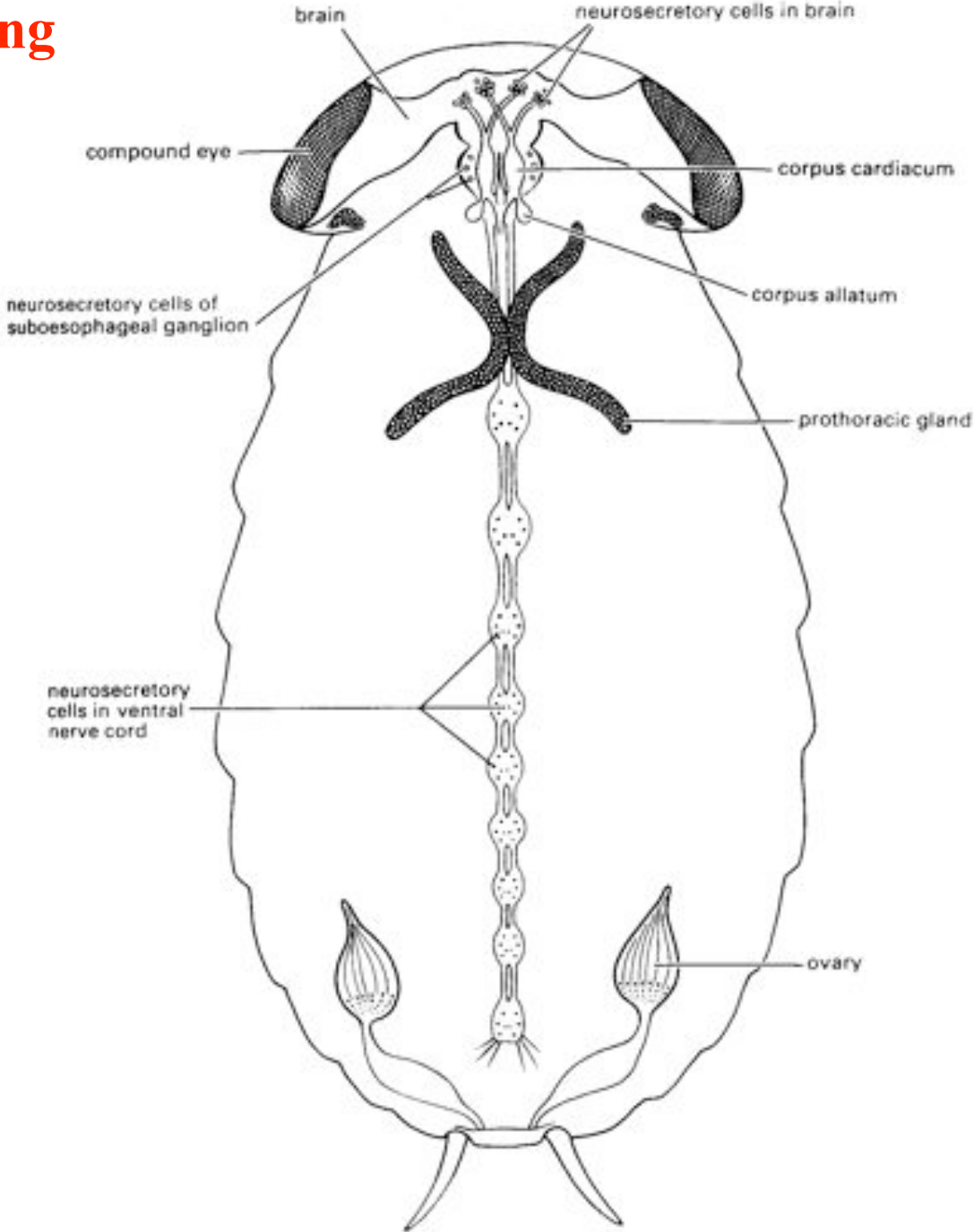




**Parabiosis**-Connecting the blood supply of two individuals by using various techniques.



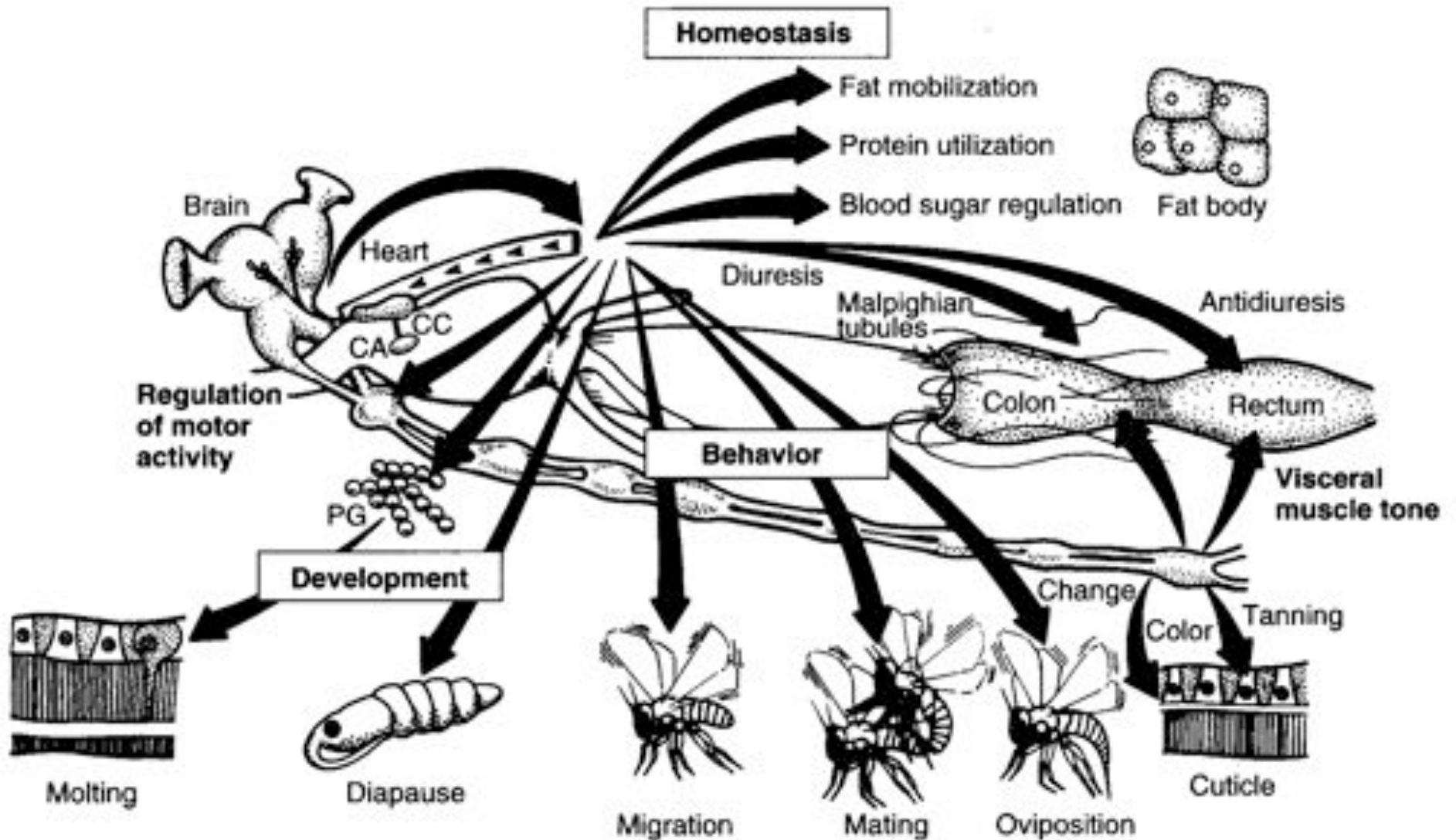
**Generalized scheme showing the location of various endocrine glands and neurosecretory cells in an insect**



## **Areas of insect biology that hormones play a major role**

1. Regulation of molting
2. Determination of form at metamorphosis
3. Effects on polymorphism
4. Regulation of diapause
5. Involvement in reproduction
6. Regulation of metabolic activities and general body functions
7. Regulation of behavior
8. Regulation of preprogrammed cell death

# Major physiological functions regulated by neurohormones





# **Insect endocrine glands & neurosecretory cells & location**

<u>Active Principle</u>	<u>Origin</u>	<u>Target</u>	<u>Role/function</u>
I. Nonneural hormones			
A. Immature insects			
Ecdysone	ecdysial gland	epidermis	initiates molt
Juvenile hormone	corpora allata	epidermis	controls or directs fate of metamorphosis at molt
B. Adult insects			
Ovarian hormone (ecdysteroids)	ovarian tissue-follicle cells	fat body	initiates + regulates the production of vitellogenin (VG)
Juvenile hormone	corpora allata	fat body	primes fat body to become competent to produce vitellogenin

# **Insect endocrine glands & neurosecretory cells & location**

<u>Active Principle</u>	<u>Origin</u>	<u>Target</u>	<u>Role/function</u>
Juvenile hormone	corpora allata	ARG's	affects development and production of glandular secretions
Juvenile hormone	corpora allata	follicle cells	activates patency and uptake of VG by the follicle cells

# Insect endocrine glands & neurosecretory cells & location

<u>Active Principle</u>	<u>Origin</u>	<u>Target</u>	<u>Role/function</u>
II. Neural hormones and peptide hormones			
A. Ecdysiotropin (PTTH) (=prothoracicotropic hormone).	brain hormone protocerebrum	ecdysial glands	developmental-stimulates and regulates production and release of ecdysone
B. Bursicon	MNSC and thoracicoabd. ganglion of flies	epidermis	dev.-stimulates sclerotization and melanization of cuticle
C. Eclosion hormone	brain of pre-ecdysis moths	abdominal ganglion	behavior-synchron. of eclosion with photoperiod
D. Ecdysis-triggering hormone	epitracheal glands (ventrolateral tracheal tube near each spiracle)	CNS (abdomin. ganglia)	Beh.-synchron. of eclosion
E. Allatostatins	Brain(lateral nsc)	corpora allata	dev./beh/homeostasis inhibits JH production
F. Allatotropin	Brain	corpora allata	dev./beh/homeostasis stimulates JH production
G. Diuretic hormones	brain/cc and thoracic ganglia	Malpig. tubules	homeostasis-controls diuresis or fluid secretion



# Insect endocrine glands & neurosecretory cells & location

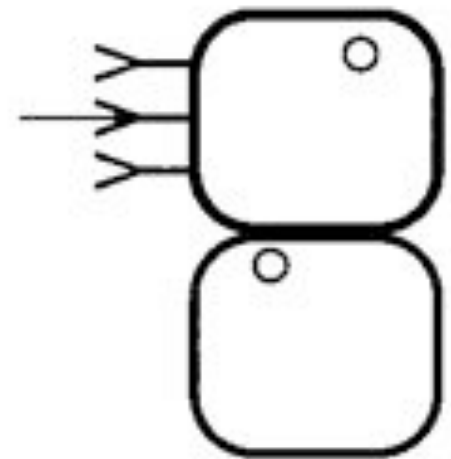
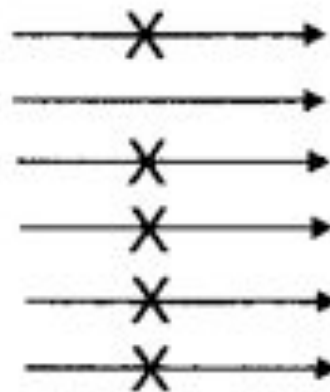
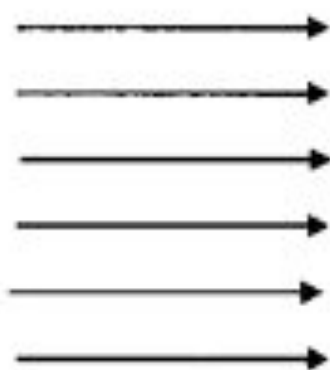
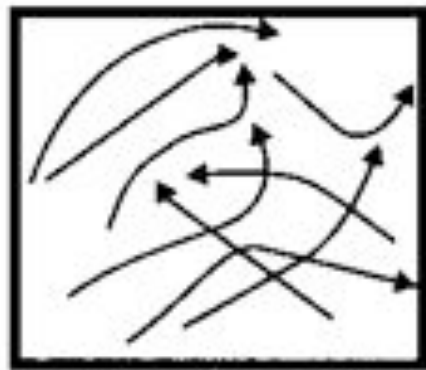
<u>Active Principle</u>	<u>Origin</u>	<u>Target</u>	<u>Role/function</u>
H. Mating inhibition	ARG of male	female's brain	beh.-prevents remating
I. Oviposition initiation	ARG of male	oviduct?	beh.-initiations egg laying
J. Cardioaccelerator	brain/CC	myocardium	Homeostasis-increase in freq. + amplitude of muscle contraction
K. Proctolin	brain/CC	hindgut and poss. visceral muscle in general	homeo.-muscles contraction, defecation, egg-laying, + heartbeat
L. Dromyosuppresin	brain/CC	muscles of crop	inhibits muscle contract
M. Ovarian ecdysteroidogenic hormone (OEH) (also know as EDNH) (these may be similar to PTTH)	brain	ovaries	stimulate ovarian tissue to produce ecdysteroids
N. Hypo- + hyper-glycemic hormones	brain/CC	fat body	conversion of glycogen to trehalose + maintain level of blood sugar

**HORMONES**-Chemicals produced in a gland that are released into the blood and have their effect somewhere else in the animal

**Hormonal activity in the blood is influenced by**

- 1. Hormone synthesis-The effective titer must be reached to work**
- 2. Hormone release**
- 3. Hormone degradation**
- 4. Receptors on the target cells-These change in number in particular tissues and at a particular time when needed**

Endocrine gland



Hormone synthesis

Hormone release

Hormone degradation

Receptors on target cells

## LOCATION OF ENDOCRINE GLANDS IN DIFFERENT INSECTS

Not only does the location of the glands differ in different insect orders but, the hormones used for various functions may also vary.

Prothoracic glands-----Produce ecdysone

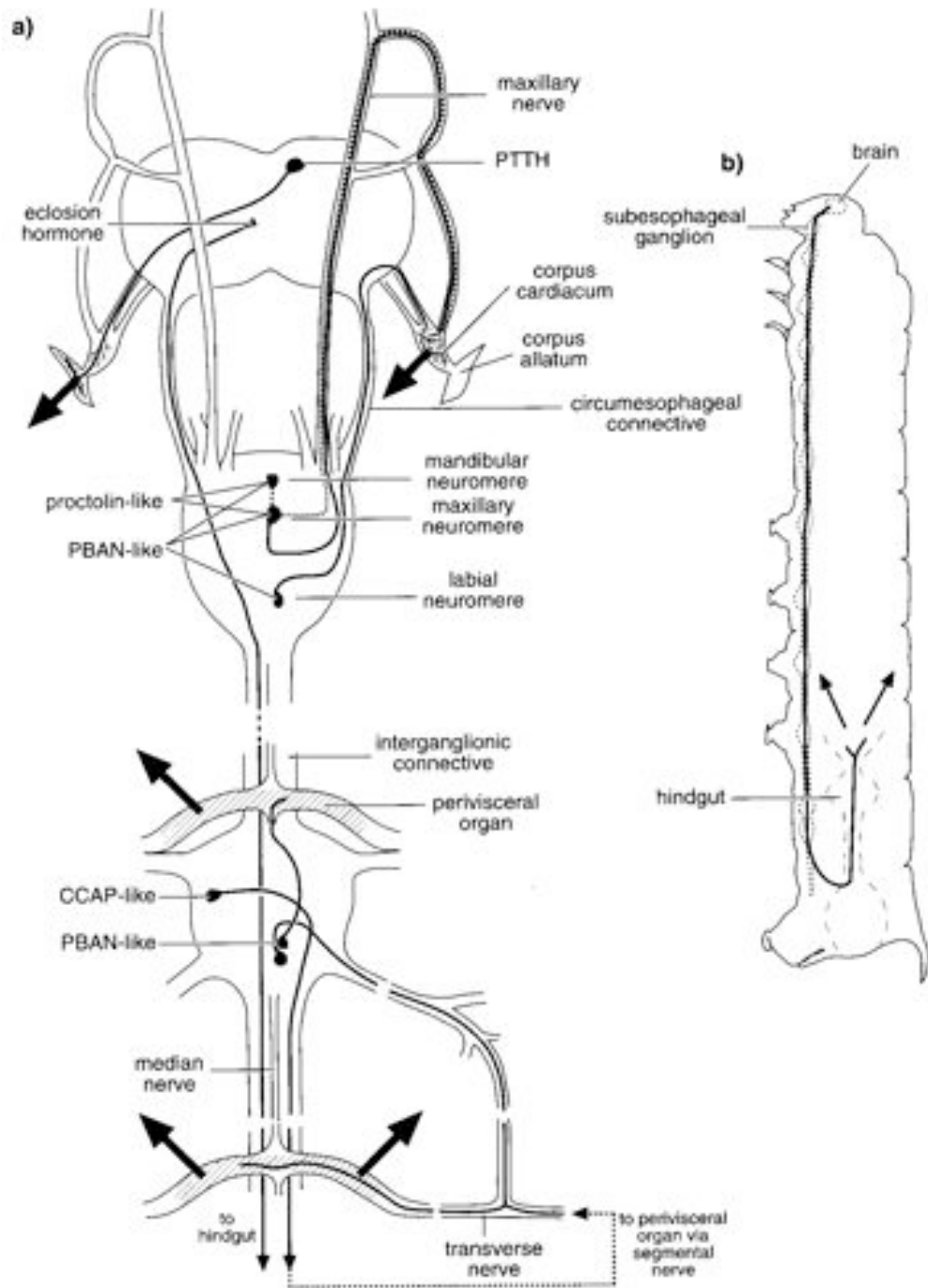
Corpora allata-----Produces JH

Corpora cardiaca-----Stores and releases brain hormones. Also produces and releases some peptides such as adipokinetic hormones

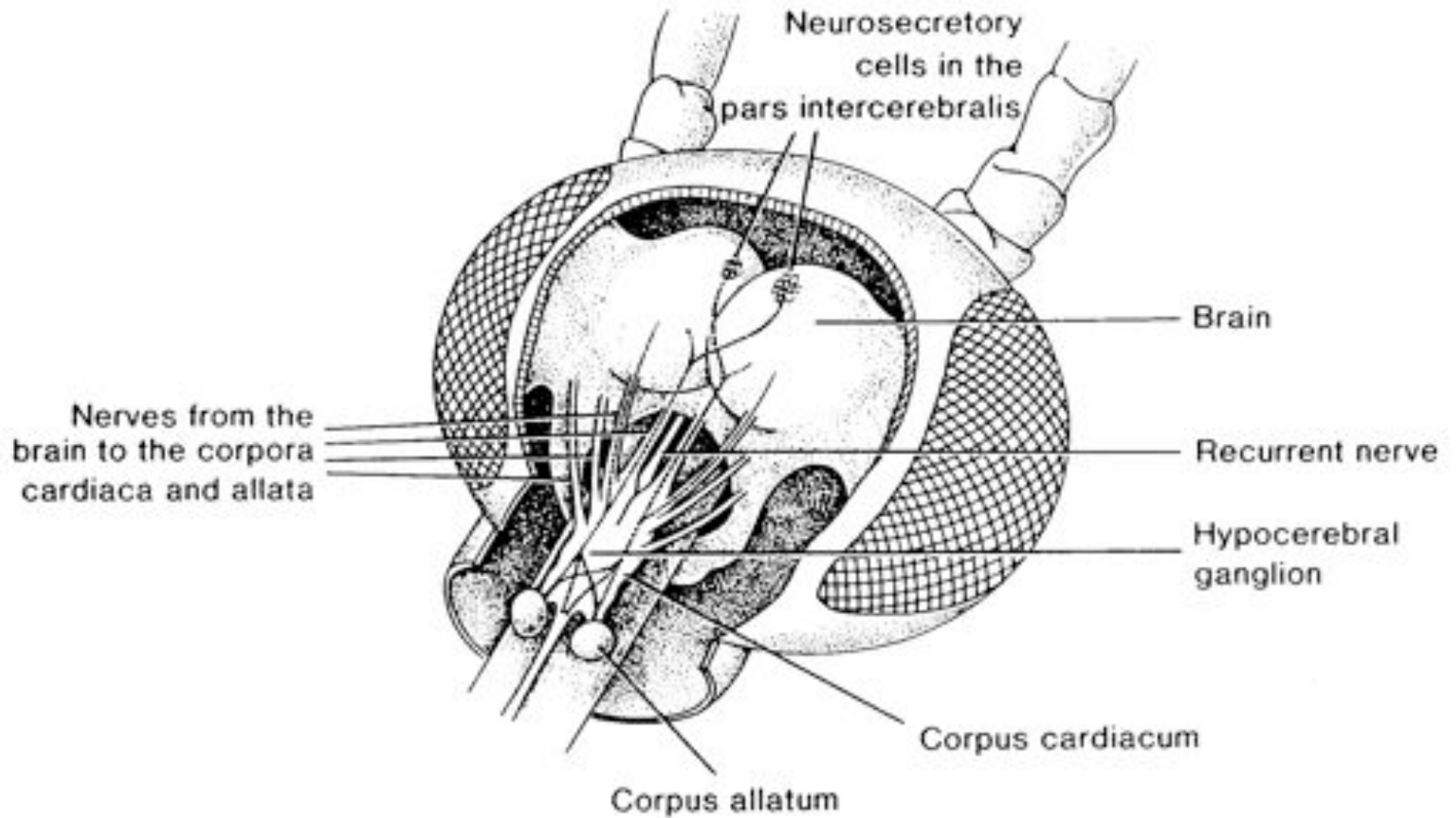
Midgut endocrine cells-Produce various peptides. Open + closed cell types.

Epitracheal glands-----Produce the ecdysis triggering hormone in Lepidoptera

Neurosecretory cells----Produce neurosecretion (peptides or biogenic amines); located in the various ganglia of CNS

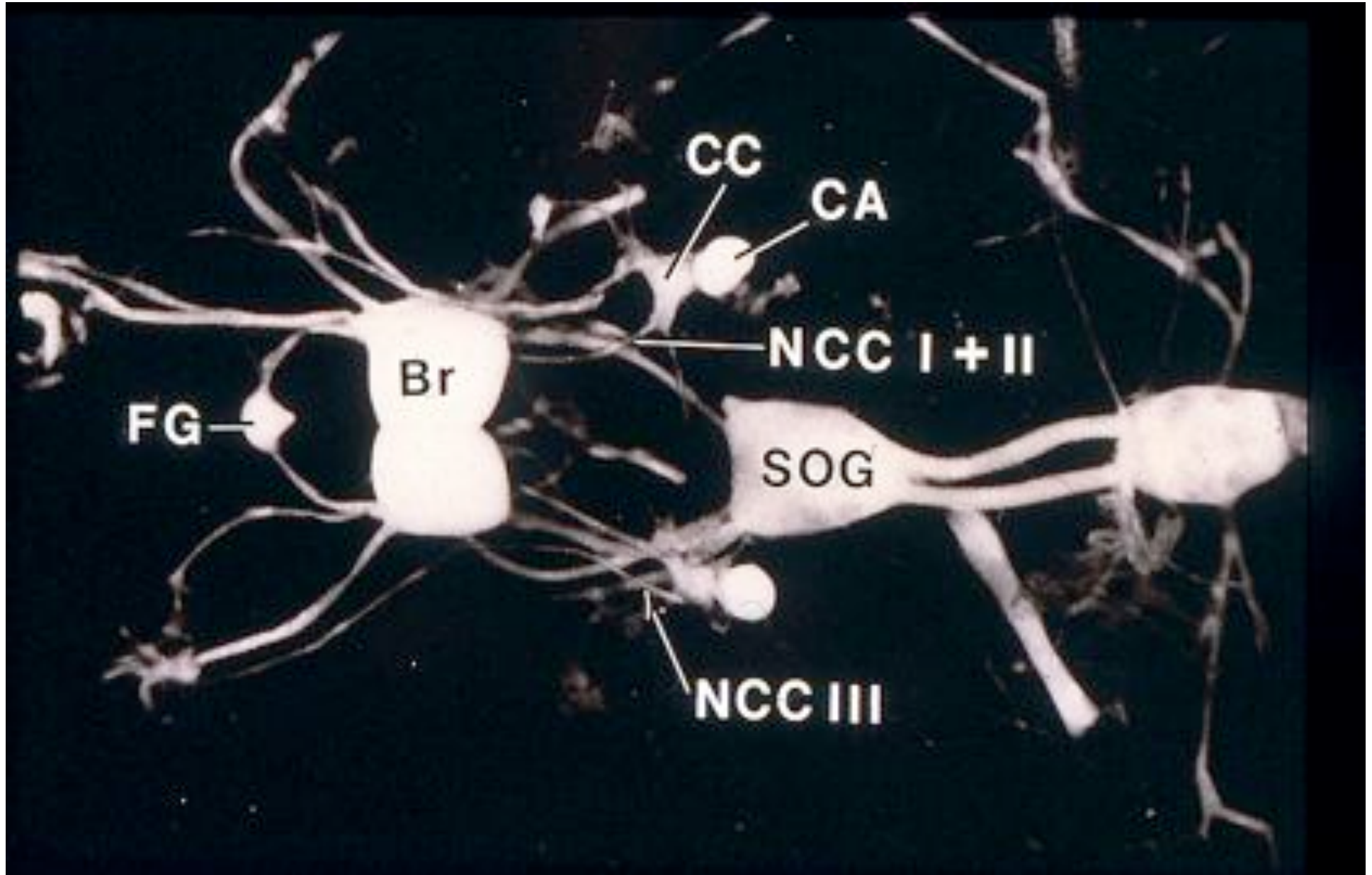


# Orthoptera



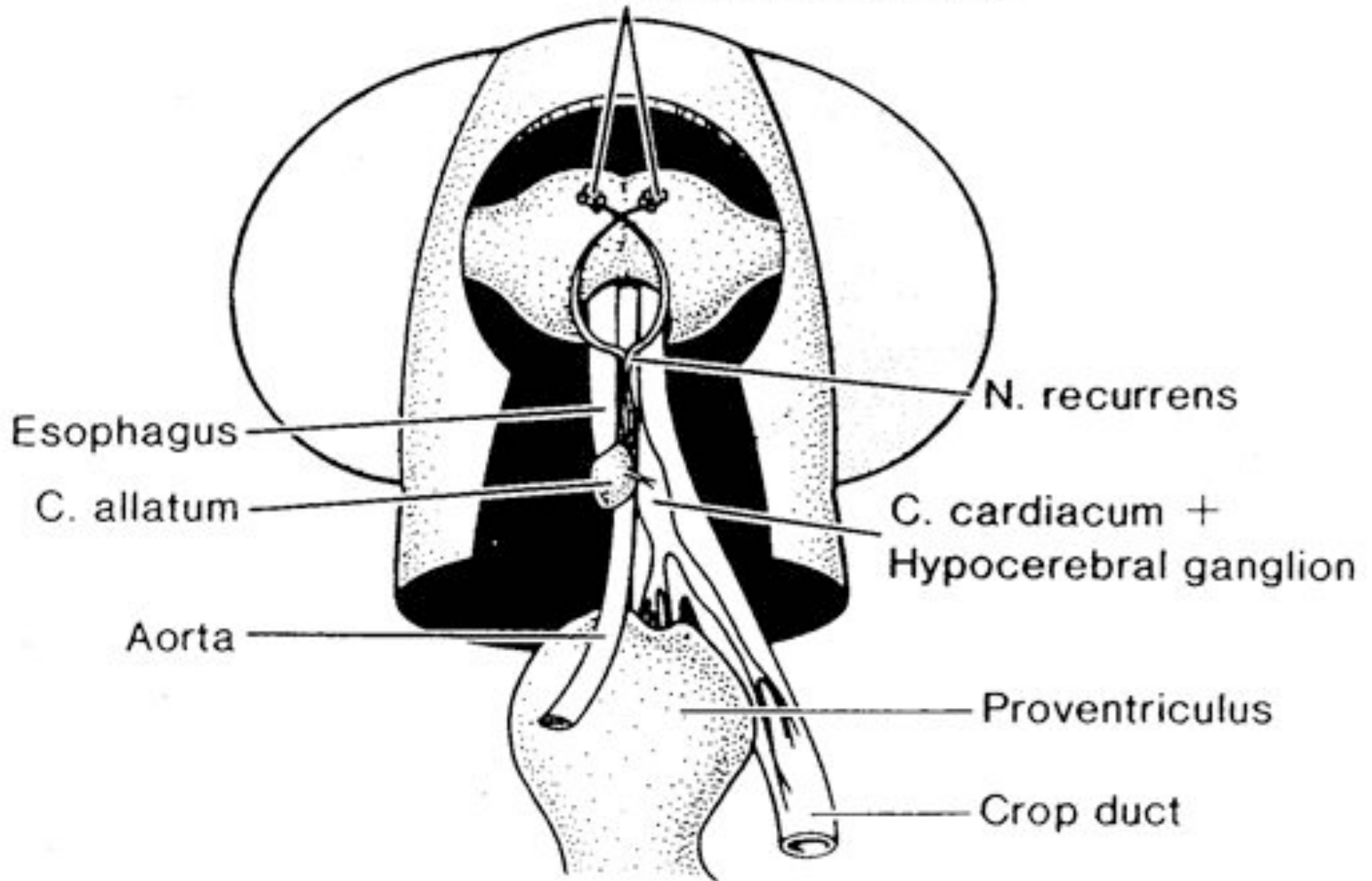


## Whole mount of nervous/endocrine systems of gypsy moth larva



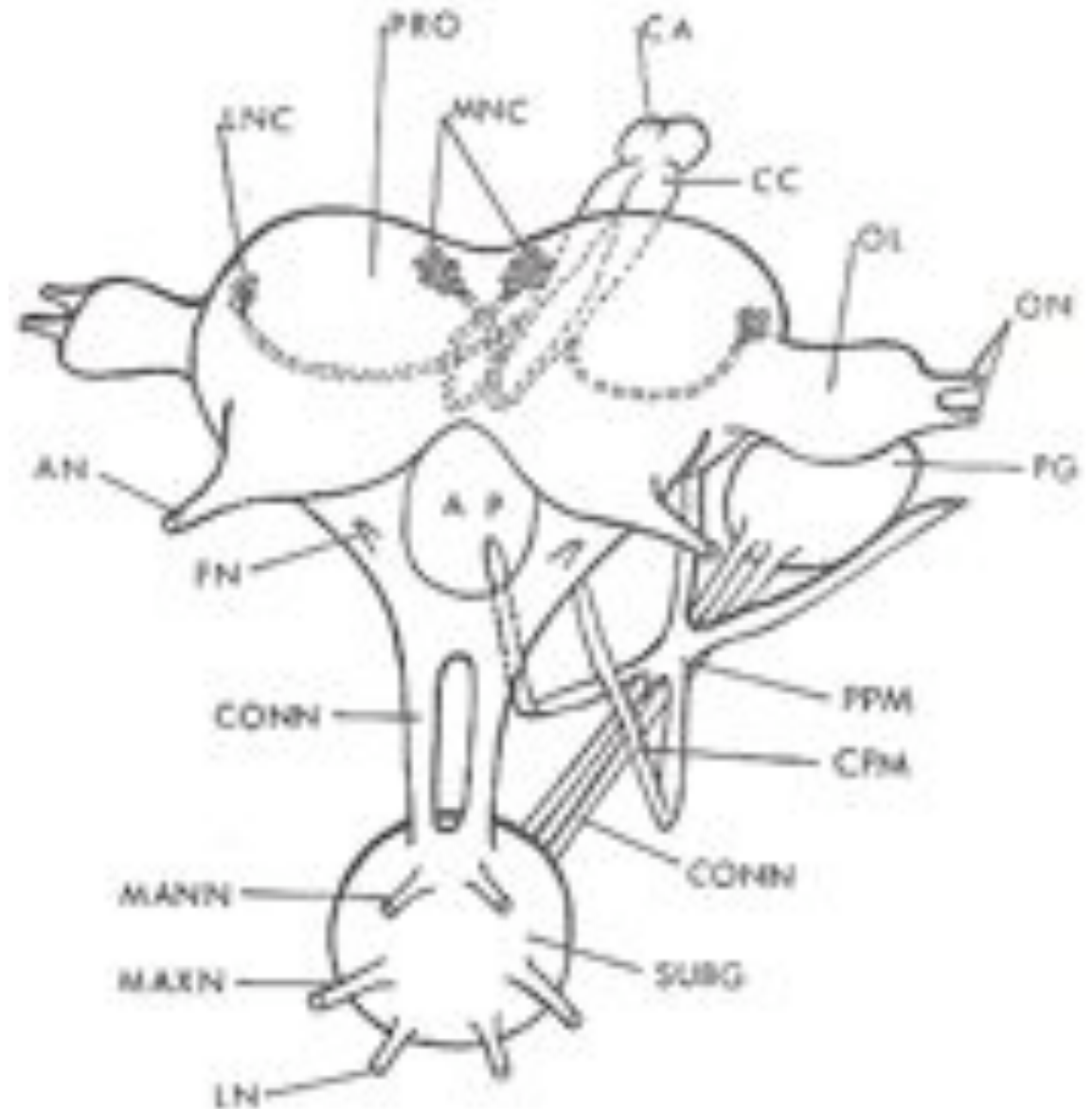
# Diptera

Pars intercerebralis

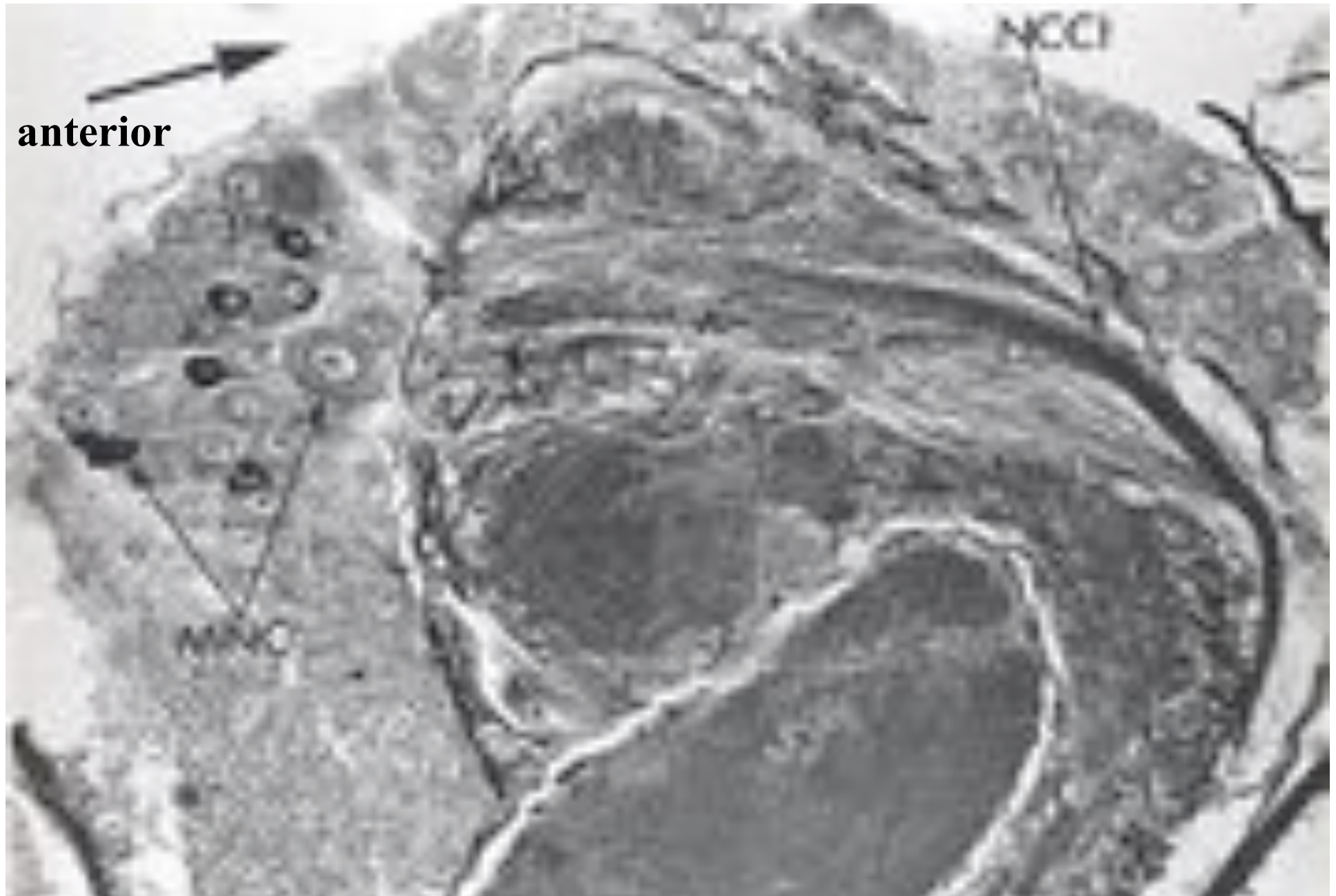




LNC=lateral neurosecretory cell  
 PRO=protocerebrum  
 MNC=median neurosecretory cell  
 CA=corpus allatum  
 OL=Optic lobe  
 ON=Optic nerve  
 PG=prothoracic gland  
 CPM=cephaic portion of the PG  
 CONN=Connectives  
 SUBG=suboesophageal gland  
 LN=labial nerve  
 MAXN=macillary

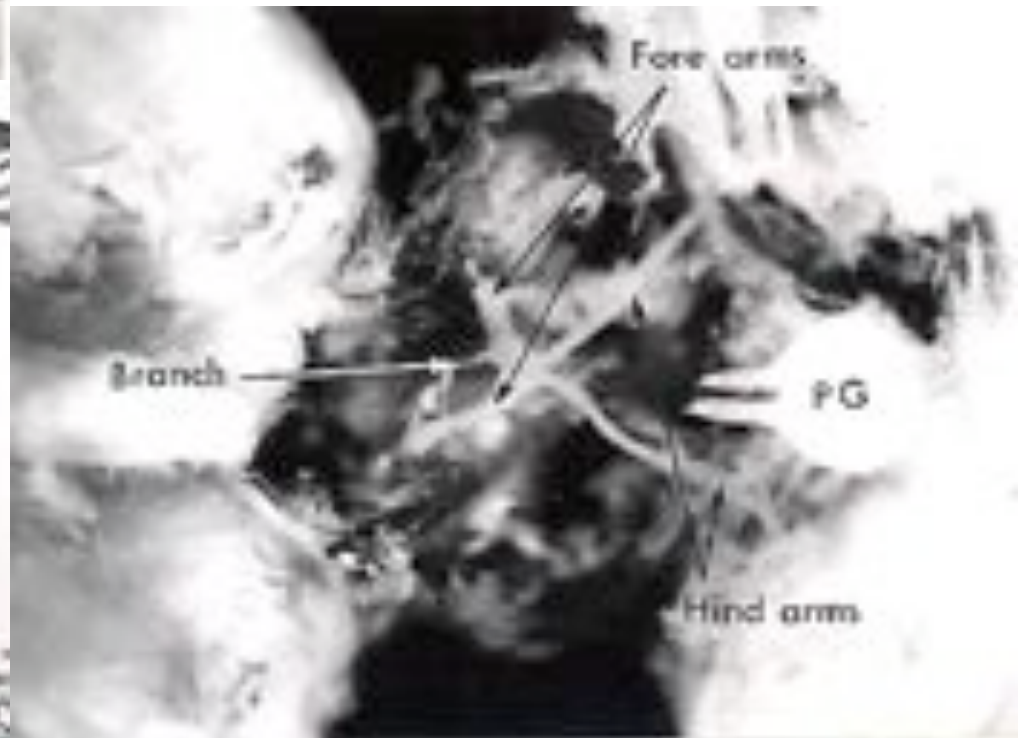
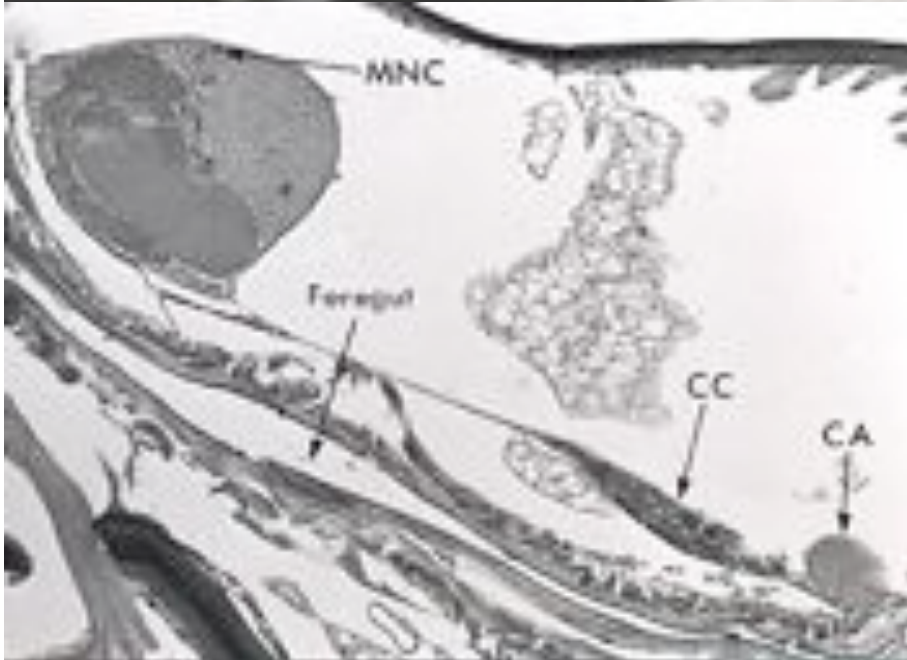
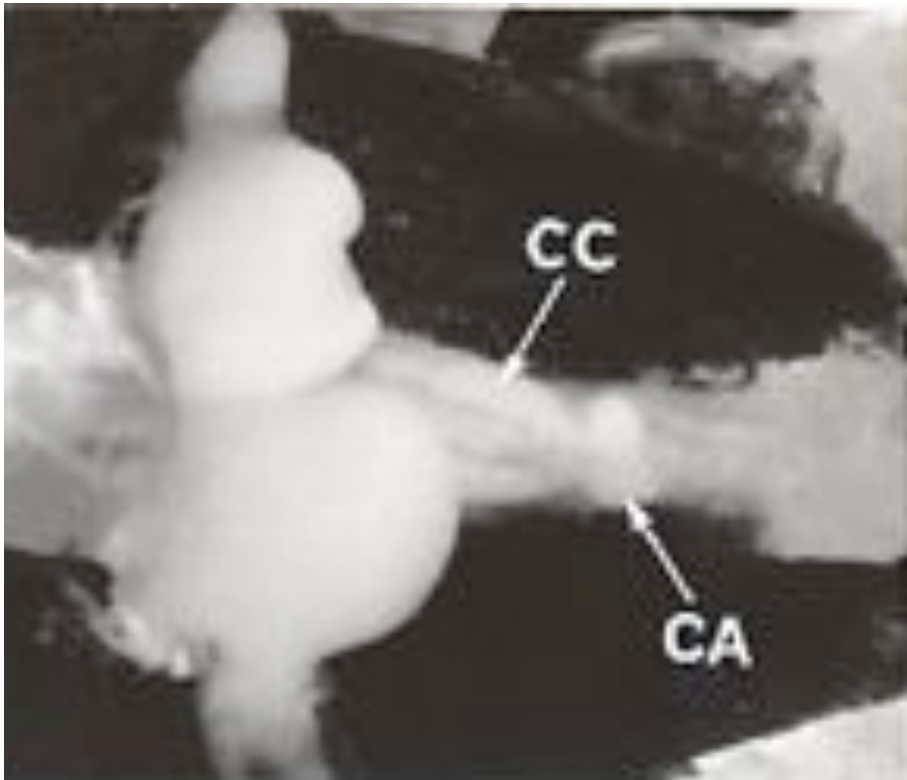


Endocrine glands in relation to head central nervous system in  
*Zootermopstis angusticollis*. From Yin. 1972. PhD. Dissertation



**Longitudinal section through pars intercerebralis of 8<sup>th</sup> instar female termite larva showing the median neurosecretory cells and their axons using a PAF stain. NCCI=nervus corporis cardiacum interni**

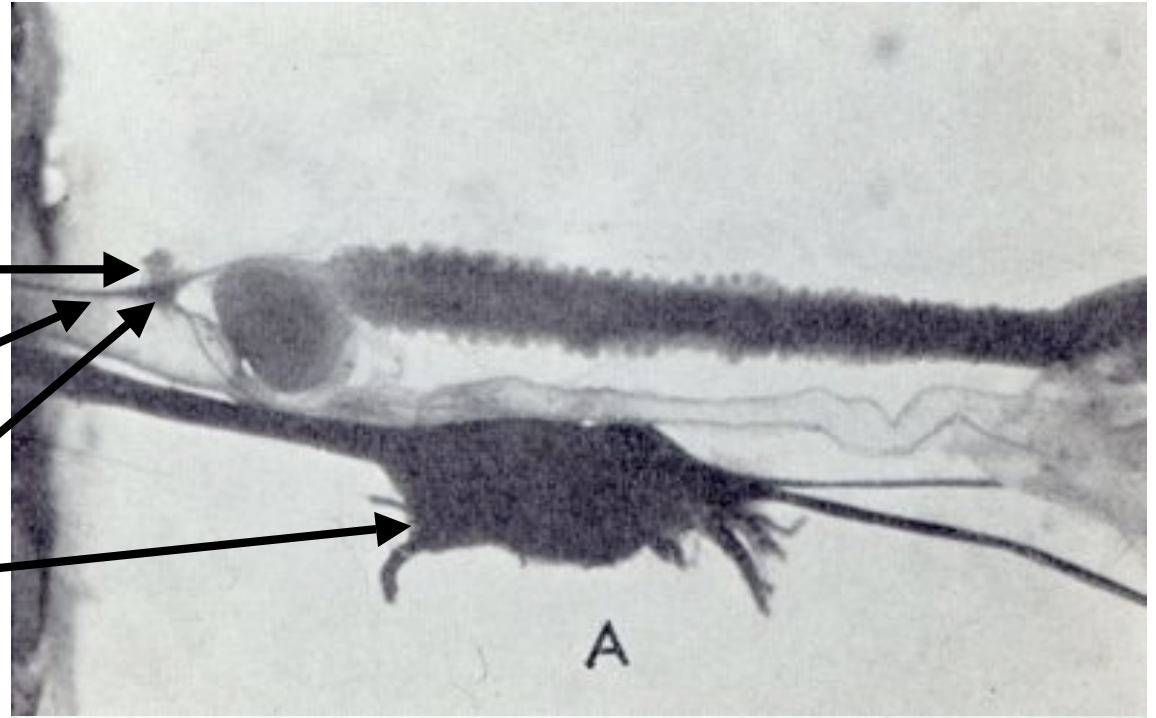
Whole mount on left and histological section showing the CA, CC, MNC and brain. Below is a whole mount of a 7<sup>th</sup> instar male *Zootermopsis* showing the arms and branches of the ecdysial gland, also known as the prothoracic gland. PG is the prothoracic ganglion.



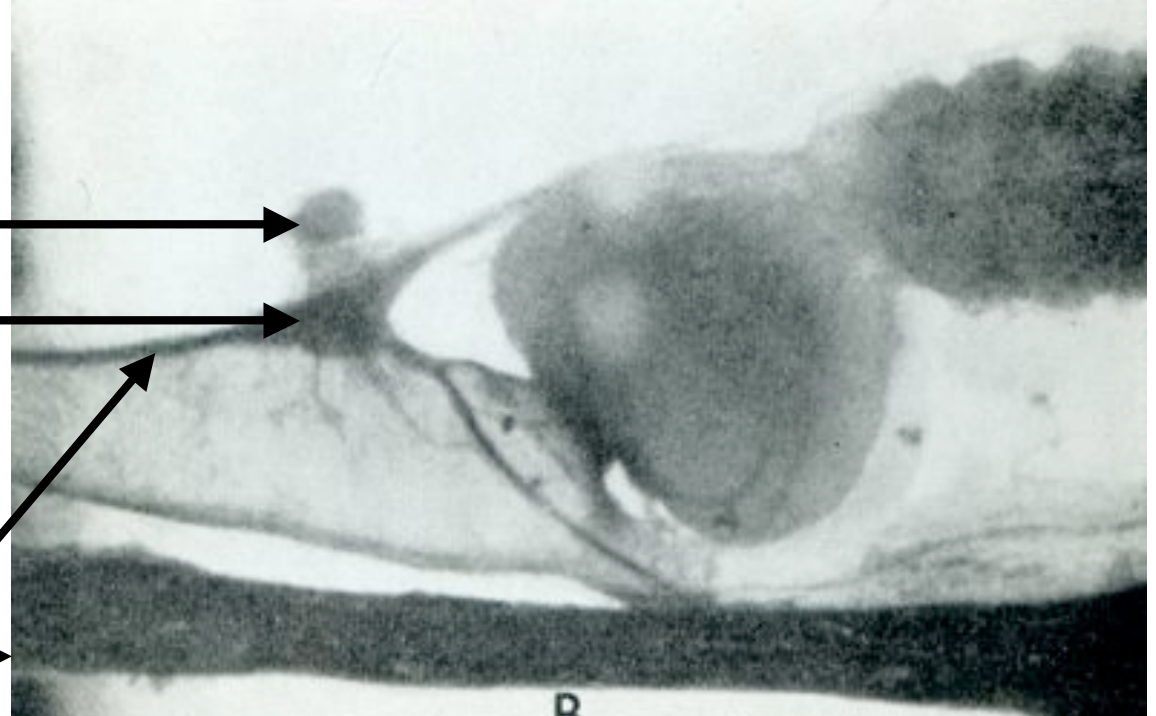


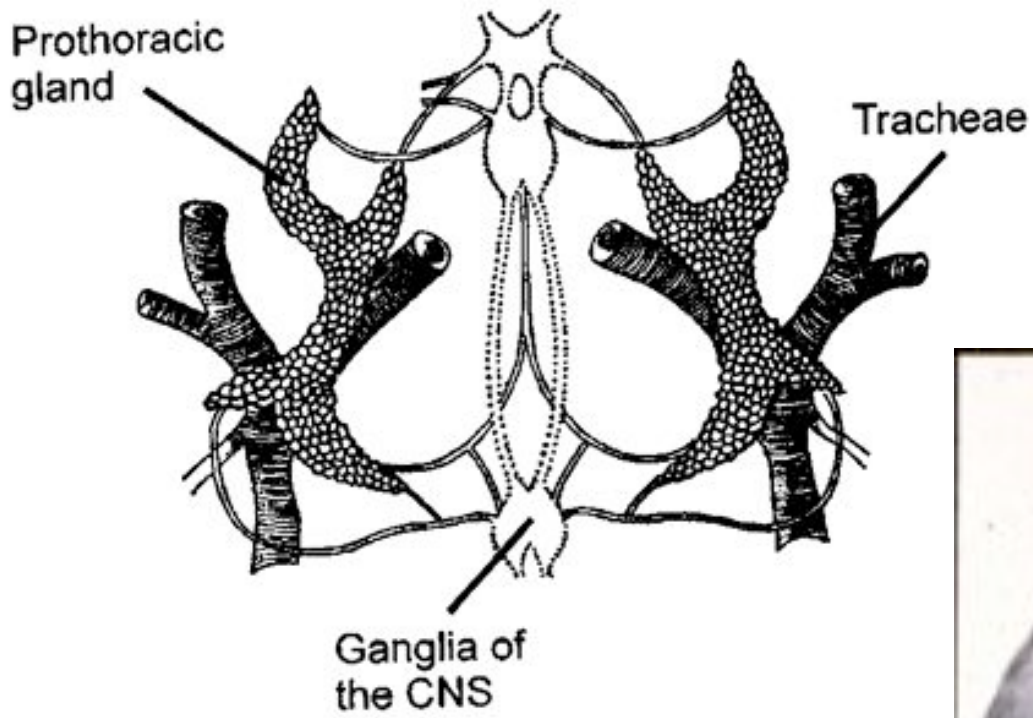
**Adult *Phormia regina***

- Corpus allatum
- Recurrent nerve/N CC1+2
- CC/hypocerebral ganglion
- Thoracoabdominal ganglion

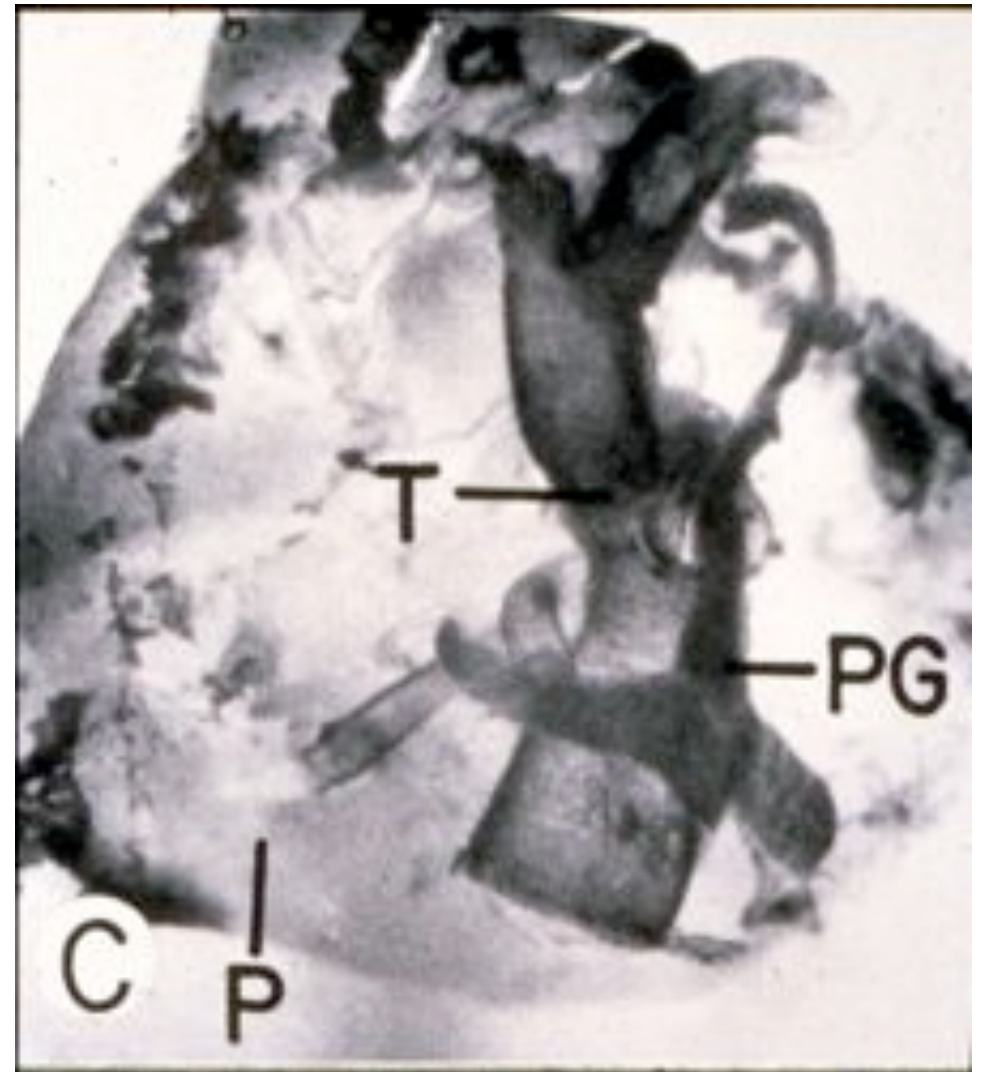


- Corpus allatum
- CC/hypocerebral ganglion
- Recurrent nerve/N CC1+2
- Ventral nerve





Prothoracic glands of Lepidoptera.  
PG=prothoracic gland  
T=trachea



REMEMBER-What happens to the ecdysial or prothoracic glands in almost all adult insects?

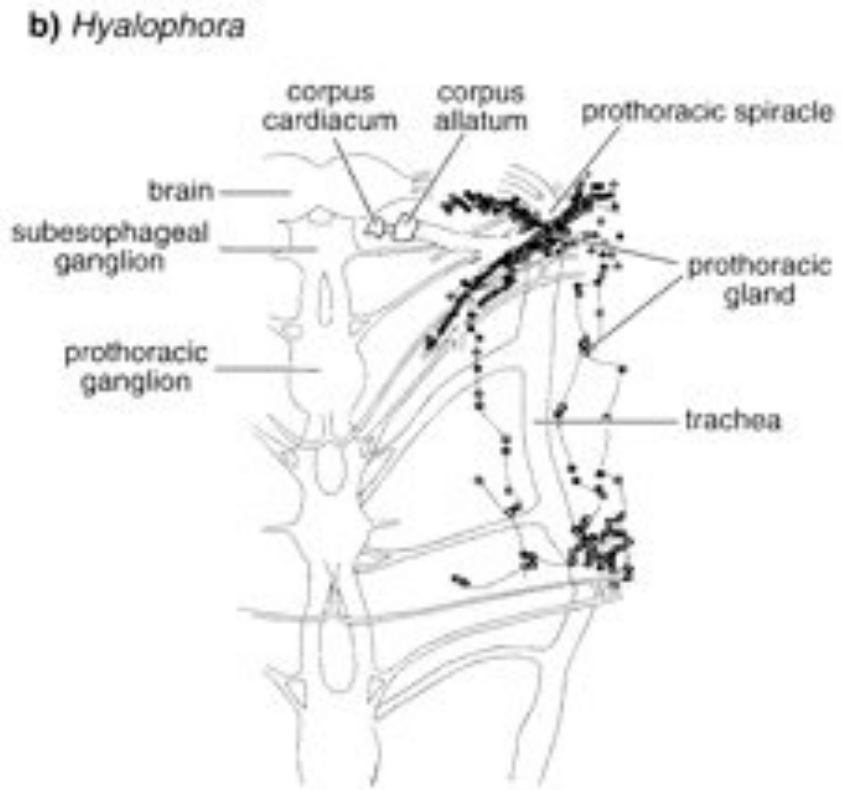
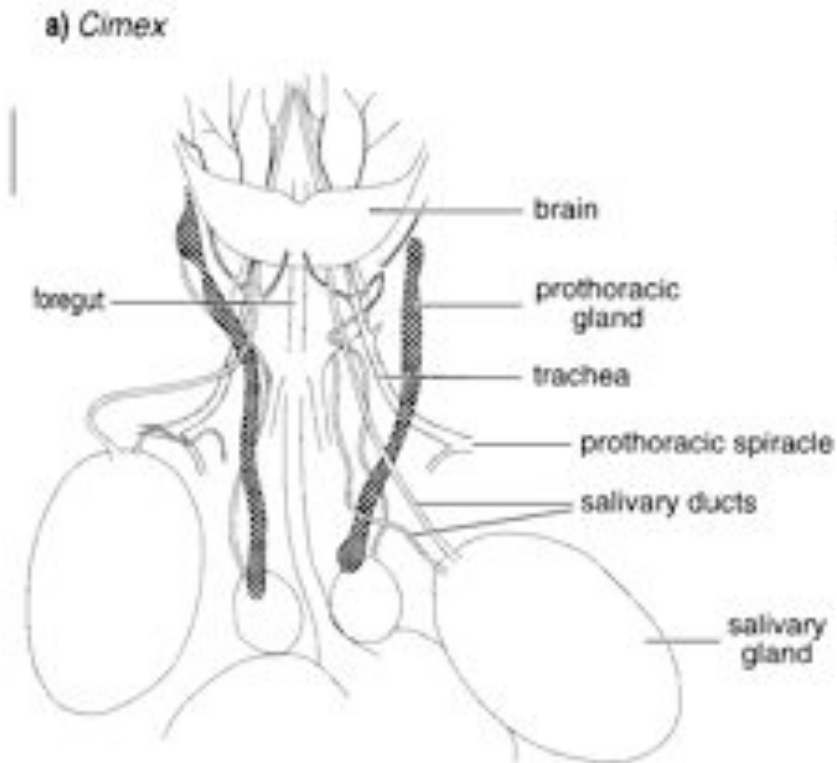
What group do they remain during adulthood and keep on molting?

## The Apterygota-especially the Thysanura

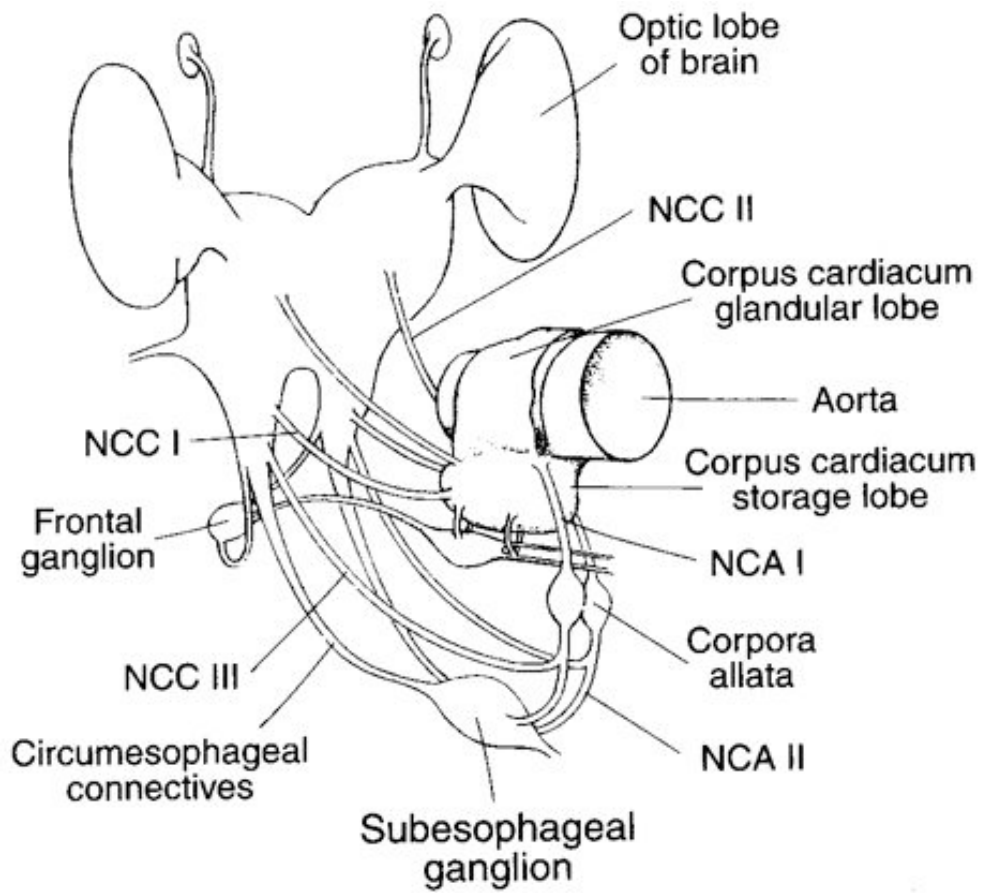
What signal is essential for their destruction or

histolysis?

## The absence of JH hormone









# ECDYSTEROIDS-

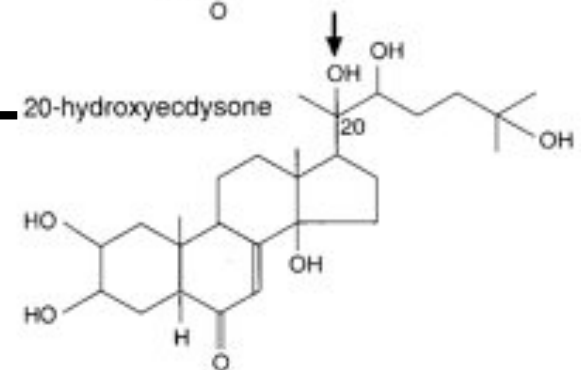
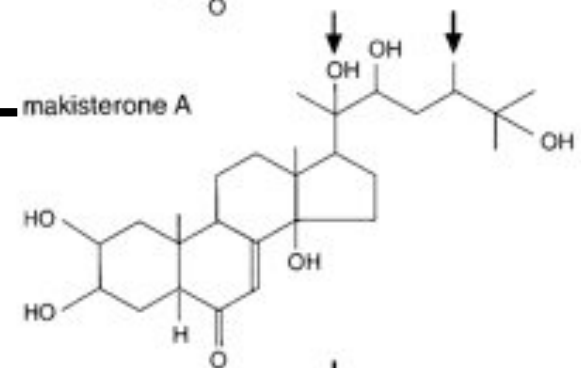
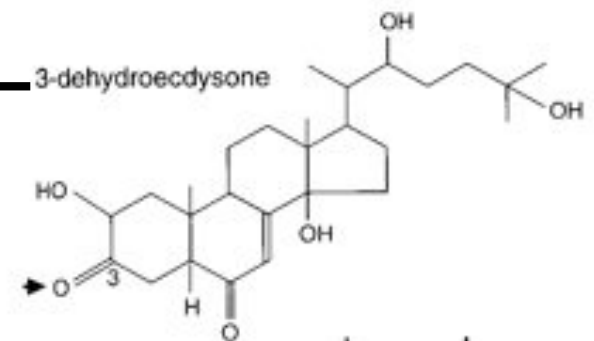
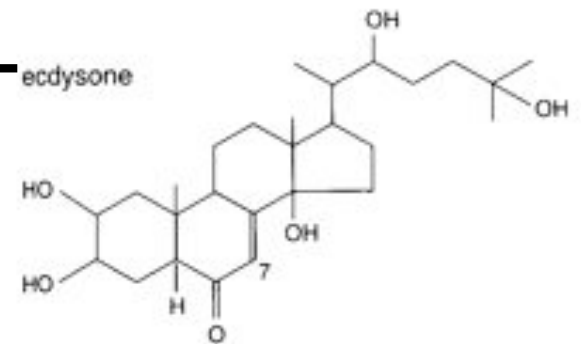
1. Ecdysone is a steroid hormone
2. Insects cannot synthesize sterols, they must get them in their diet (cholesterol or phytosterol)
3. There are several forms of the active molecule and it depends on the insect group which one is used.

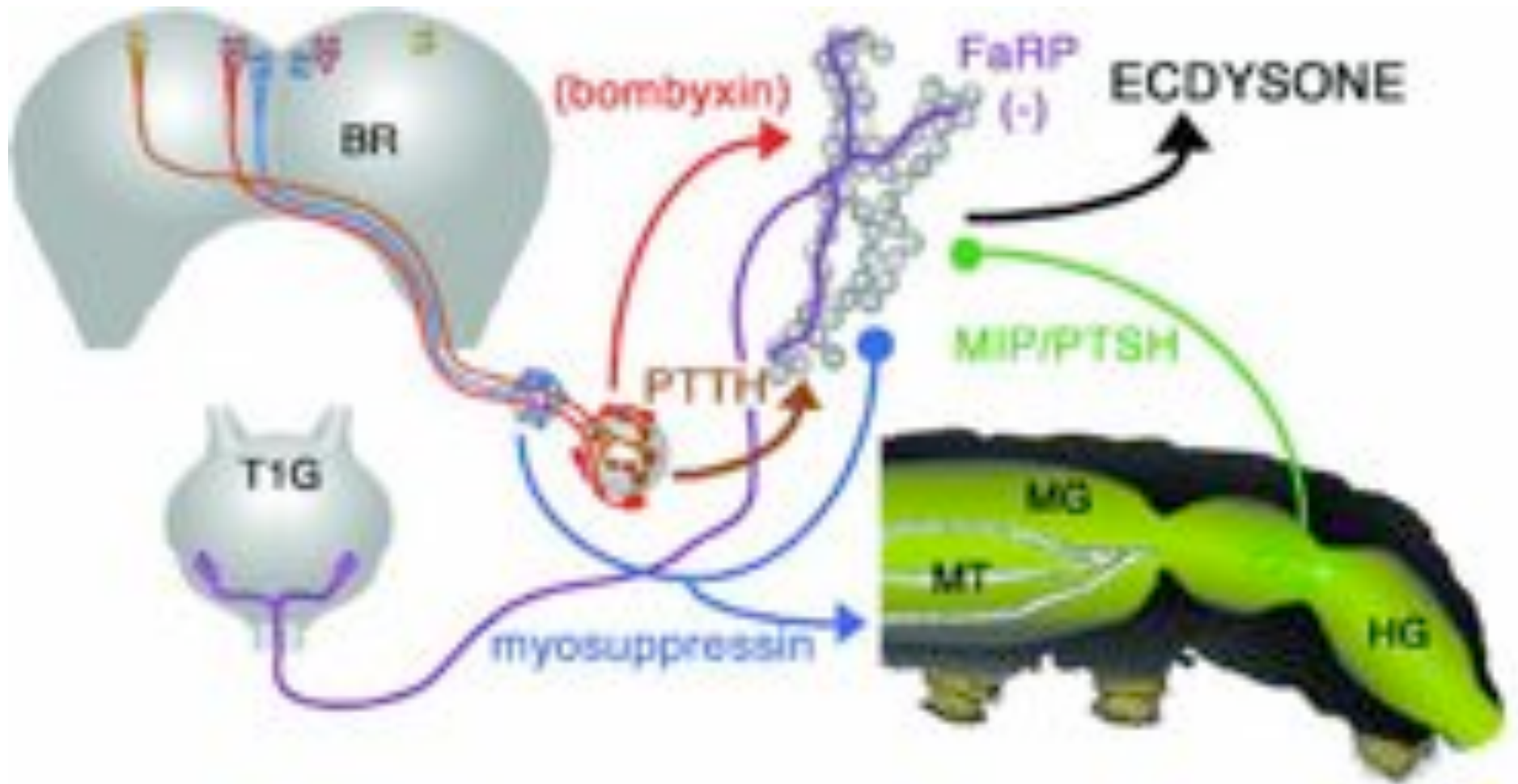
**Ecdysone is the most common ecdysteroid produced in insects**

Some larval leps. Use 3-dehydroecdysone that is covered to ecdysone by enzymes in the hemolymph

In the honeybee and Heteroptera, makisterone is the main ecdysteroid produced and used

In the Diptera is release from the ovaries and stimulates the fat body to produce vitellogenin. Ecdysone converted to 20-hydroxyecdysone in fat body.





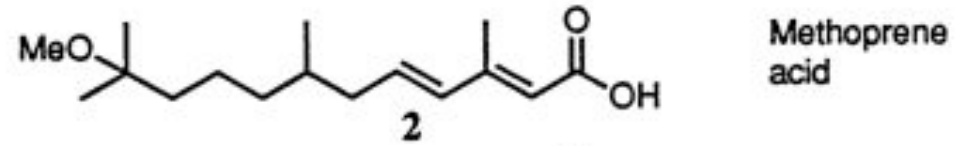
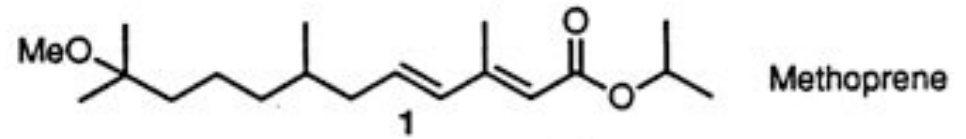
**Current view of the control of ecdysone secretion by the prothoracic glands. The prothoracicotropic hormone (PTTH) from the brain (BR) provides the primary drive for steroid secretion, but the steroid profile is sculpted by the inhibitory effects of circulating myosuppressin from the brain and myoinhibitory peptide/prothoracicostatic peptide (MIP/PTSP) from the hindgut (HG). FMRamide-related peptides (FaRPs) from regulatory neurons in the first thoracic ganglion (T1G) also directly suppress ecdysone secretion. Bombyxin may have an indirect effect on steroidogenesis through stimulation of gland growth. Midgut (MG) and Malpighian tubules (MT) are other targets of the myosuppressin. Taken from Truman, 2006. Steroid hormone secretion in insects comes of age. Proc. Natl. Acad. Sci. USA 103(24):8909-8910.**

# JUVENILE HORMONES

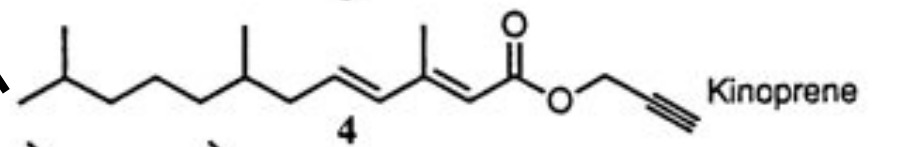
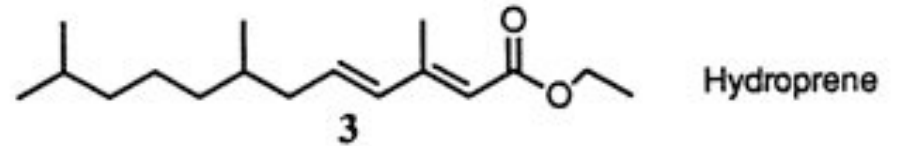
Are sesquiterpenes produced by the corpora allata. Several different forms have now been discovered (see next slide).

Analogues of JH, especially methoprene have been successfully used in insect control. Used against mosquito larvae and fleas (Siphotrol).

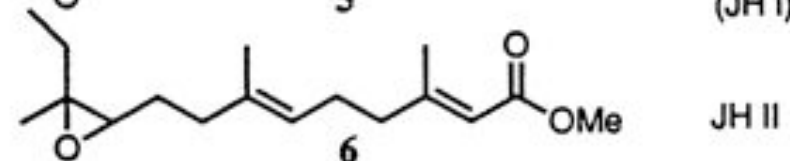
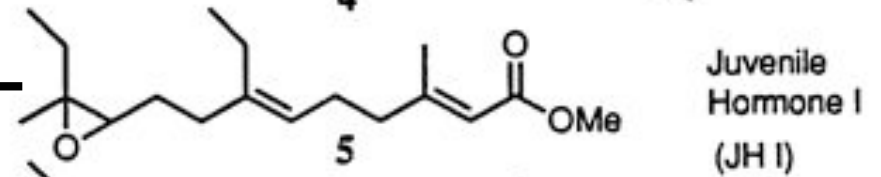
Methoprene is a JH analogue and is used in insect control and experiments



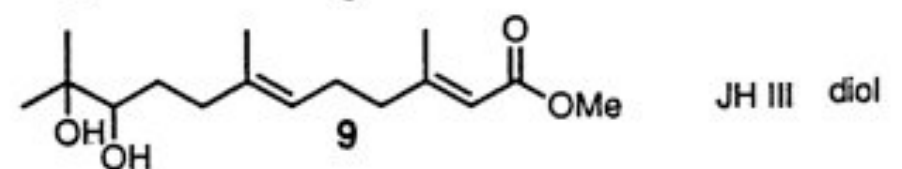
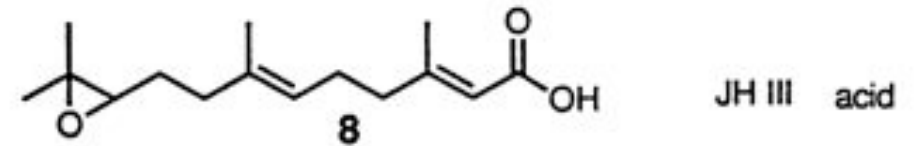
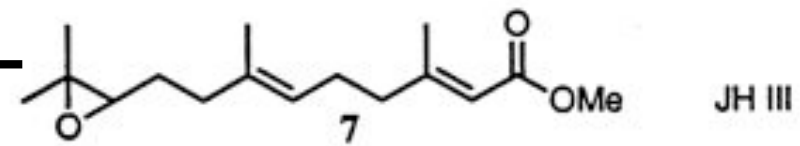
Hydroprene and kinoprene are JH analogues and are used in insect control



JH I is found mainly in the Lepidoptera



JH III was discovered by Dr. Yin (tell story about its discovery, etc.). Found mainly in the Cyclorrhaphous dipterans.

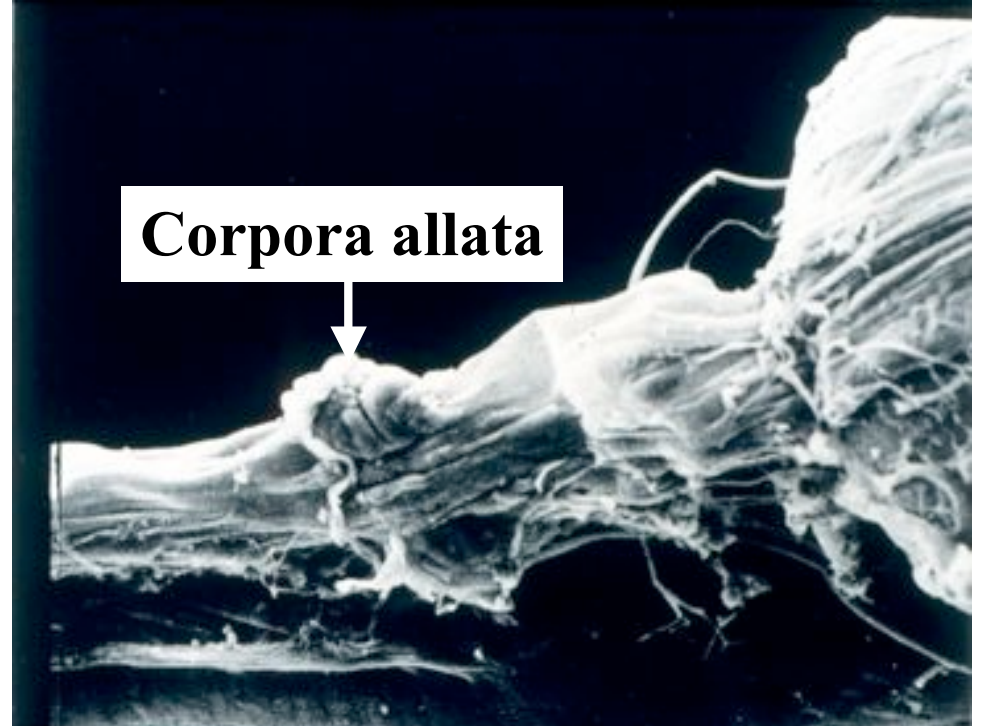
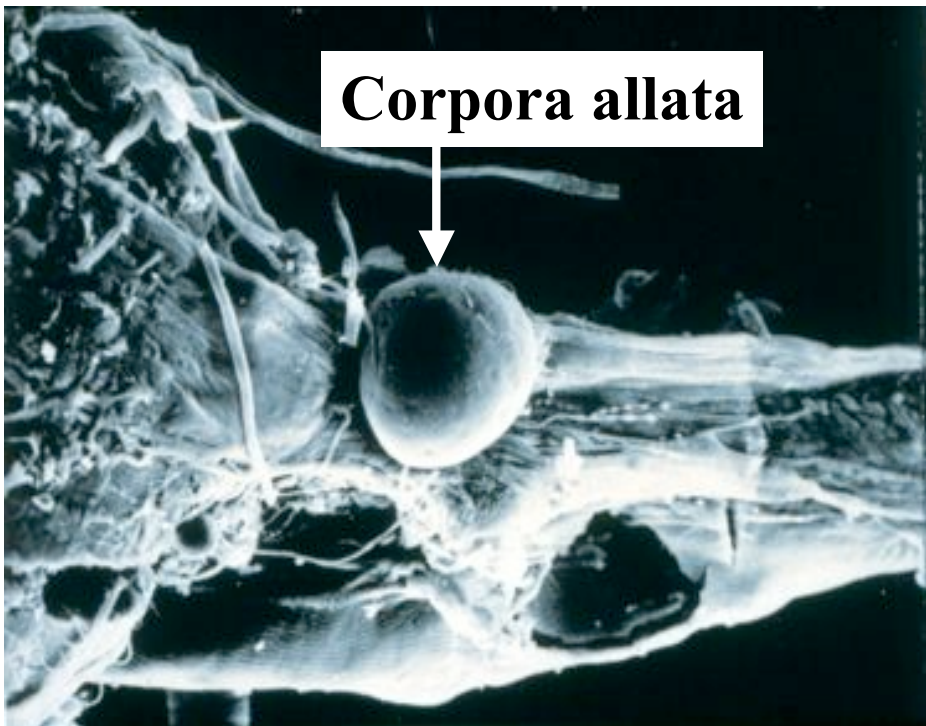


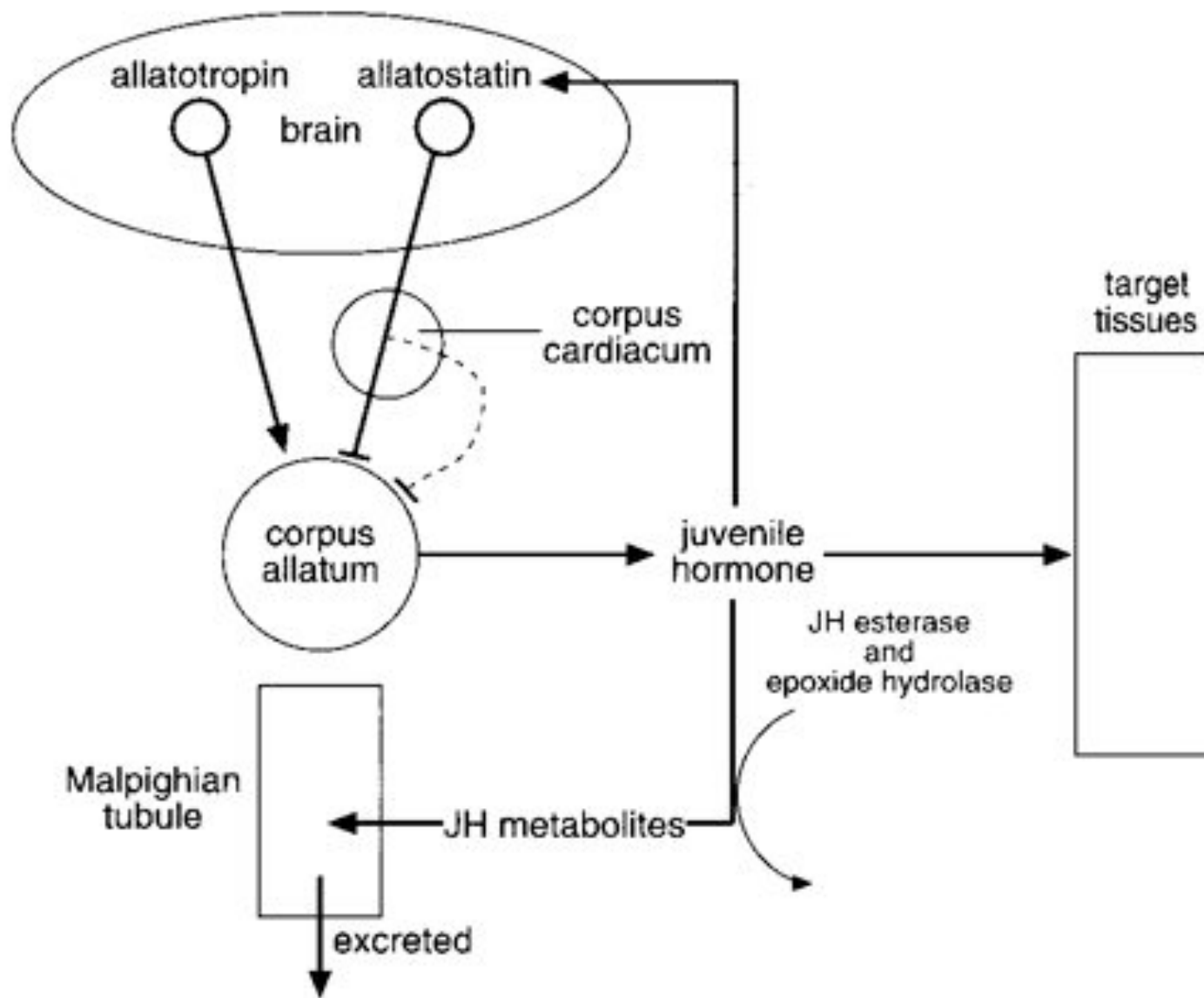


Precocene-from the common bedding plant, *Ageratum houstonianum*



An extract of this plant produces a chemical that selectively kills the cells of the corpora allata, thus no JH is produced. Below on the left is a photo showing the healthy, normal CA in adult *Phormia regina* while on the right is the effect of precocene injection. Notice CA size change.





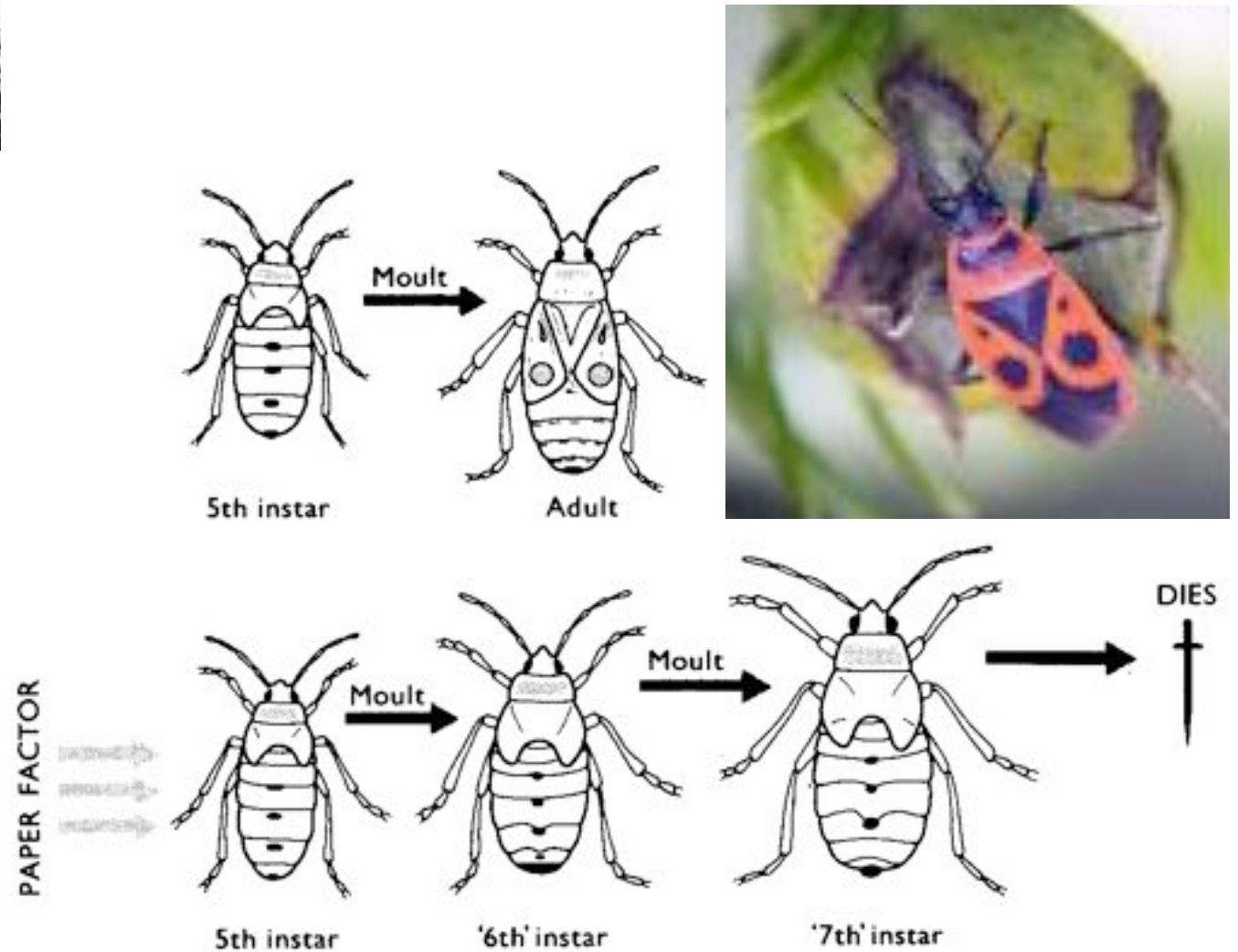
**Fig. 21.11.** Juvenile hormone. Regulation of hemolymph titer involves the balance between synthesis in the corpora allata and degradation and excretion by the Malpighian tubules.



Carroll Williams and John Law getting the paper factor from brown paper towels

## PAPER FACTOR

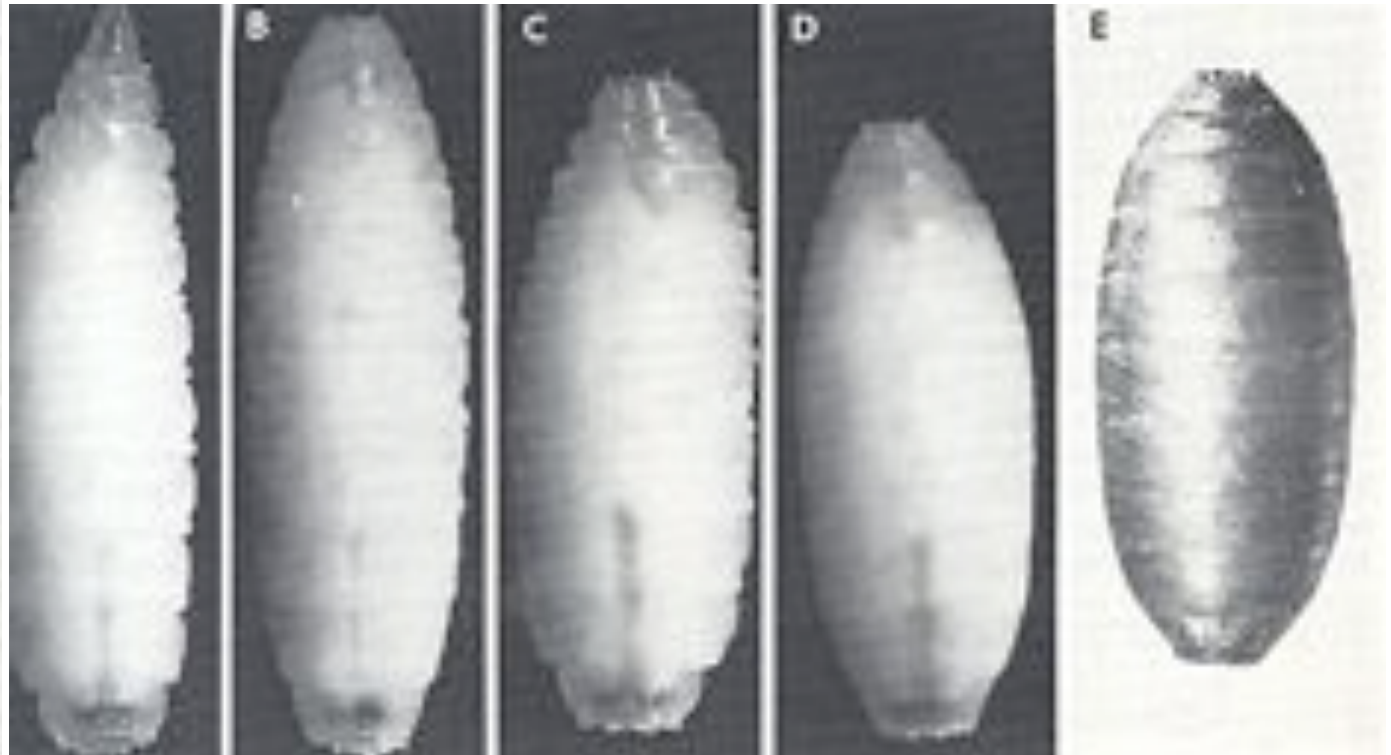
1. Beginning of idea for using hormone analogs to control insects-Zoecon started





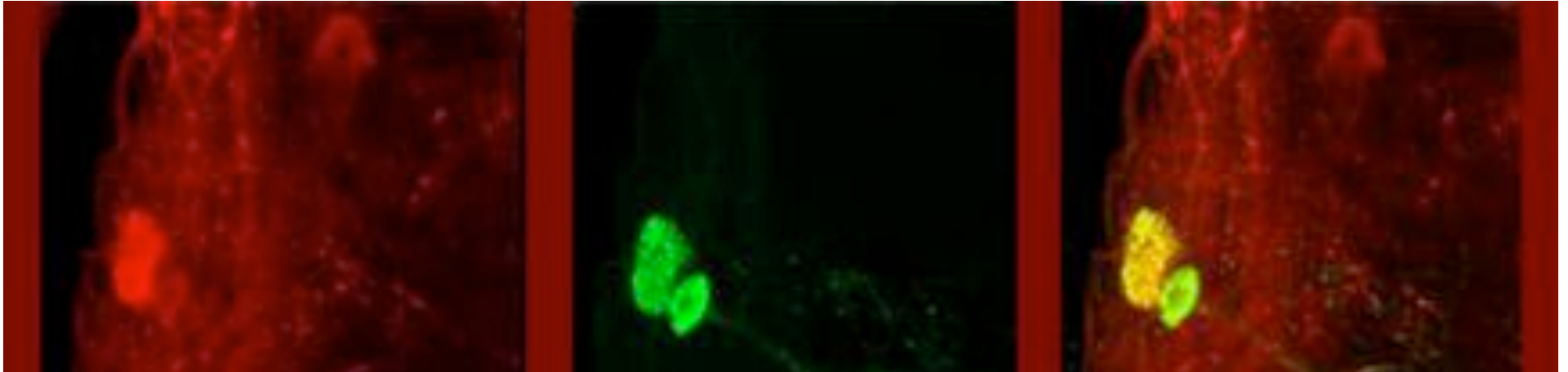
## PUPARIATION IN THE CYCLORRHAPHOUS DIPTERA-

1. Cuticle of last larval instar becomes the pupal case or puparium.
2. Injecting JH into the last larval instar does not lead to a supernumary larva like in some other insects.
3. What causes the tanning and darkening of the cuticle?



- BURSICON**-Neurosecretory hormone that controls tanning (sclerotization) and mechanical properties of the cuticle during and after a molt. Found in most ganglia of the CNS.
1. Cuticle of newly emerged adult fly is soft and plasticized.
  2. Soon, however, it becomes smooth and rigid due to the tanning process and also it becomes darkened due to the melanization process.
  3. Ecdysone or eclosion hormone causes the release of bursicon, which is the neurohormone that causes both plasticization and melanization.





Major point here is that:

Neuropeptides can coexist and be found in the same neurosecretory cell

## **Vertebrate hormones in insects-**

Insulin-like peptide. In 1975, T. Normann suggested that an insulin-like activity in decapitated blowfly, *Calliphora*, was due to a lack of a hypotrehalosemic hormone of cephalic origin. He suggested that the neuroendocrine gland complex, the corpus cardiacum-corpora allata was the likely site for its release. Chen and Friedman (1977) also made a similar assumption using *Phormia regina* and pointed to the CC-CA as the site of the compound.

**OTHER VERTEBRATE PEPTIDES ARE BEING FOUND IN INSECTS**

# Bioinformatics - GENOMICS

The goal of *Genomics* is to promote the understanding of the structure, function, and evolution of genomes in all kingdoms of life and the application of genome sciences and technologies to challenging problems in biology and medicine.

- **Comparative genomics analysis that yields valuable insights into conserved and divergent aspects of function, regulation, and evolution**
- **Bioinformatics and computational biology with particular emphasis on data mining and improvements in data annotation and integration**
- **Functional genomics approaches involving the use of large-scale and/or high-throughput methods to understand genome-scale function and regulation of transcriptomes and proteomes**
- **Identification of genes involved in disease and complex traits, including responses to drugs and other xenobiotics**
- **Significant advances in genetic and genomics technologies and their applications, including chemical genomics**

**Taken from site below:**

[http://www.elsevier.com/wps/find/journaldescription.cws\\_home/622838/description#description](http://www.elsevier.com/wps/find/journaldescription.cws_home/622838/description#description)

## **Bioinformatics - PROTEONOMICS**

**Proteomics is often considered the next step in the study of bioinformatics, after genomics.**

**Proteomics is the large-scale study of proteins, particularly their structures and functions. Proteins are vital in living organisms, as they are the main components of the physiological pathways of cells. The term "proteomics" was coined to make an analogy with genomics, the study of the genes. The proteome of an organism is the set of proteins produced by it during its life, and its genome is its set of genes.**



# BIOLOGICAL PATHWAYS

## A. Signal transduction pathways

## B. Metabolic pathways

**The pathway, starting from the genes involved, the molecules involved in signal transduction (i.e., signal transducers), and the various proteins, etc., involved in a pathway that leads to a biological function such as the production of a hormone (i.e., insulin) or a pathway involved in *Drosophila* immunity.**

See the website below and click onto BIOCYC  
[bioinformatics](http://bioinformatics)

<http://bioinfo.utmb.edu/biopathways.html>

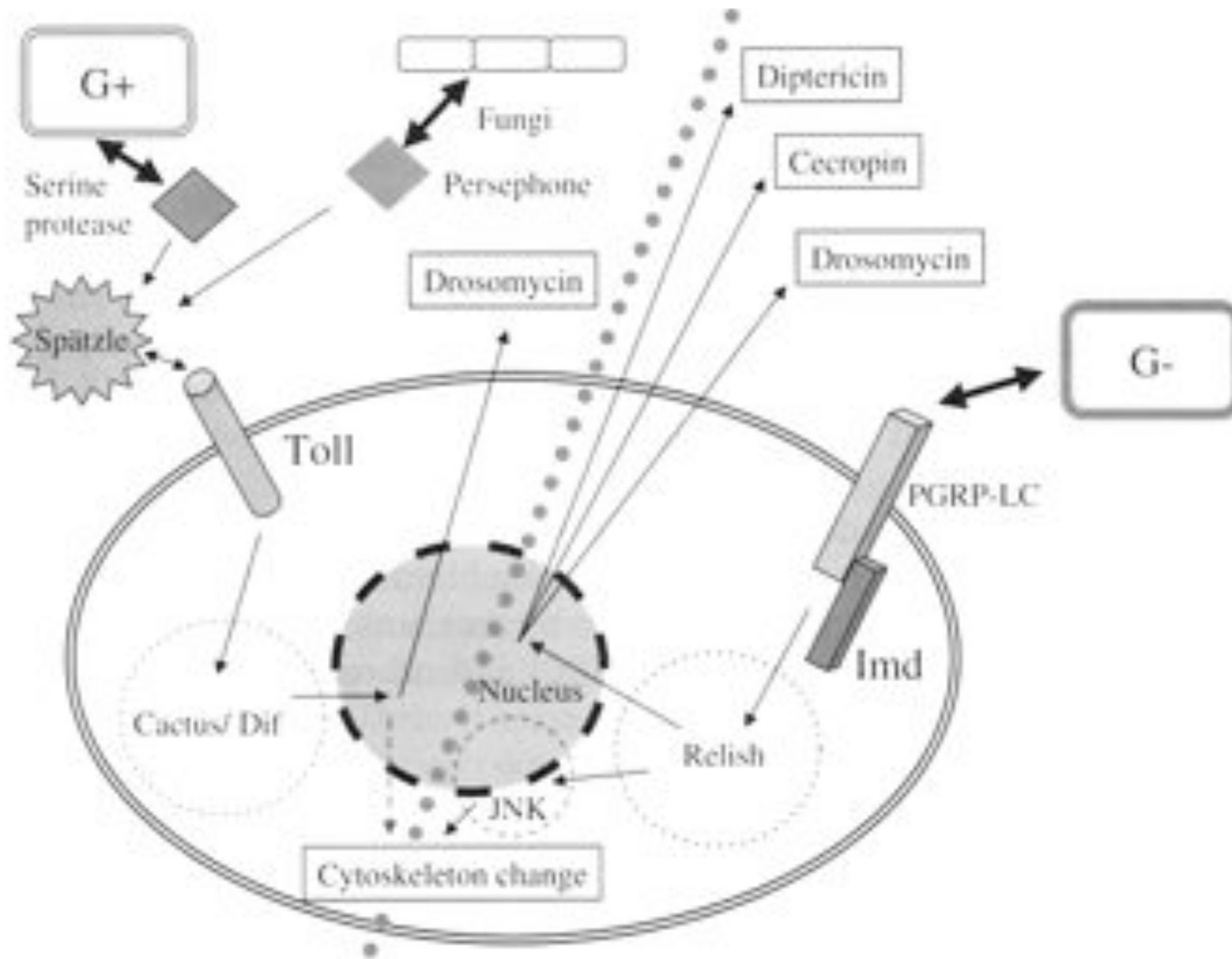
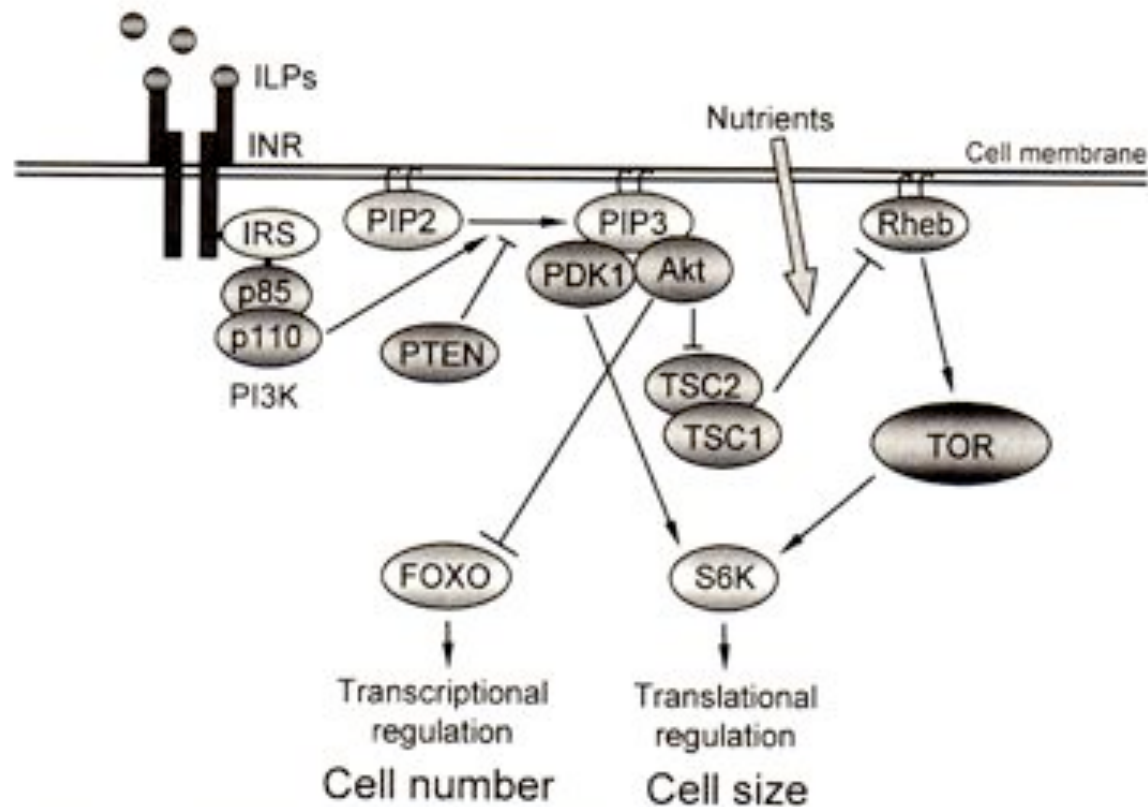


FIG. 3 A simplified schematic of the activation of the Toll and Imd pathways in *Drosophila* immunity.

Taken from Siva-Jothy et al. 2005. Insect Immunity. Adv. Ins. Phys.



**Figure 1** Conserved insulin signaling pathway in insects. Homologs for the key components illustrated here have been characterized genetically and biochemically in *Drosophila melanogaster* and in a few other insects. Abbreviations: Akt, protein kinase B; FOXO, forkhead box-containing protein, O subfamily; ILPs, insulin-like peptides; INR, insulin receptor; IRS, insulin receptor substrate; p110, catalytic subunit of PI3K; p85, adaptor subunit of PI3K; PDK1, phosphatidylinositol-dependent kinase 1; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PTEN, phosphatase and tensin homolog; Rheb, Ras-homolog expressed in brain; S6K, p70 ribosomal S6 kinase; TOR, target of rapamycin; TSC, tuberous sclerosis complex. See text for details.

Taken from Wu and Brown. 2006. *Ann. Rev. Entomol.* 51: 1-24

“Insulin-like peptides (ILPs) exist in insects and are encoded by multigene families that are expressed in the brain and other tissues. Upon secretion, these peptides likely serve as hormones, neurotransmitters, and growth factors, but to date few direct functions have been demonstrated.

In *Drosophila melanogaster*, molecular genetic studies have revealed elements of a **conserved insulin signaling pathway**, and as in other animals models, it appears to play a key role in metabolism, growth, reproduction and aging.”

1. From *D. melanogaster* genome database found 7 ILP genes.
2. Insulin receptor in *Drosophila* is identical to that in humans (i.e. same physical and enzymatic properties).
3. Where would one expect to find an ILP in insects if it was involved in the regulation of blood sugar levels?

**The fat body since it is where glycogen is stored and converted to trehalose when needed. Trehalose and glucose levels in the blood are regulated by hyper- and hypoglycaemic hormones from the brain and stored in CC.**

**Above taken from:**

**Wu, Q. and M.R. Brown. 2006. Signaling and function of insulin-like peptides in insects. Ann. Rev. Entomol. 51: 1-24.**

## Multiple insulin-like peptides (ILPs) + its signaling pathway

coordinates the regulation of



Metabolism



Growth



Reproduction



Longevity



and some behavioral events in insects

**HEPING LIN<sup>1</sup>, CHIH-MING YIN<sup>1</sup>, JOHN G. STOFFOLANO, JR.<sup>1</sup>, AND ROBERT S. GAROFALO<sup>2</sup>. 2005. Immunological localization of mosquito ovary ecdysteroidogenic hormone I and fruit fly insulin receptor in adult *Phormia regina* (Diptera: Calliphoridae). *Ann. Entomol. Soc. Amer.* 98: 329-335.**

**Cao, C.; Brown, M.R. 2001. Localization of an insulin-like peptide in brains of two flies. *Cell Tissue Res.* 304: 317-321.**

**Chen, C.; Jack, J.; Garofalo, R.S. 1996. The *Drosophila* insulin receptor is required for normal growth. *Endocrinology* 137: 846-856.**

**Ruan, Y.; Chen, C.; Cao, Y.; Garofalo, R.S. 1995. The *Drosophila* insulin receptor contains a novel carboxyl-terminal extension likely to play an important role in signal transduction. *J. Biol. Chem.* 270: 4236-4243.**

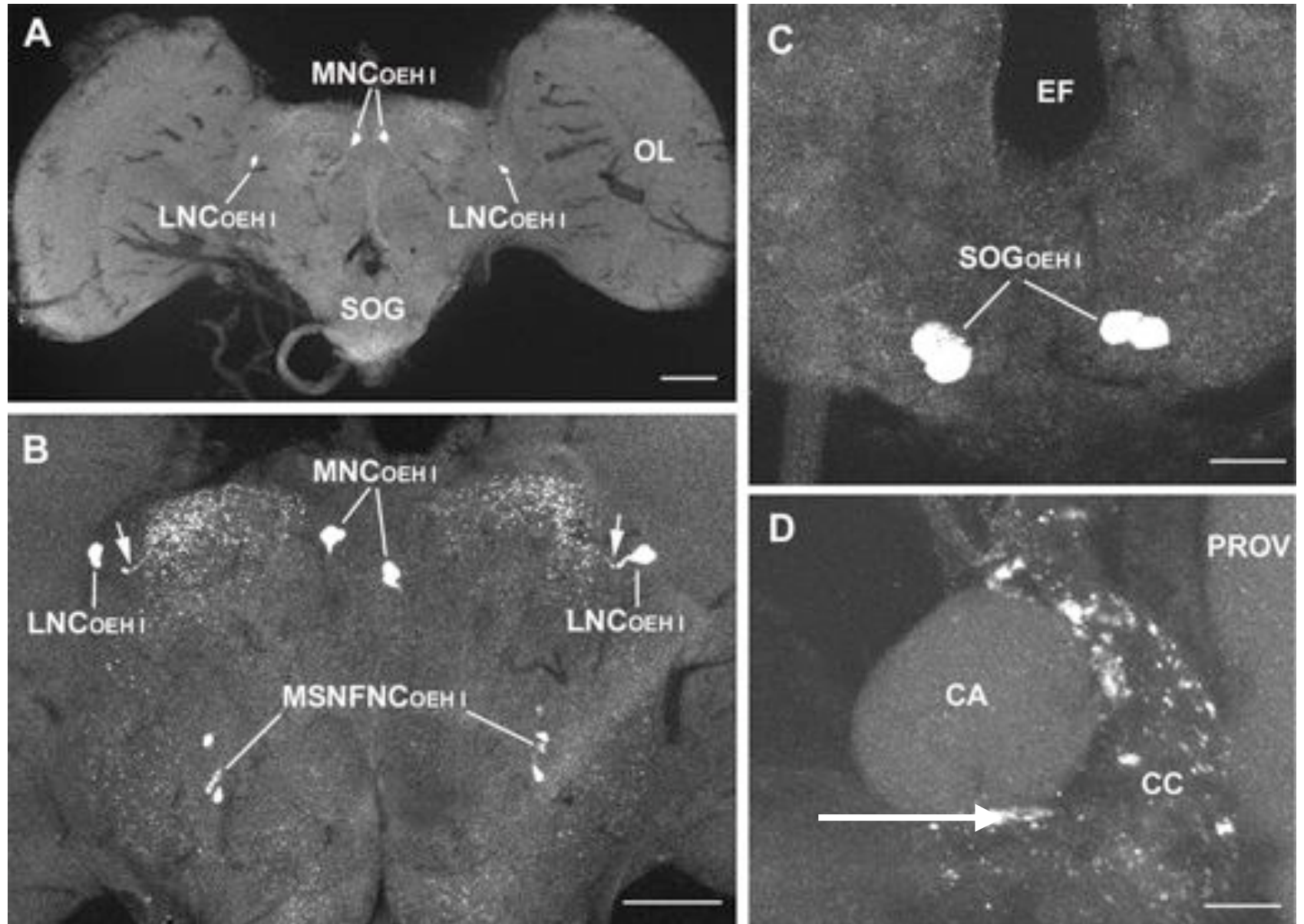
**Rulifson, E.J.; Kim, S.K.; Nusse, R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296: 1118-1120.**

**Wu, Q. and M.R. Brown. 2006. Signaling and function of insulin-like peptides in insects. *Ann. Rev. Entomol.* 51: 1-24.**

**RULIFSON ET. AL. SUGGESTED THE RECEPTOR IS IN THE CC/CA COMPLEX BUT NOW EVIDENCE IS SHOWN IN *PHORMIA REGINA* (SEE NEXT SLIDE)**

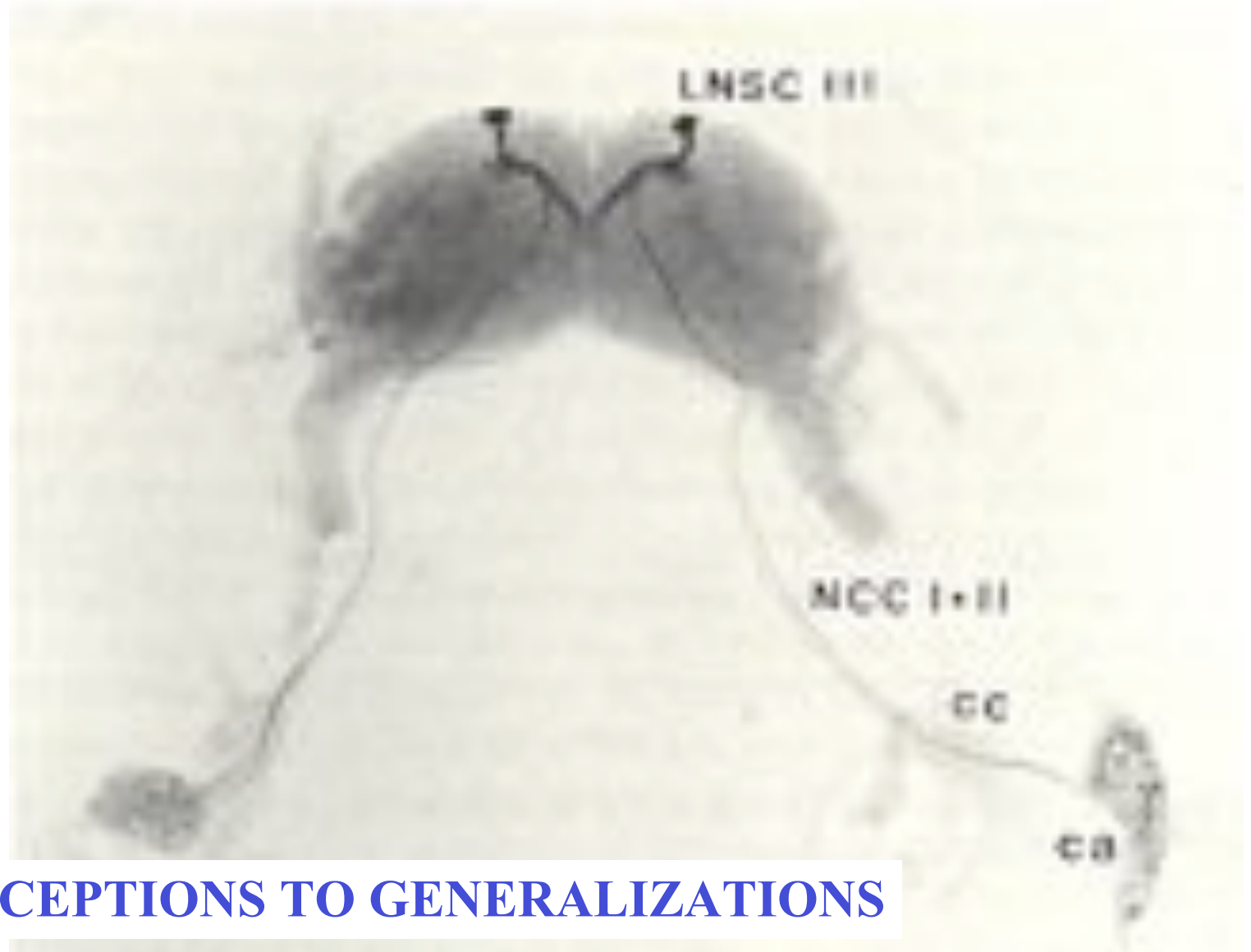


Brain of *Phormia regina* showing the presence of *Aedes aegypti* ovary ecdysteroidogenic hormone I (OEH) in the brain of female (A), male (B), and (C) suboesophageal ganglion of the female. **Fig. D shows the insulin-receptor immunopositive signals in the CC-CA complex of female *Phormia regina* (see white arrow in D).**



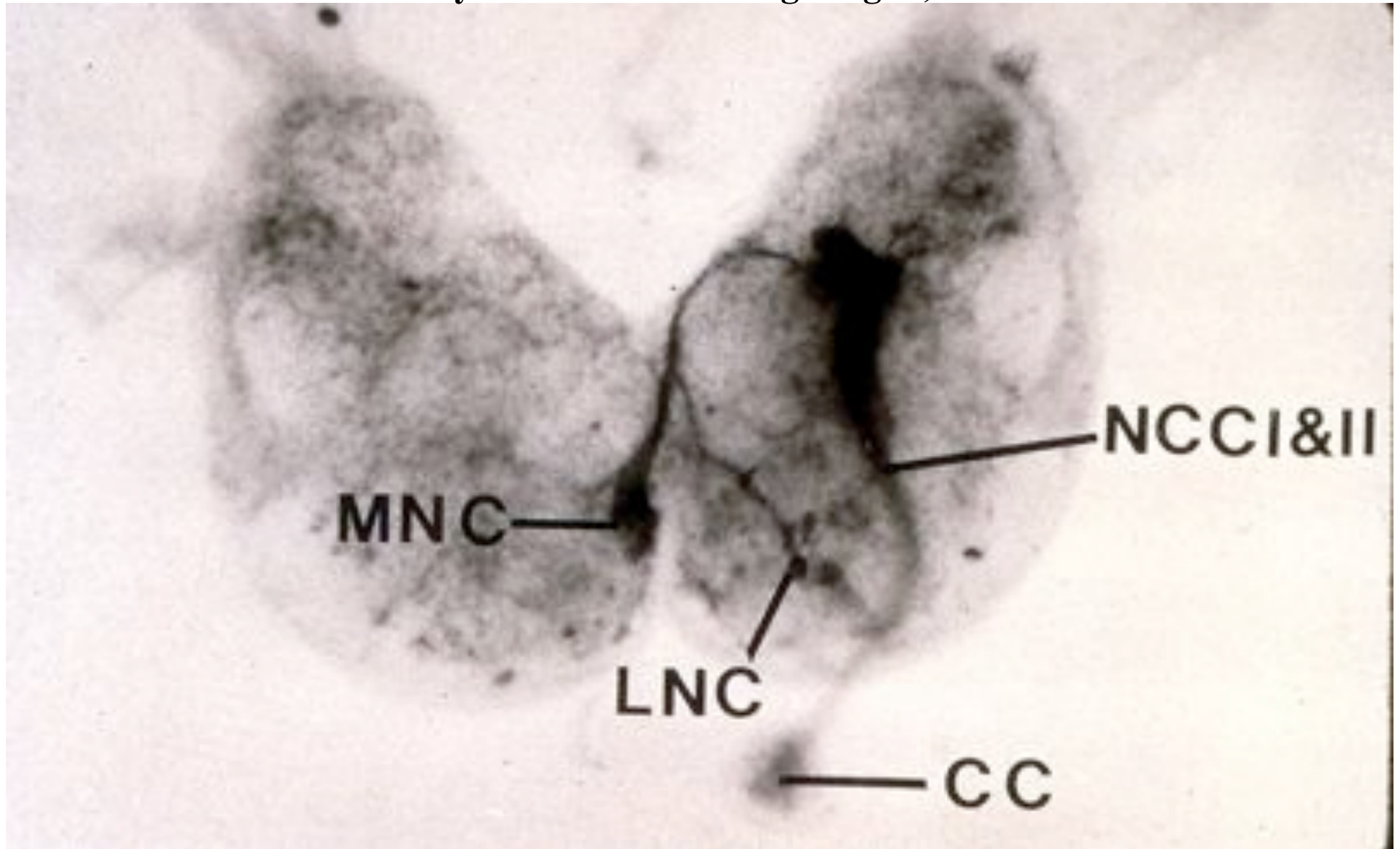
PTTH-Used an immunohistochemical stain with an antibody against PTTH. PTTH is produced in the LNSC III cells in the brain. Travels down the NCC I+II through the CC and is released from the CA

5<sup>th</sup> instar of *Manduca sexta*. In most insects the CC is the release site of PTTH but, in Leps., it is the CA where release occurs.



**ALWAYS EXCEPTIONS TO GENERALIZATIONS**

**Cobalt backfilling of 6<sup>th</sup> instar larva of gypsy moth showing the median neurosecretory cells (MNC), lateral NSC (LNC), the nervi corpora cardiaca (NCCI&II) and the corpus cardiacum (CC). Note the passage of the dye between the neurosecretory cells and the storage organ, the CC.**





**Neurosecretory cells**-Specialized cells that are both nervous and secretory. Identified basically by specific staining techniques and electron microscopy. In transmitted light they appear blue because of the Tyndall effect of light scattering due to the fine droplets of neurosecretion found in the cells. The droplets are electron dense (See below right photo)

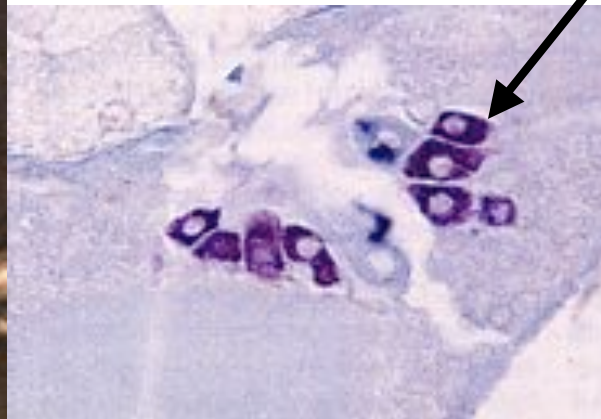
Tyndall blue effect



*Aedes aegypti* brain

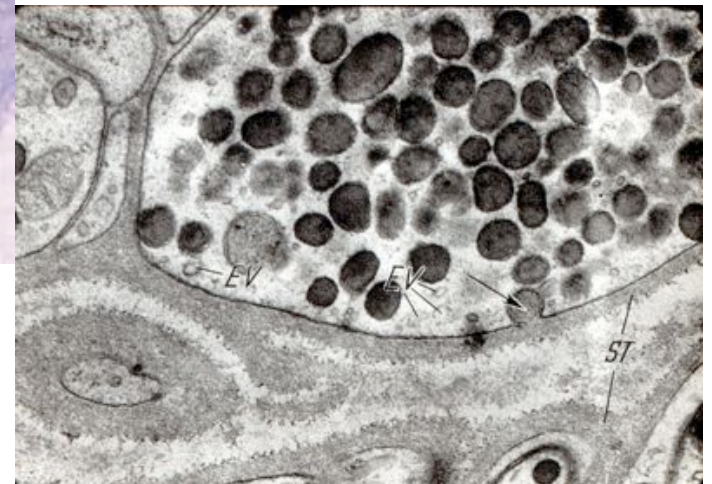
Medial neurosecretory cells

Paraldehyde fuchsin

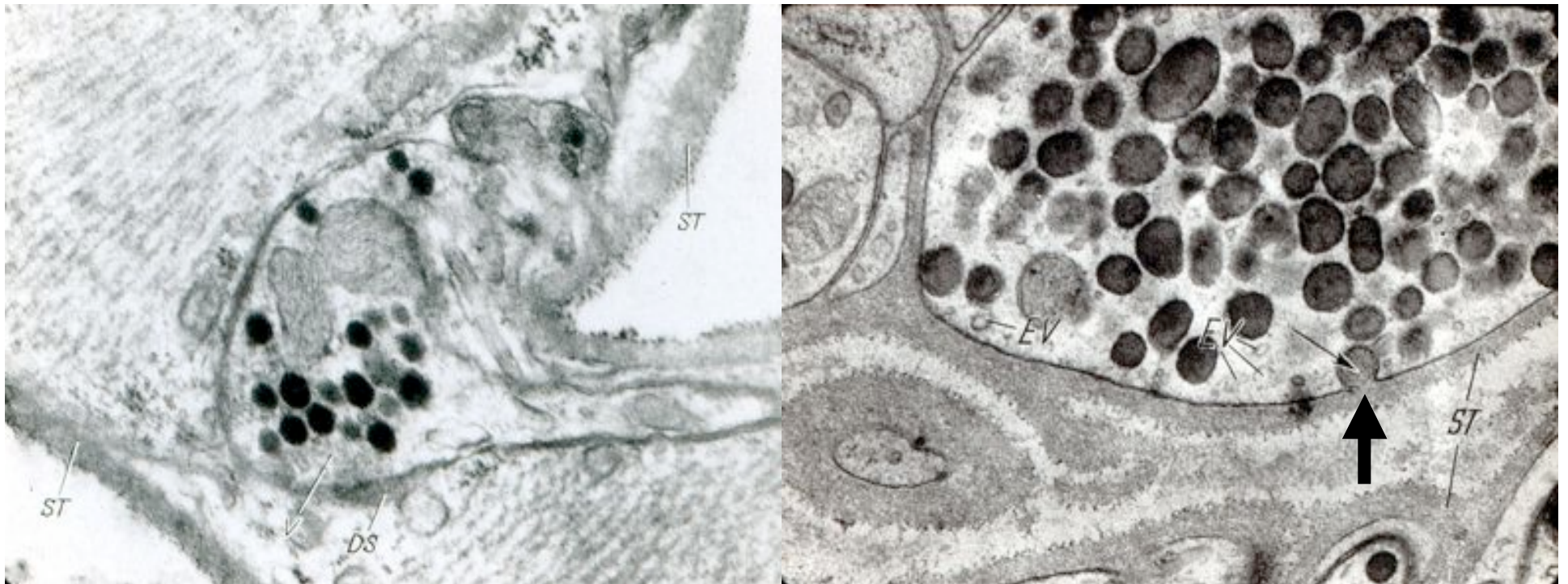


Brain of *Phormia regina*

Electron microscopy



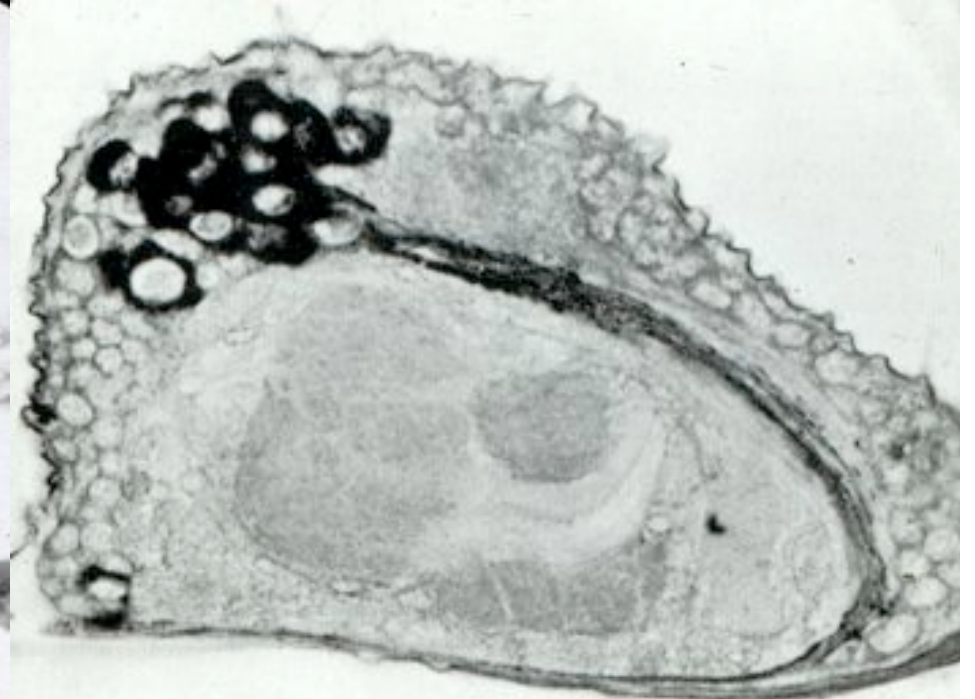
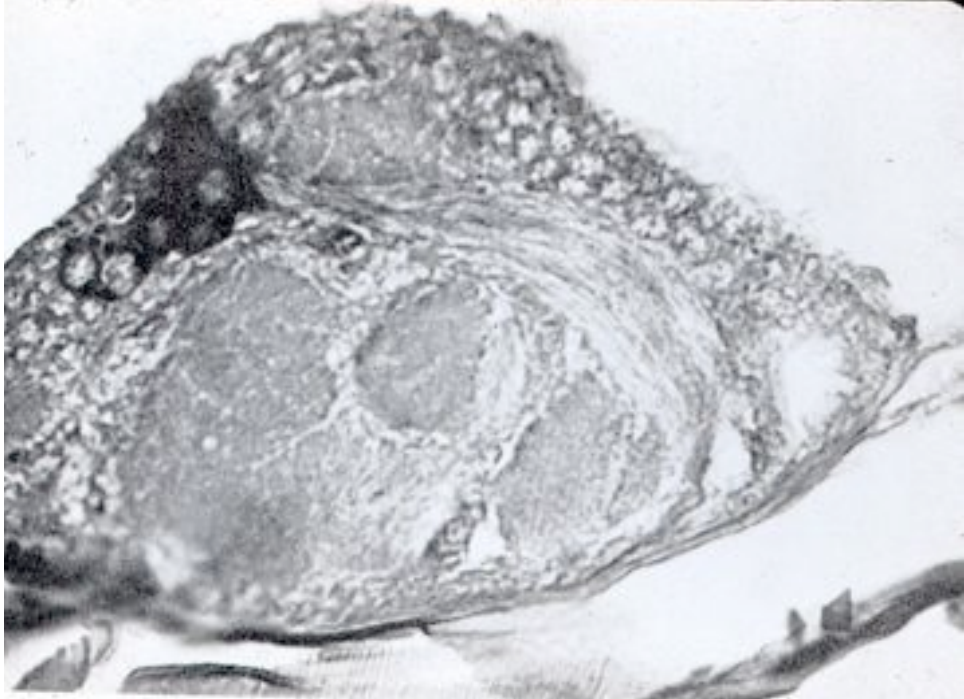
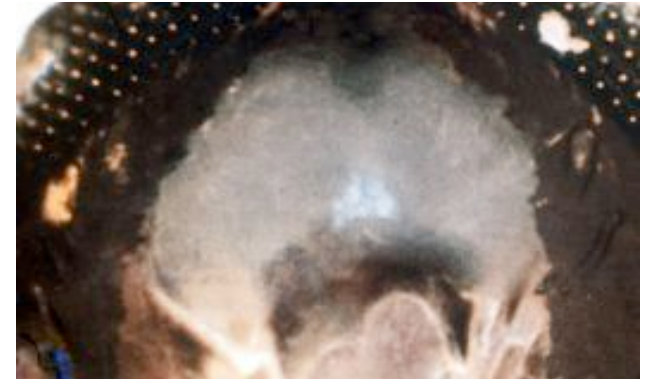
Neurosecretory cells can release their secretion directly onto tissue, as seen in bottom left with the material being released onto heart muscle. Release occurs as a result of fusion of the membrane of the vesicles of neurosecretion with the membrane of the cell through exocytosis (see bottom right bold arrow).





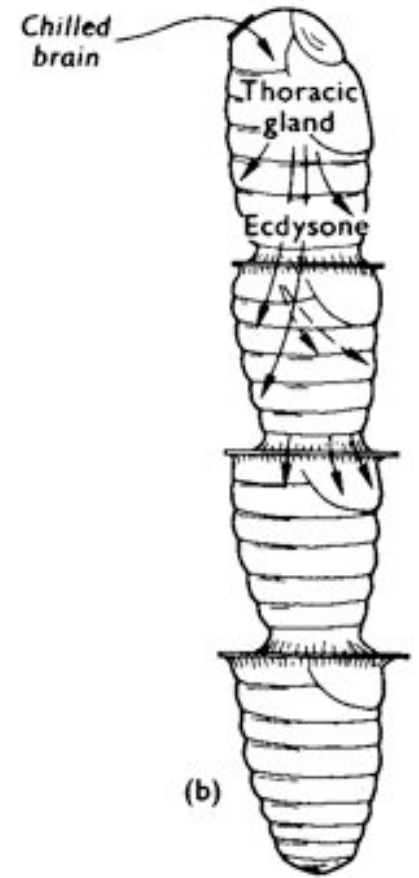
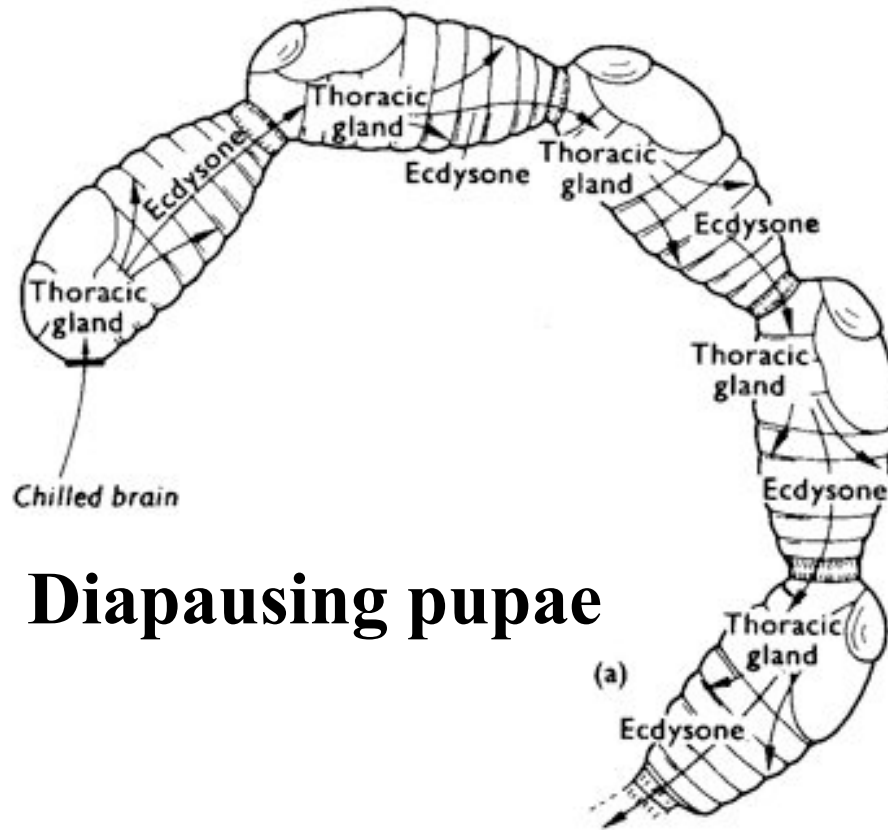


Brain neurosecretory cells in *Aedes aegypti*.  
Seen as blue cells because of Tyndall blue effect  
caused by droplets of neurosecretion. Note  
bottom left cells, using PAF to show no release  
but in bottom right you can see it in the axon.

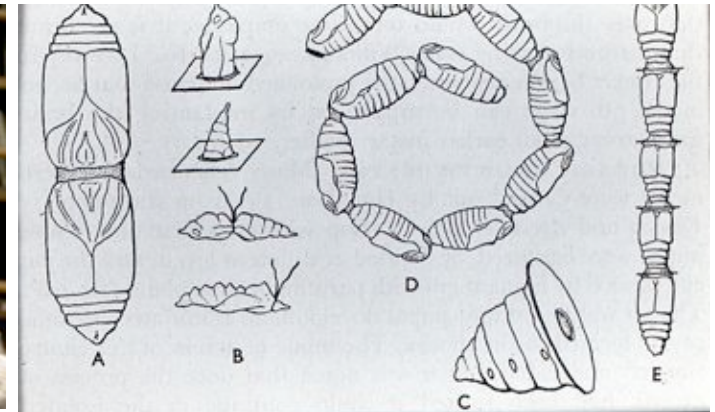
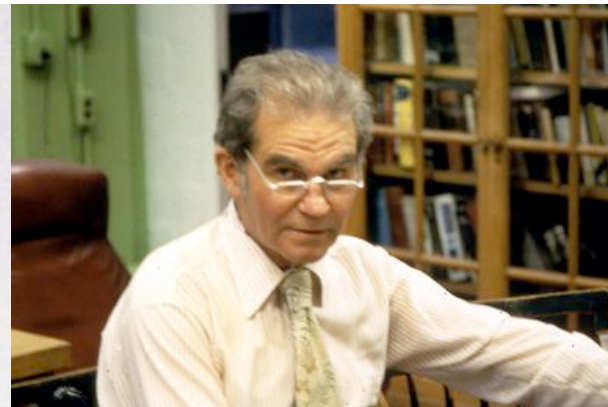
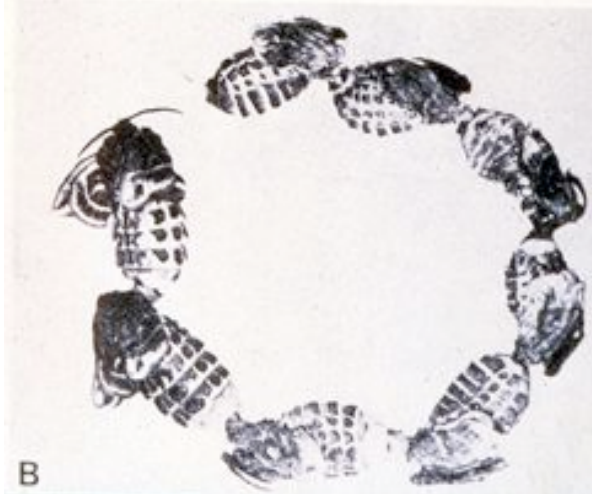




# THE BRAIN HORMONE



## Diapausing pupae





## Transport of hormones in hemolymph-

Ecdysteroids are relatively insoluble in water. Transported by binding proteins

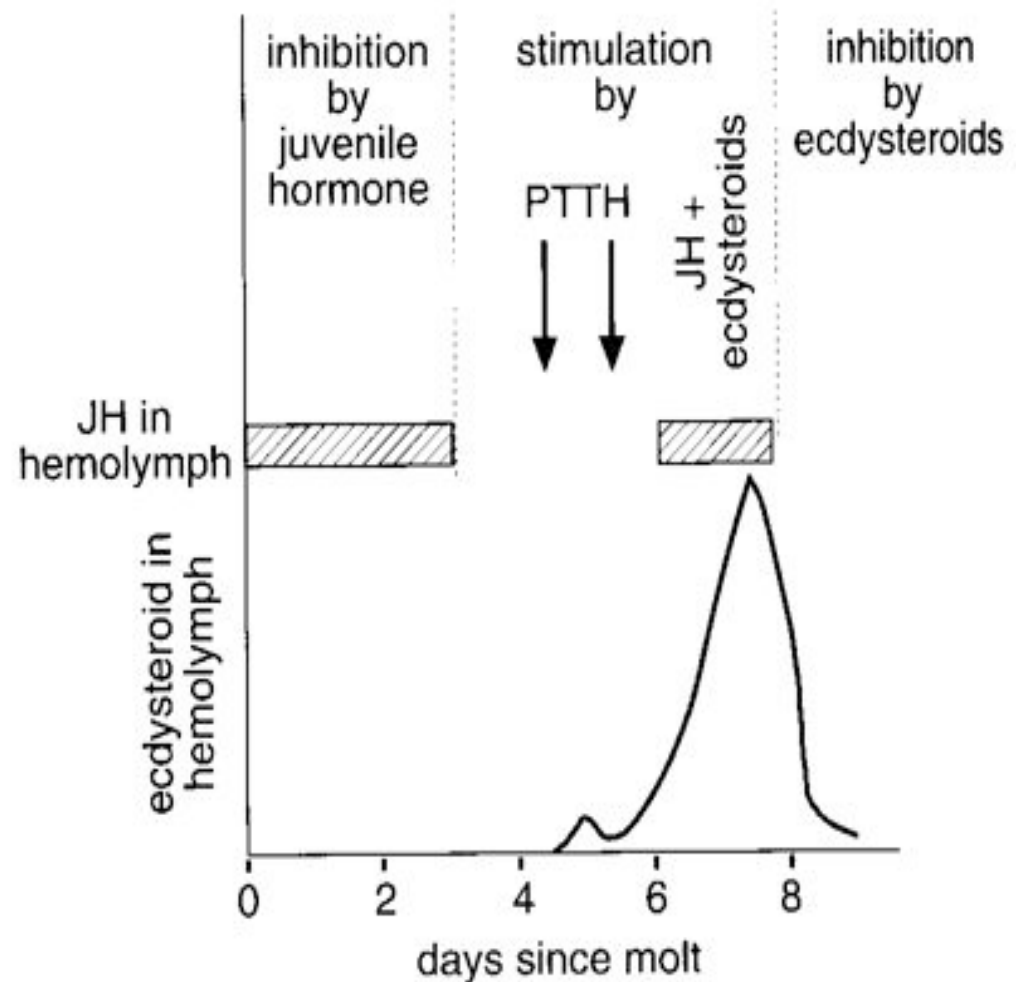
JH is slightly soluble in water + is also transported by binding proteins

Peptides are soluble in water and need no binding protein for transport

## Control of titers in hemolymph-

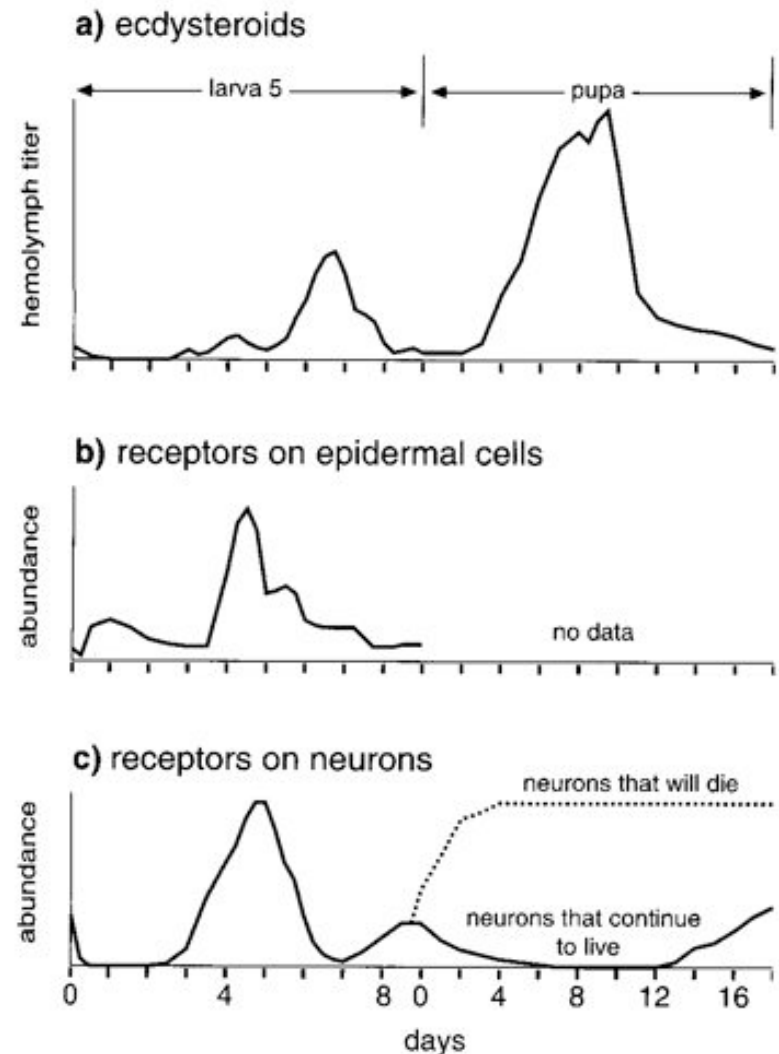
**Critical titer**-the concentration of the hormone in the hemolymph where it can produce an effect on its target site.

**Critical window**-the time frame in which the hormone can actually have its effect on the target site or tissue. This probably depends on the presence of appropriate receptors.



## Mode of action of hormones-

1. Activity within a cell depends on specific receptors for that hormone
2. The response of different tissues depends on the presence + number of receptors. This varies with development. Thus, different tissues will respond at different times
3. Receptors are in the cell membrane or within the cell
4. Both Ecdy. + JH are lipophilic so they pass through the cell membrane and have their effect within the cell
5. Cause inactive genes to become active or can inactivate other genes
6. In immature insects, JH has no effect by itself but it modifies the responses to ecdysteroids. In adult insects JH can produce an effect by itself.



## **Mode of action of hormones-**

7. Peptide hormones and biogenic amines are lipophobic, thus they will not pass through the cell membrane. Specific receptor proteins for these substances are present in the cell membranes. Activation of these receptors activates secondary messengers (e.g., cAMP + cGMP) with the cells

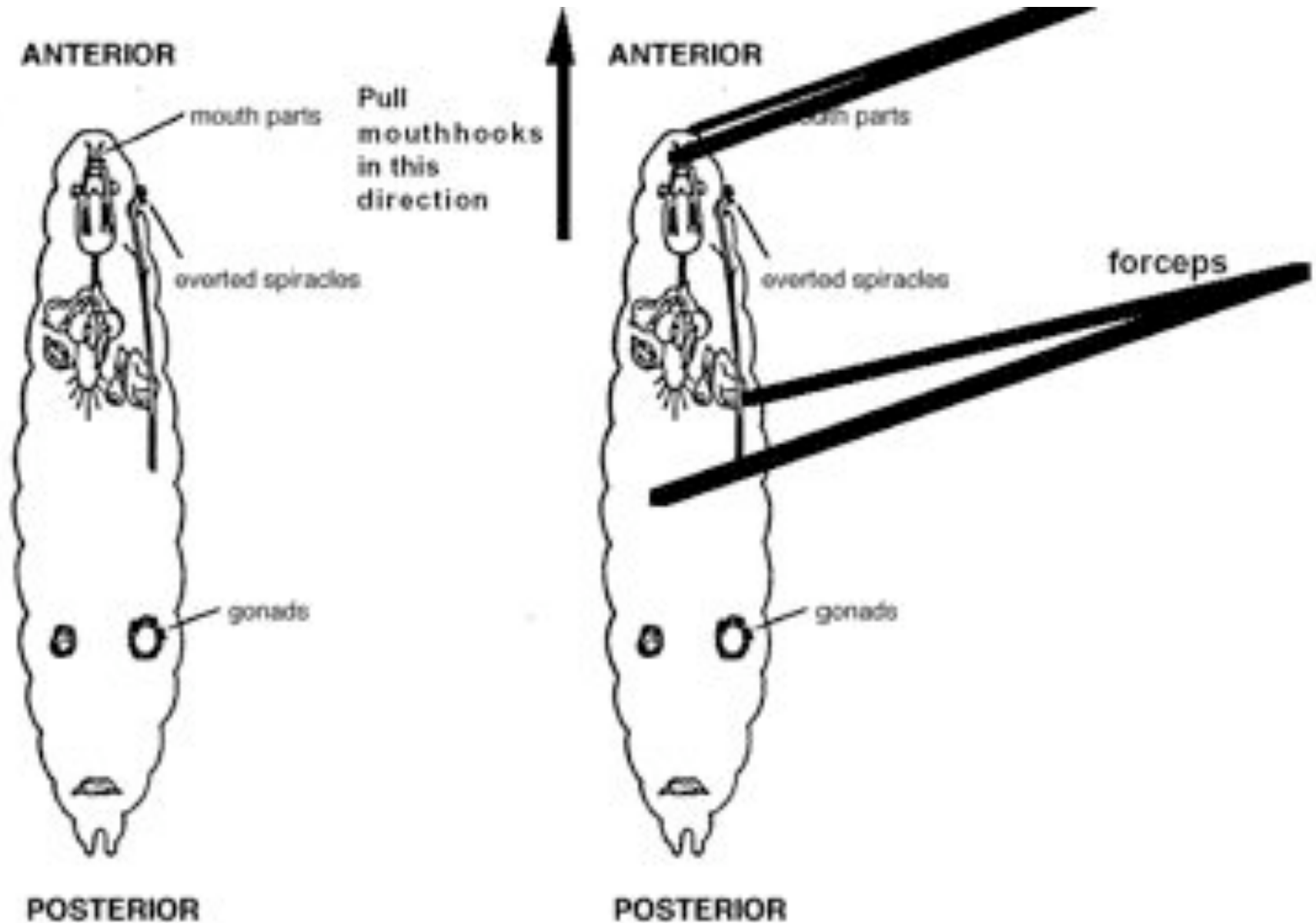


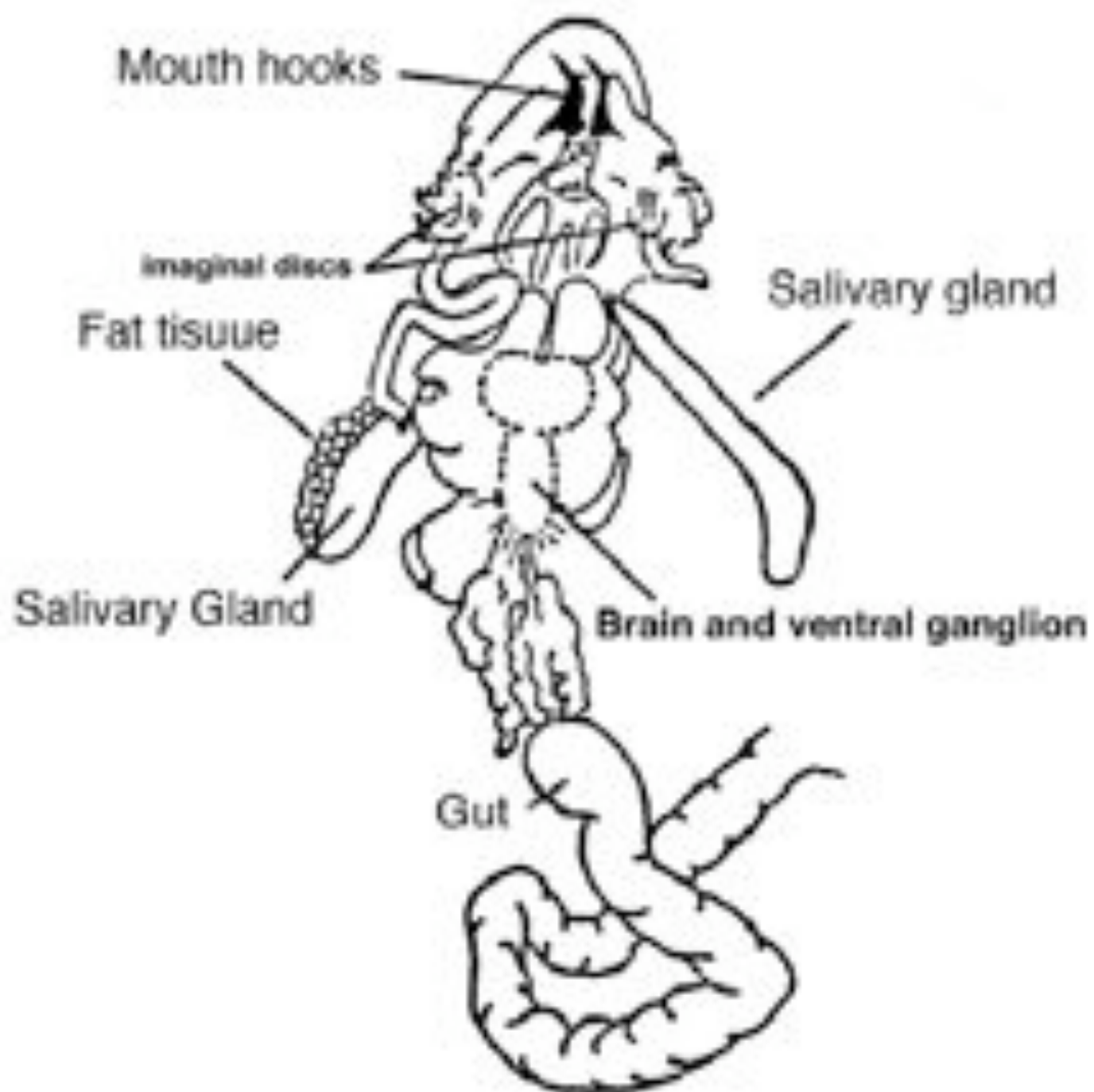
### **3 important aspects concerning gene regulation in eucaryotic cells**

- 1. There is a similarity of DNA in all nuclei of an organisms that is quantitative as well as qualitative. Each cell possesses a complete and identical set of genetic information. How do cells respond differently then?**
- 2. The availability of genetic information for transcription is restricted: 5-20% of the genome is transcribable at a time and the specific sequences expressed are different in each cell type, thus reflecting the metabolic requirements of the cell.**
- 3. Cells are able to modulate gene expression in response to specific demands. Such modifications in gene readout occur during development and differentiation, during the cell cycle, and in **response to hormones**.**
  - a. How are specific regions of the genome rendered transcribable?**
  - b. How are genes ‘turned on’ or ‘turned off’**
  - c. Specific repressor proteins called histones**

For a discussion of removing the salivary glands in *Drosophila* and looking at heat shock genes, see the following

<http://biosci191.bsd.uchicago.edu/labdocs/20191F02PolyteneLab.pdf>





## Temperature induced puffing of polytene chromosomes

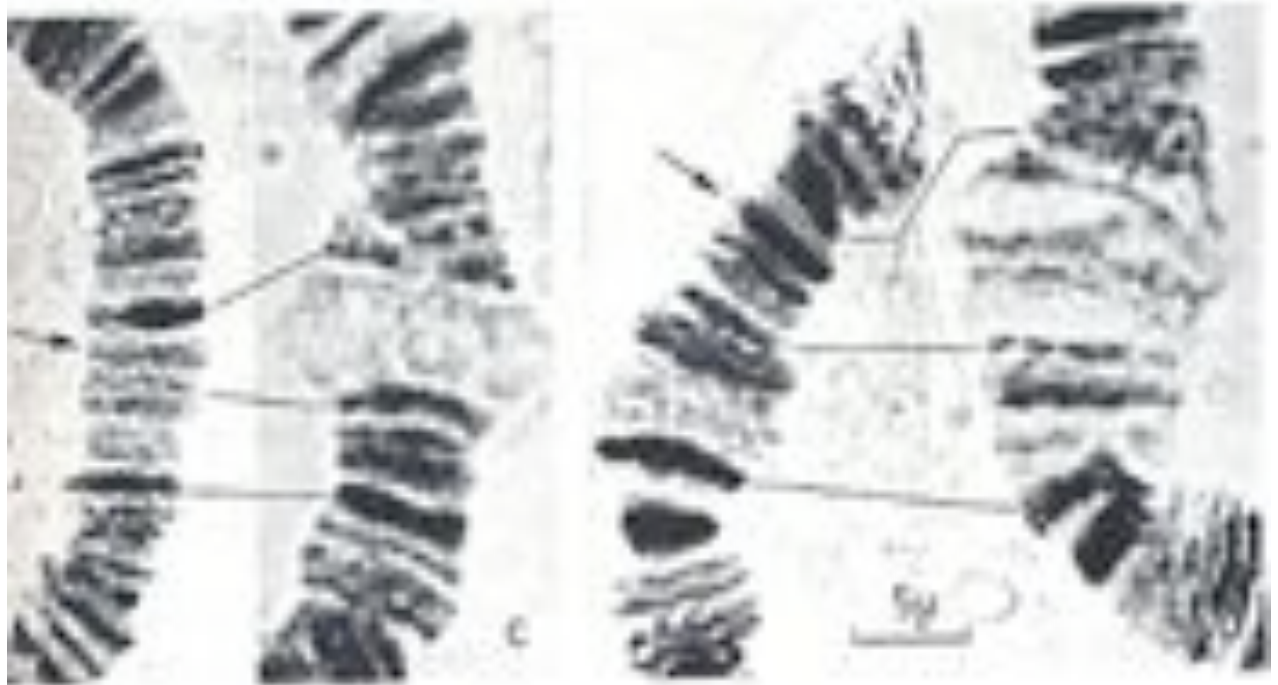
Chromosome puffing activated in *Drosophila* chromosomes due to different temperature treatments.

a. 48°C

b. 36°C

c. 81°C

d. 32°C

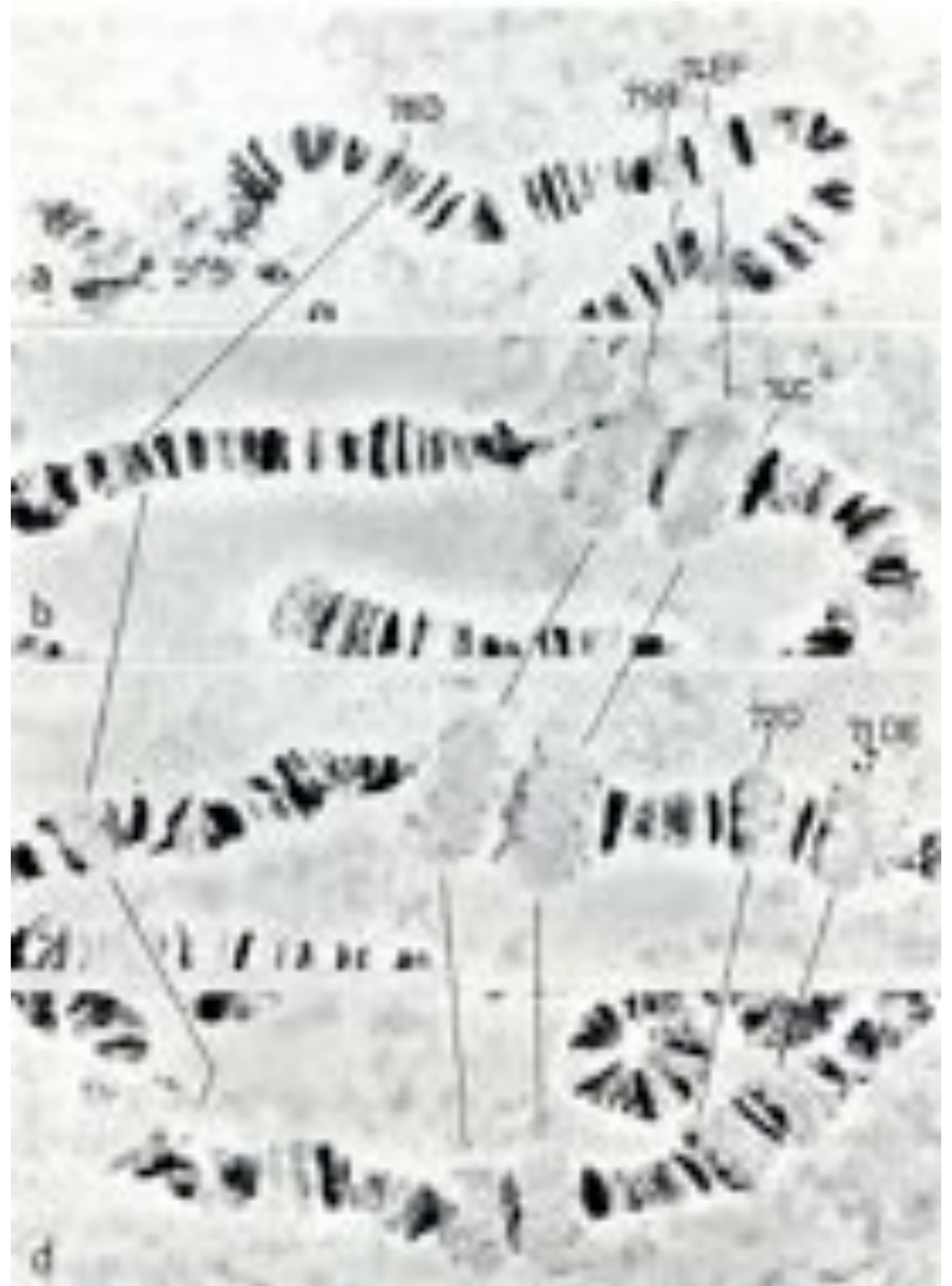




## Ecdysteroid induction of puffing patterns in *Drosophila* polytene salivary gland chromosomes

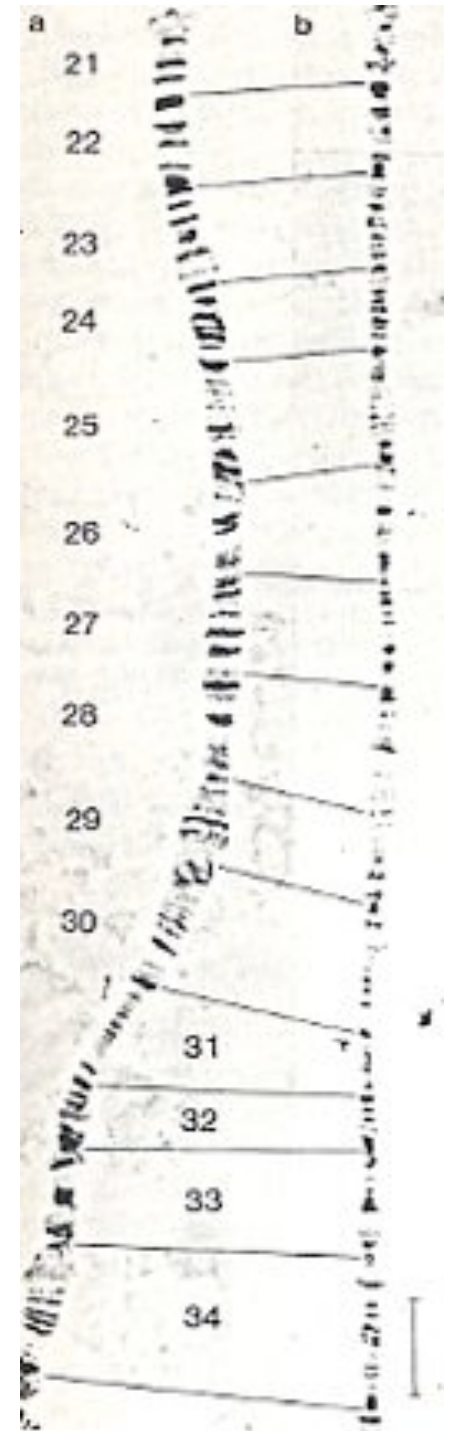
*In vitro* induction of puffing pattern changes after incubation with 20-hydroxyecdysone at 1, 2, 4, and 6 hr. after incubation

Puffing patterns change with respect to tissue, age of insect and stage of insect





Banding pattern differences of chromosome 21 of *Drosophila melanogaster* showing the difference between the puffing of the same chromosome in the salivary gland (a) and fat body (b).



Baermann's (1961) evidence that puff sites are sites of active messenger RNA synthesis.

He took 2 interbreeding species of *Chironomus*

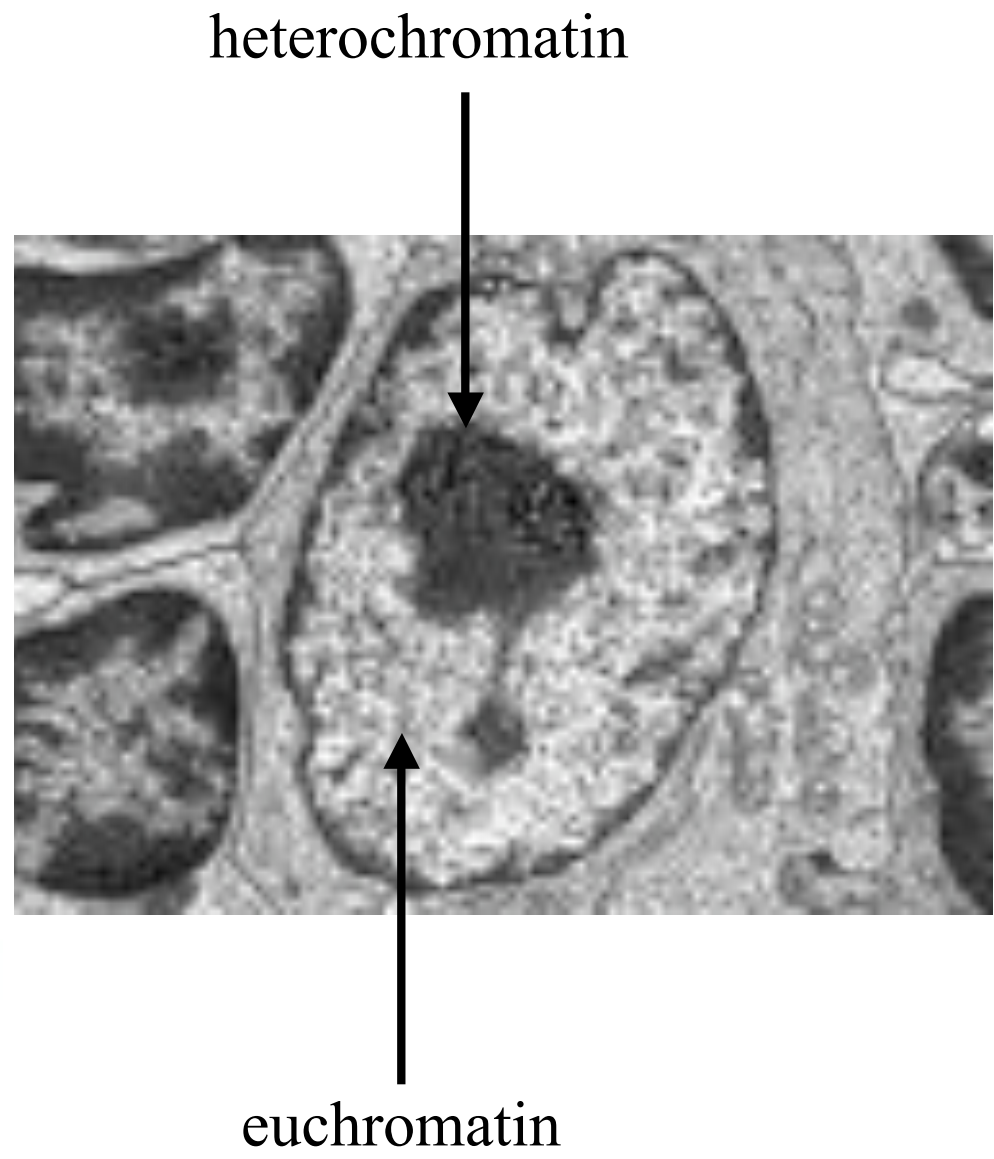
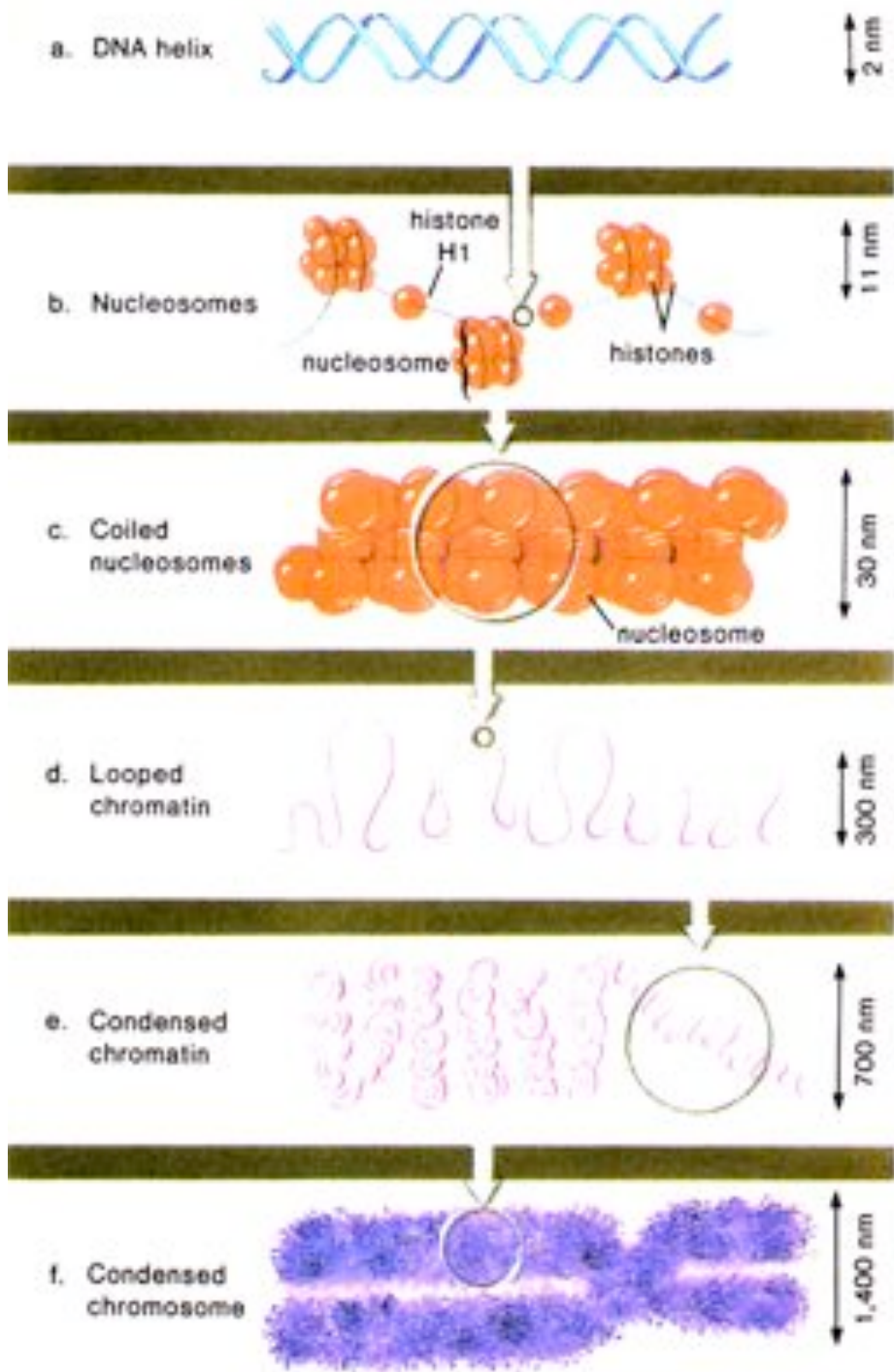
	<i>species</i>	<u>A</u>	<u>B</u>
1. Produced major salivary protein		+/+	-/-
2. Large puff present		+/+	-/-

He crossed A with B (+/-) he got hybrid that produced intermediate amounts of protein. What he saw in the puffing patterns were:

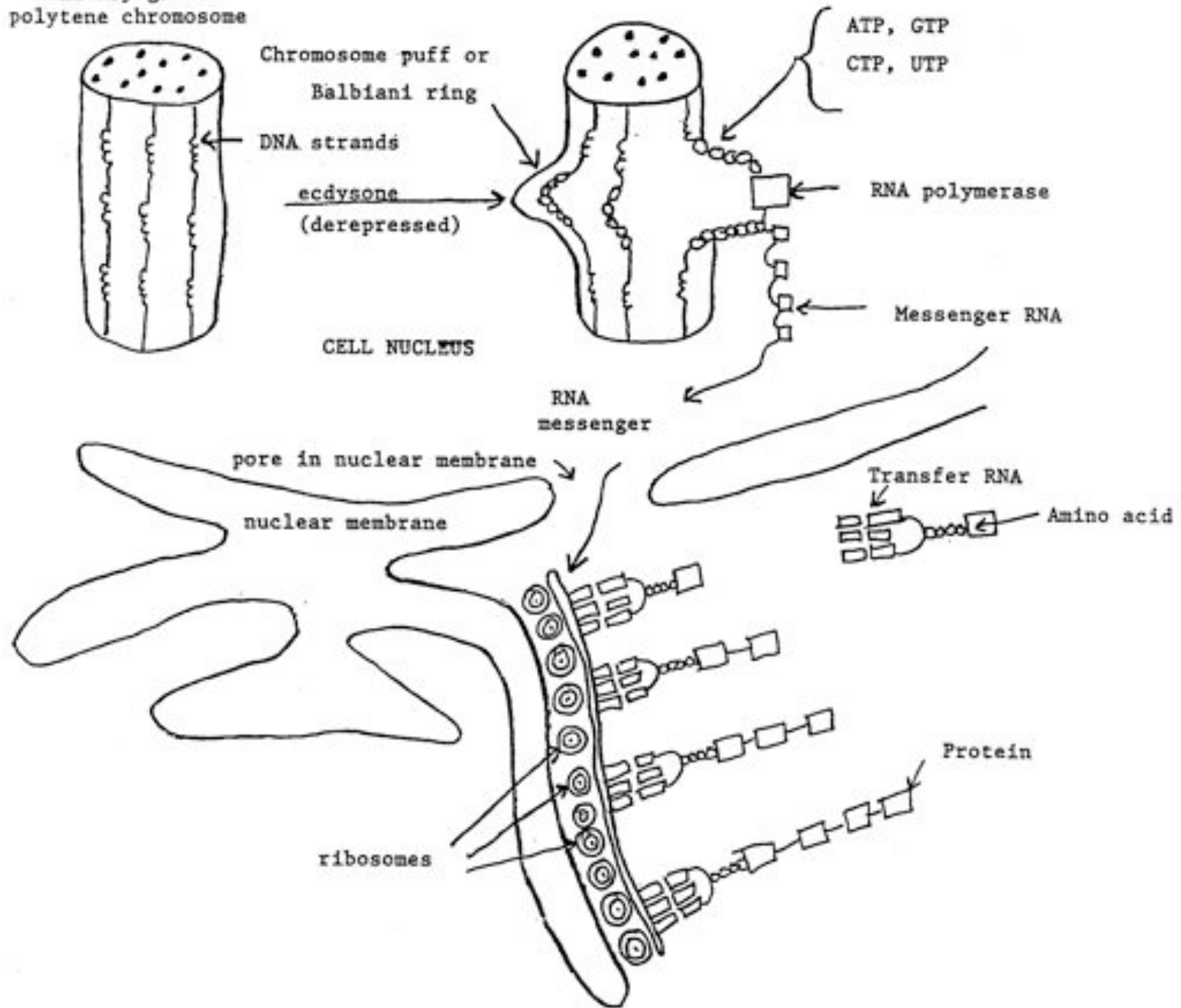
(+/-) x (+/-) crossed he got

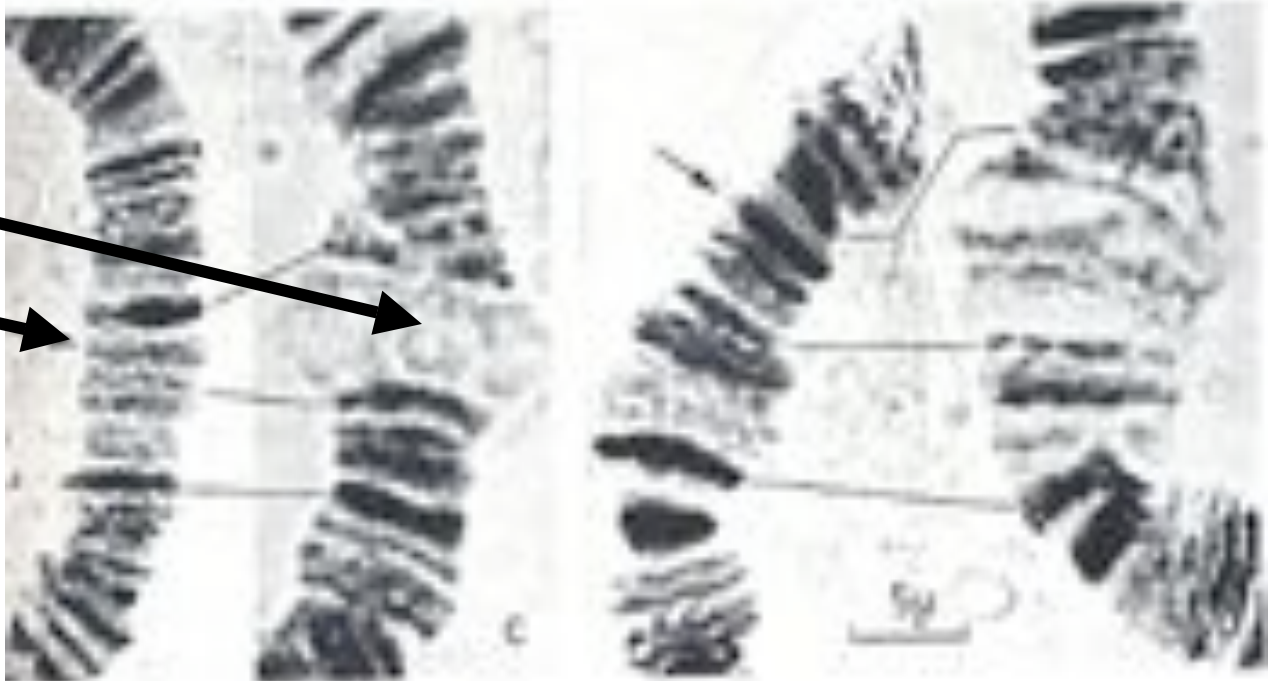
+/+	with 2 puffs at region
+/-	with 1 puff at region
-/-	with no puffs

Using Mendelian genetics he demonstrated that the puff site correlates with production of salivary protein



Salivary gland  
polytene chromosome





Derepressed area

Repressed area





Beckage, N.E. (Ed.) "Parasites and Pathogens: Effects on Host Hormones and Behavior" (Chapman and Hall) (1997).

# **RESEARCH ON THE INSECT NEUROENDOCRINE SYSTEM**

## **I. MODEL SYSTEMS**

- A. Role of hormones on behavior**
- B. Role of hormones on development**
- C. Effects of environmental factors (light and temp. and food) on physiological events acting via the endocrine system**

## **II. APPLIED ASPECTS**

- A. 3<sup>rd</sup> generation pesticides (IGR's or insect growth regulators)**
- B. JH mimics or analogs-use as insect growth regulators (e.g., methoprene)**
- C. Harvesting nature's treasures-anti-corpora allata compound from the common bedding plant (see next slide)**

<http://www.altosid.com/>



Methoprene is an Insect Growth Regulator (IGR), which is the active ingredient in the larvicide Altosid. A larvicide attacks mosquitoes in the larval stage, when they are waterborne and concentrated together, before they emerge as breeding, biting adults. Methoprene's disruption of the mosquito growth cycle allows it to be defined as a bio-rational agent, rather than a conventional pesticide. It specifically targets mosquito larvae, but does not kill them until they reach their next developmental stage, the pupae. This can be key to preserving the natural food chain, since mosquito larvae can be a minor food source for other organisms. In addition, extensive studies have shown that methoprene breaks down quickly in the environment, spares non-target organisms, and poses no hazard to humans.