BEHAVIOR AND PHYSIOLOGY

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Behaviour as physiology

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Entomological Field Station
34a Storey's Way, Cambridge, England

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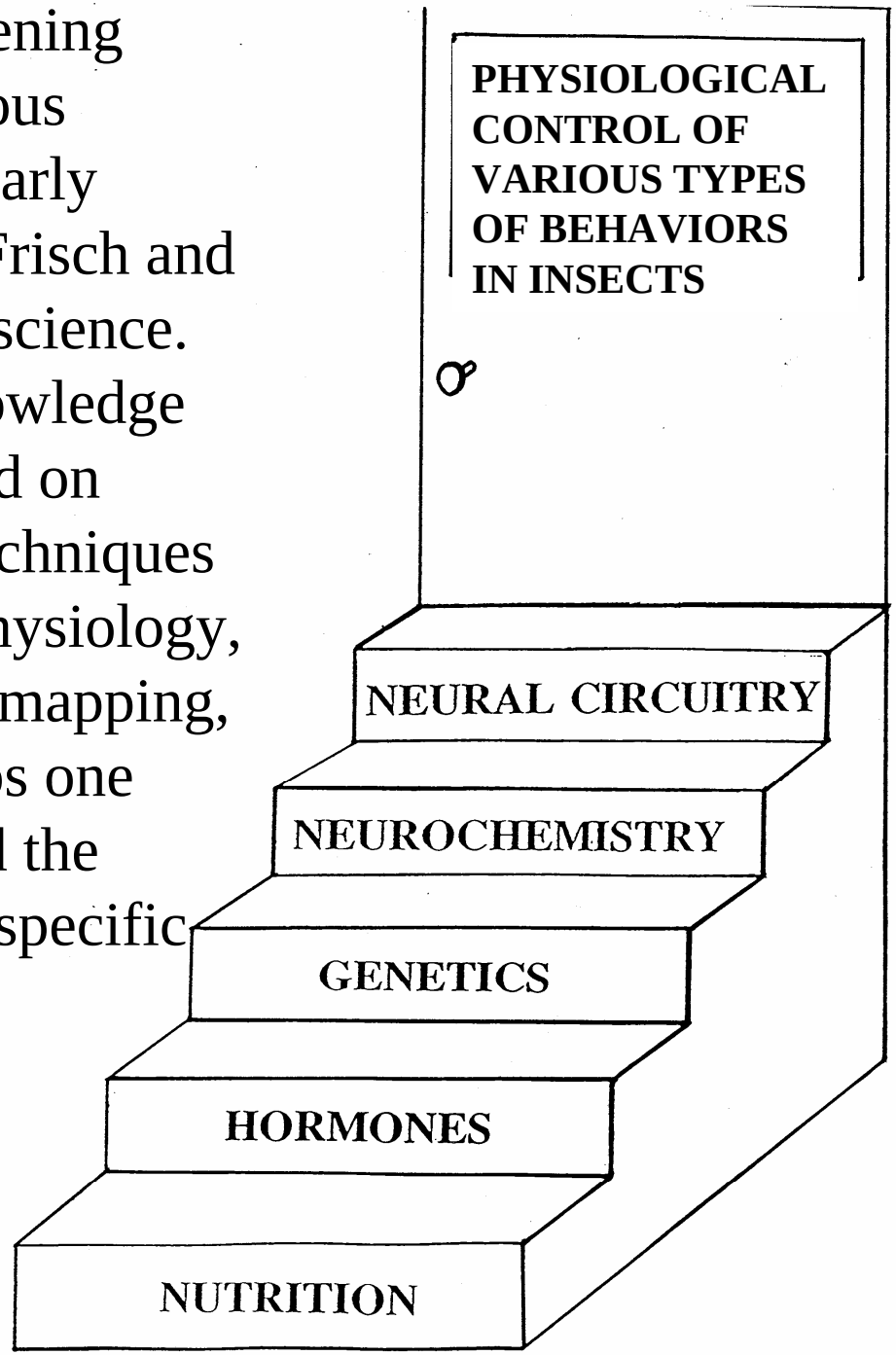
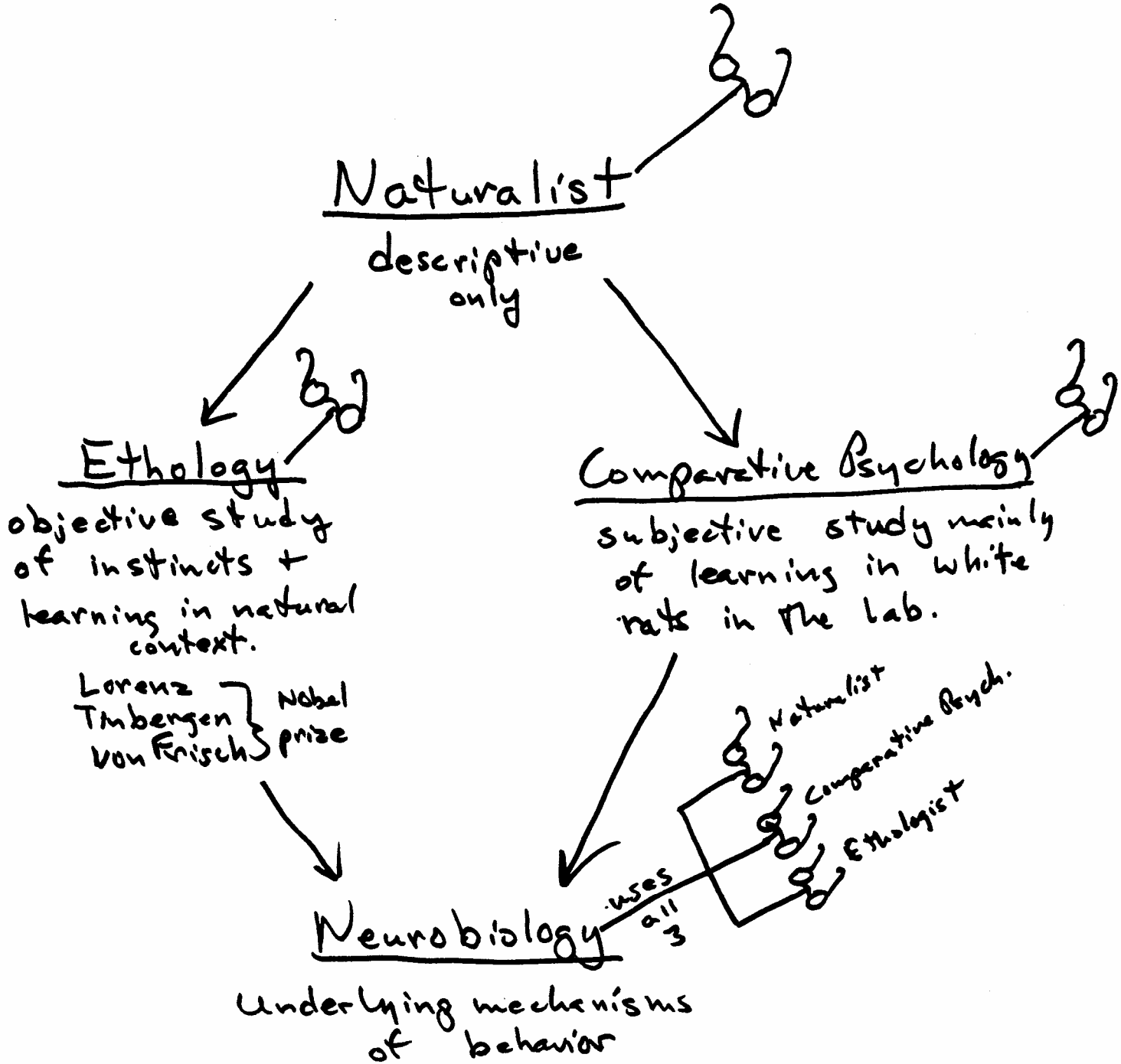
OPENING THE DOOR TO UNDERSTANDING VARIOUS INSECT BEHAVIORS

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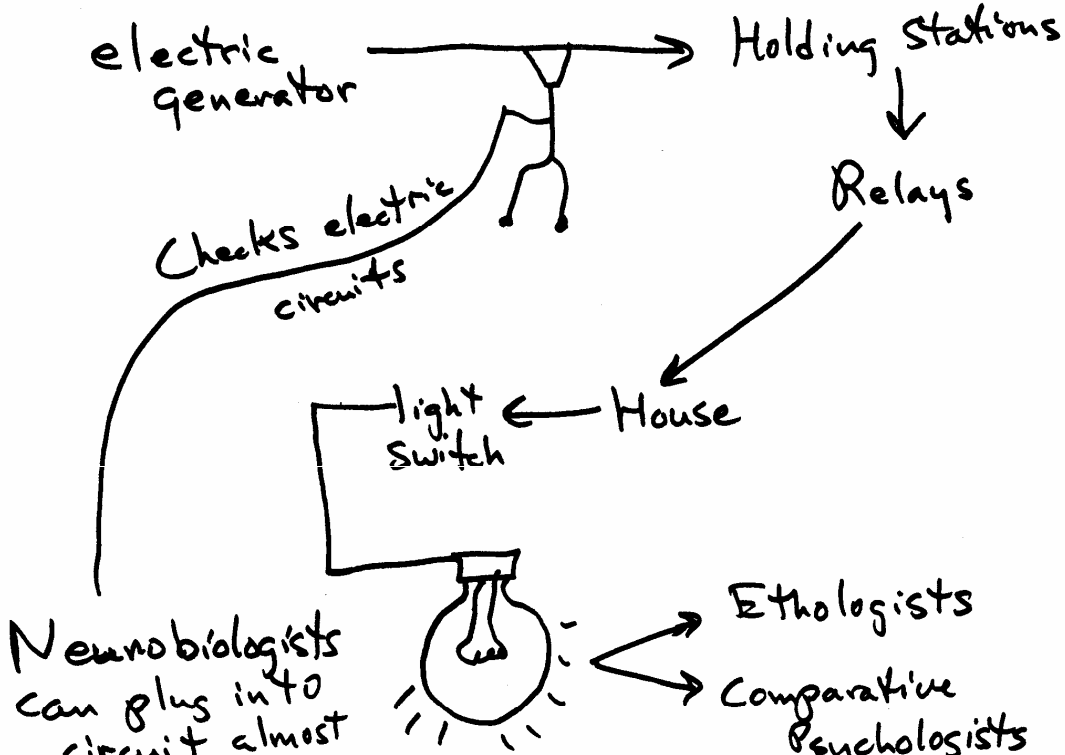
Neurobiology Circuit



Neurobiologists - Dissects out the neural circuitry

Stimulus

↓

Releaser hormone

↓

CNS

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Motor response

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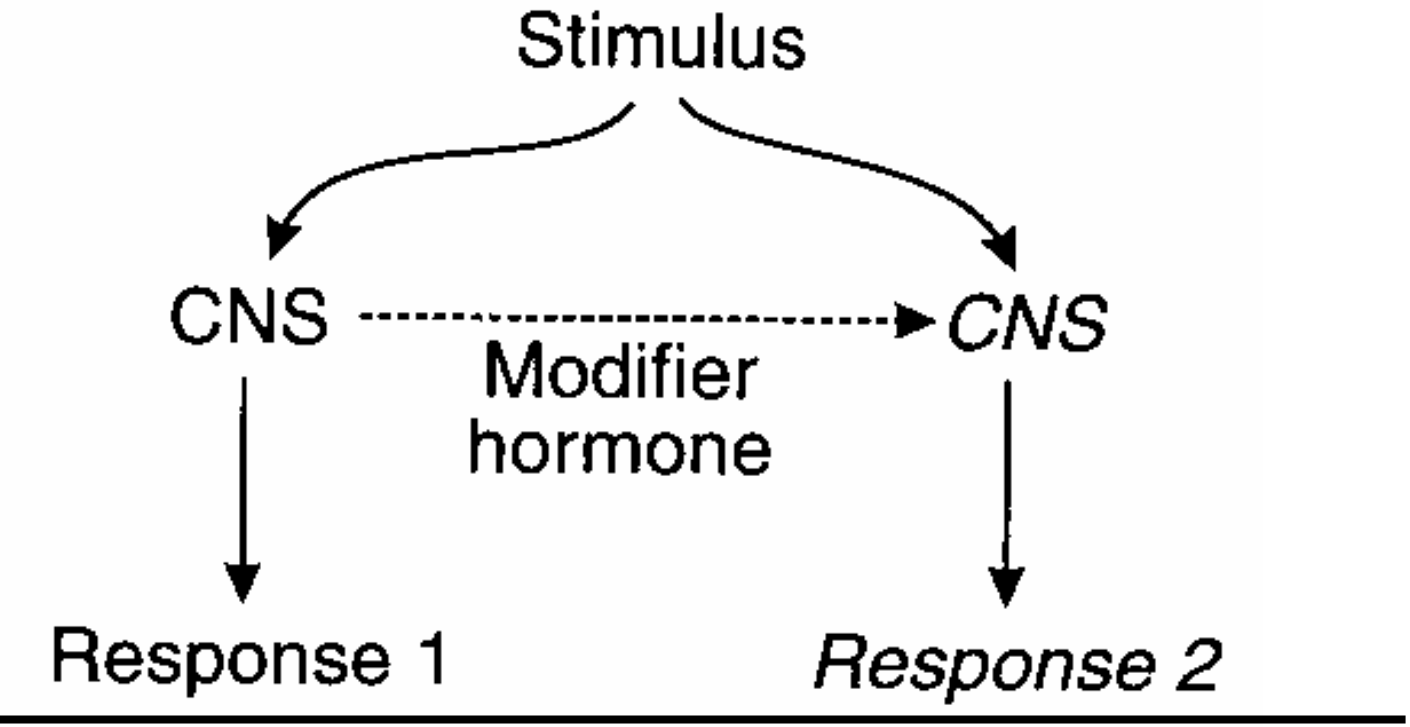

In addition to hormones, biogenic amines such as

1. Octopamine
2. Dopamine
3. Serotonin=5-HT

can act as

a. Neuromodulators
b. Neurotransmitters
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Light production	*Photuris*	OA	Carlson, 1968

OA= octopamine; DA= dopamine; 5-HT= serotonin; MEL= melatonin

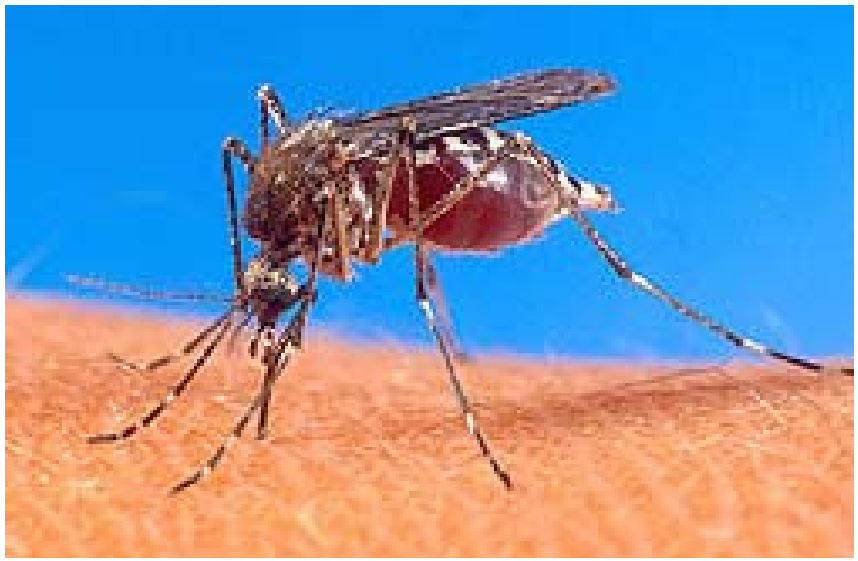
Various behaviors controlled by hormones, neurohormones and/or biogenic animes in insects and various chemicals used by the insect

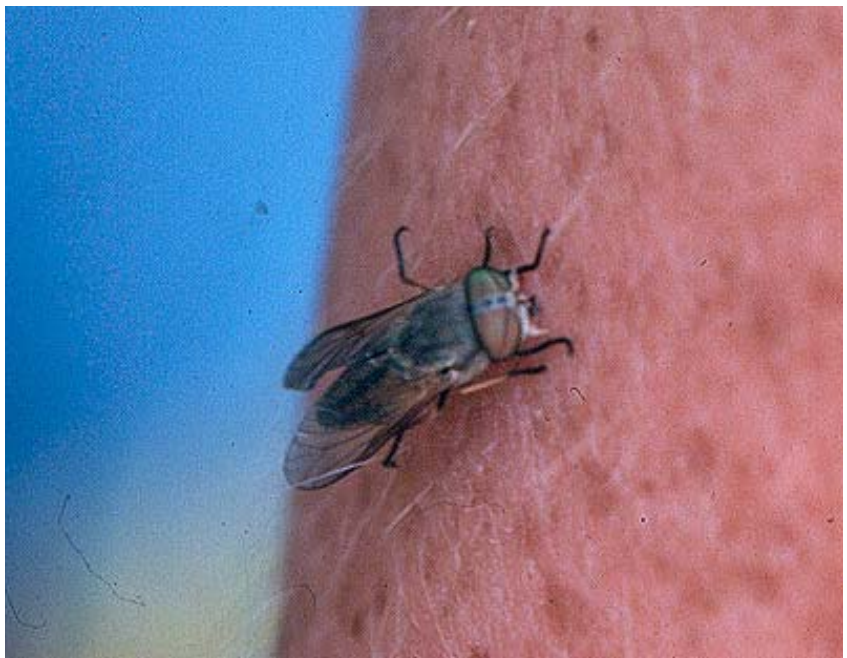
1. Mating
2. Migration and dispersal
3. Host finding behavior
4. Reproductive behavior
5. Social behavior
6. Rhythms and behavior
7. Pheromones and behavior
8. Adaptive behavior

THE 3 BEHAVIORS DISCUSSED IN THIS SECTION ARE:

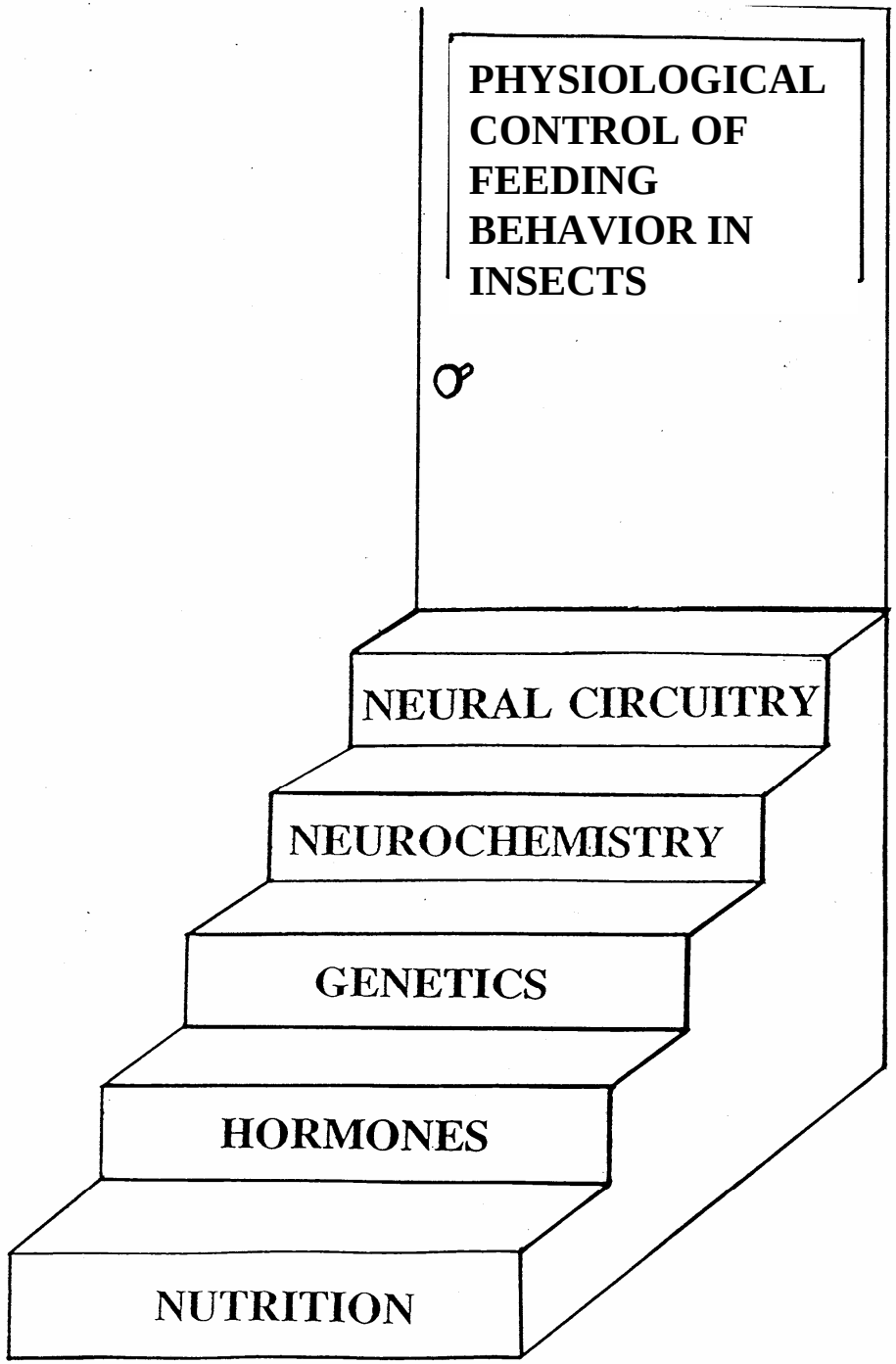
1. FEEDING BEHAVIOR
2. MATING BEHAVIOR
3. ECLOSION BEHAVIOR

FEEDING BEHAVIOR-makes insects economically and medically important

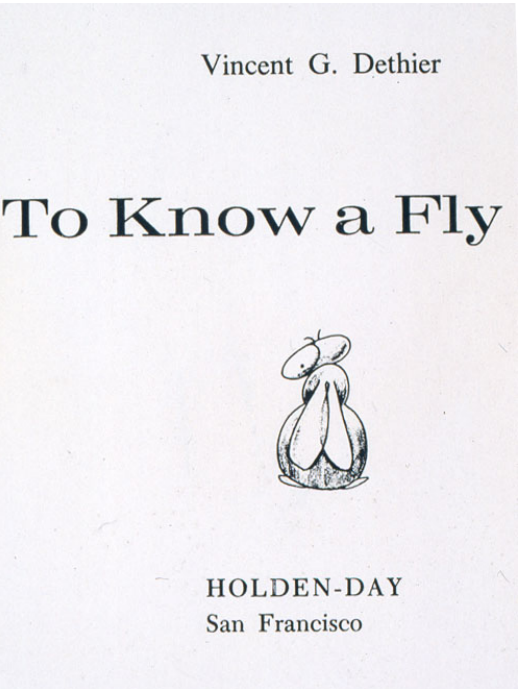




As technology and science advanced it became possible to look at a problem from a different angle. Today, in order to completely understand the problem of feeding behavior one has to draw upon research studies and protocols that involve all of the steps shown in the drawing to the right.



MECHANISMS REGULATING INTAKE AND CESSATION OF FEEDING IN THE BLOWFLY, *PHORMIA REGINA*



Vincent Gaston Dethier pioneered the area of sensory physiology in insects and in l963 published the important book, "*The Physiology of Insect Senses*." He was a genius at making experiments simple and was extremely able at taking his research to the general public. In 1962 he published the popular book, "*To Know a Fly*." He was very adept at bridging the gap between insect behavior, physiology and psychology. He also bridged the gap between studies on feeding in the blowfly and phytophagous caterpillars. In 1975, he published a definitive work on his life work, "*The Hungry Fly*."

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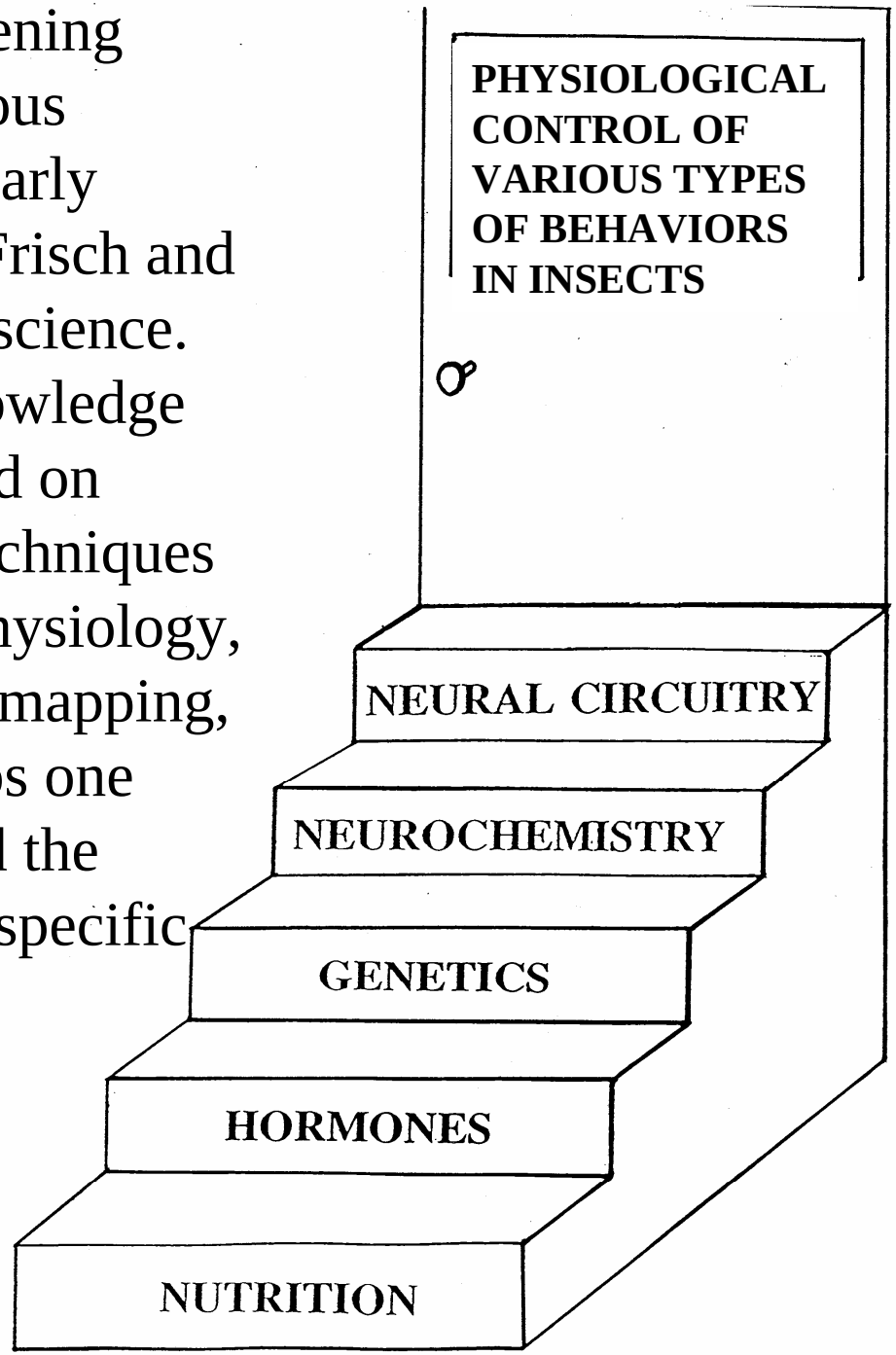

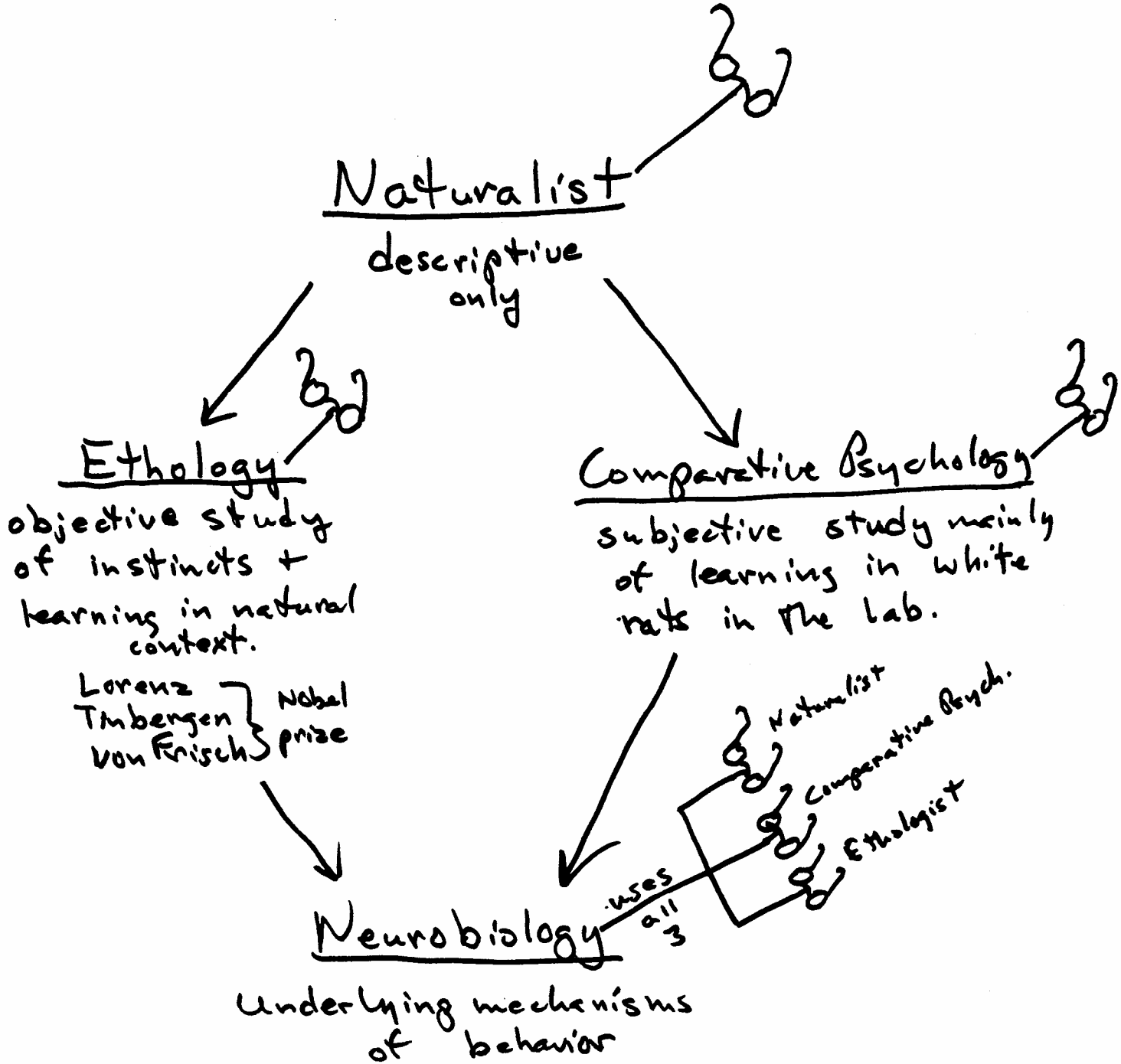
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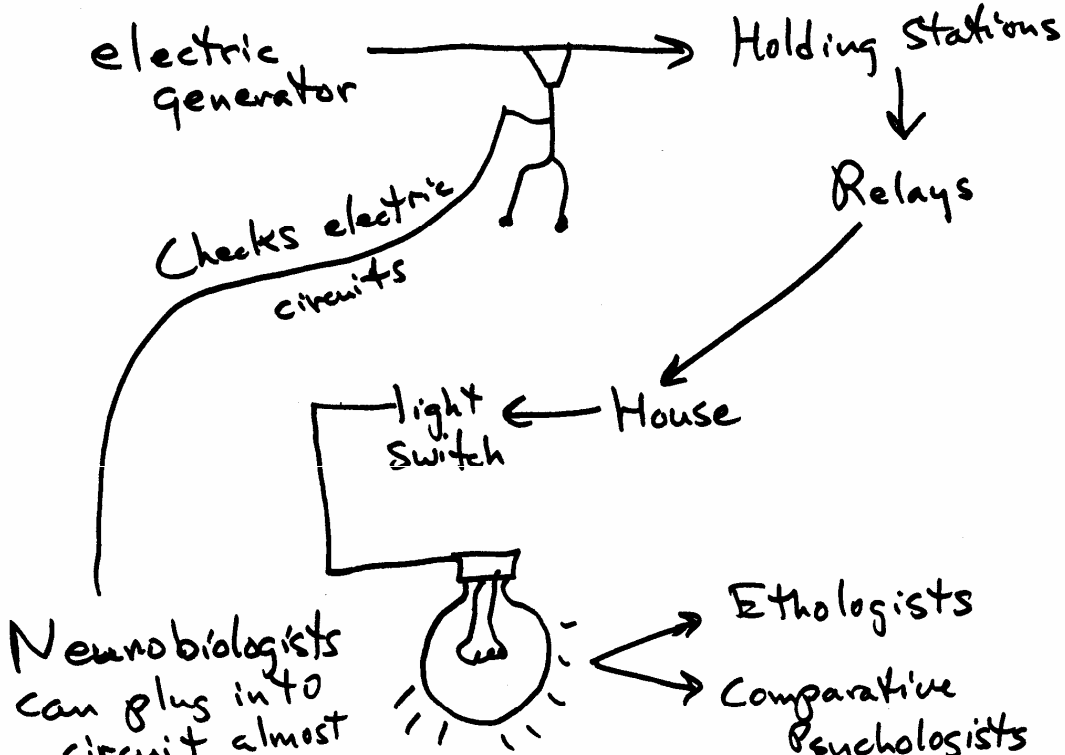

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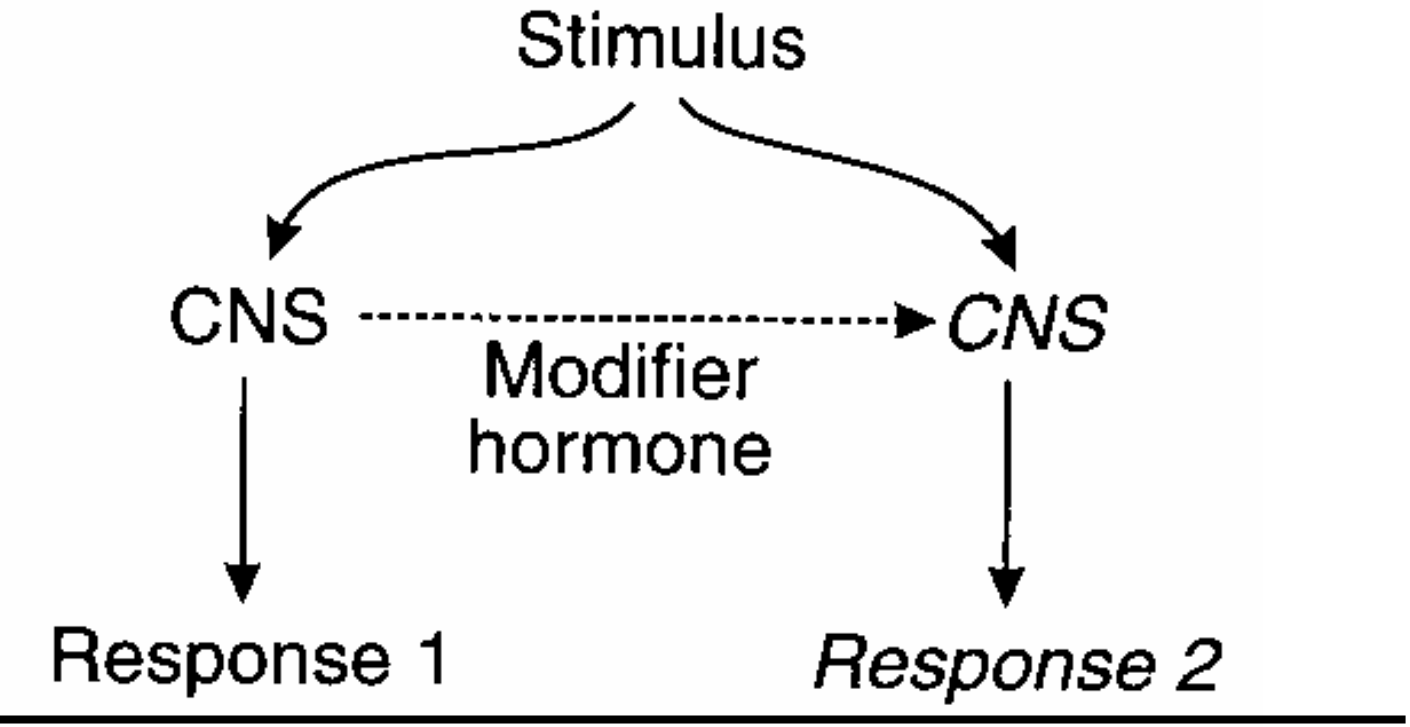

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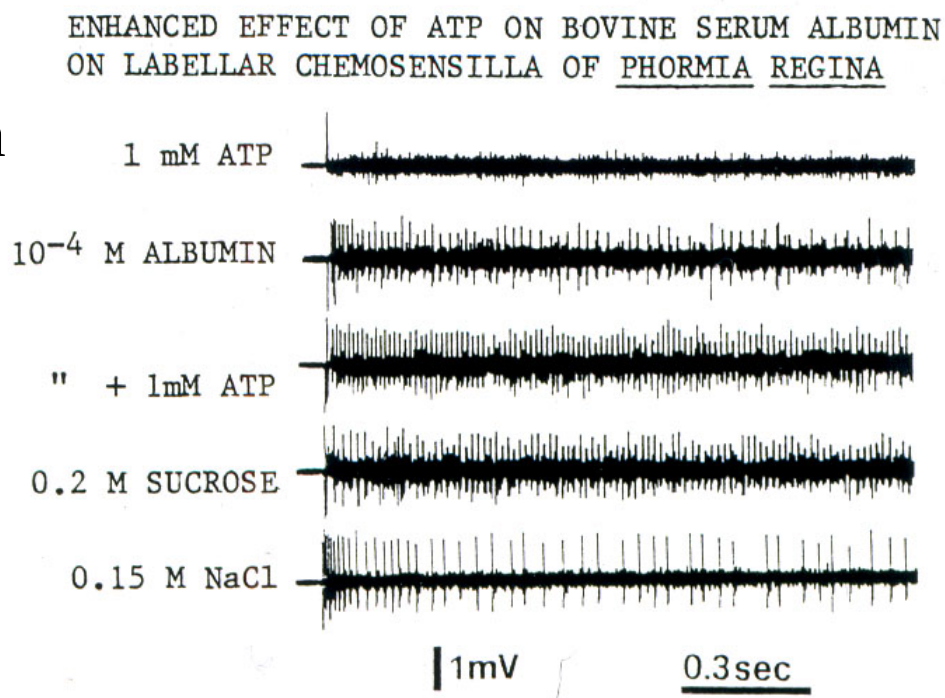
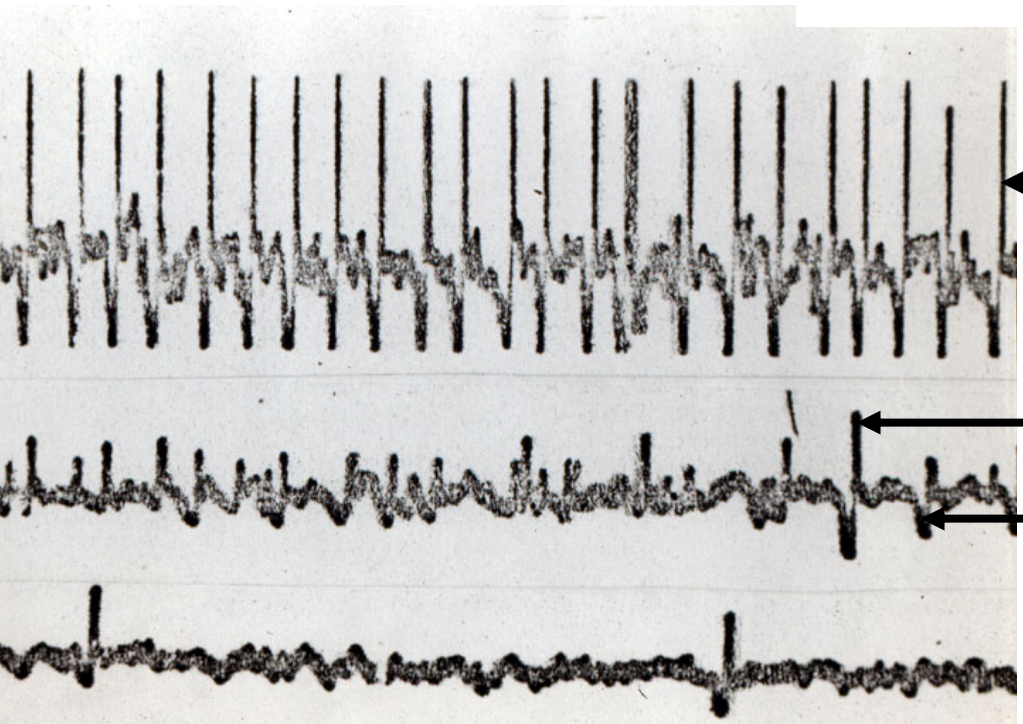
THRESHOLDS

1. Electrophysiological (receptor potential threshold)
    a. Tarsal
    b. Labellar
    c. Interpseudotracheal papillae
2. Behavioral
    a. Recognition
    b. Acceptance
        (1) Mean Tarsal Acceptance Threshold
        (2) Mean Labellar Acceptance Threshold
    c. Rejection
        (1) Mean Tarsal Acceptance Threshold
        (2) Mean Labellar Acceptance Threshold

From each of the 5 bipolar neurons in each chemosensillum one gets a different action potential from each that can be measured by the height of the action potential. The old technique was to hand count these but now computers can do the calculations.





Salt-like action potential or spike

Sugar-like action potential

Water action potential

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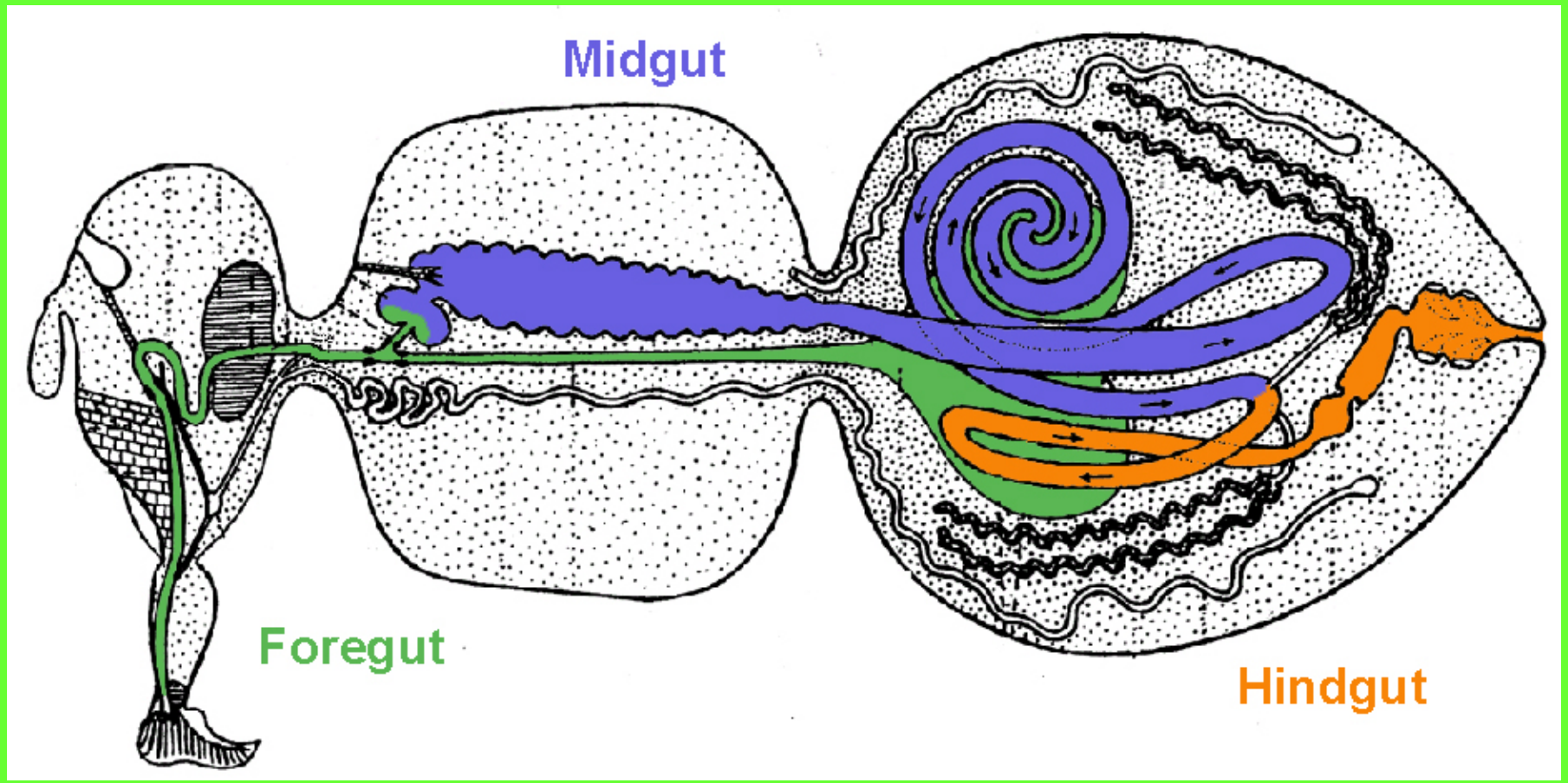
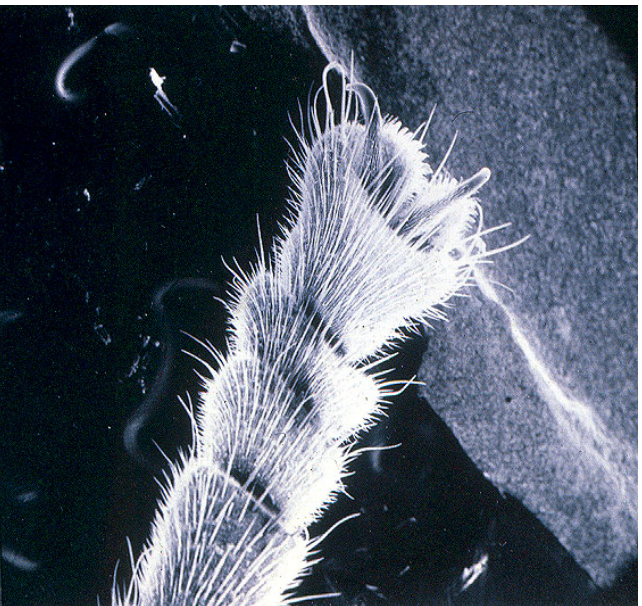
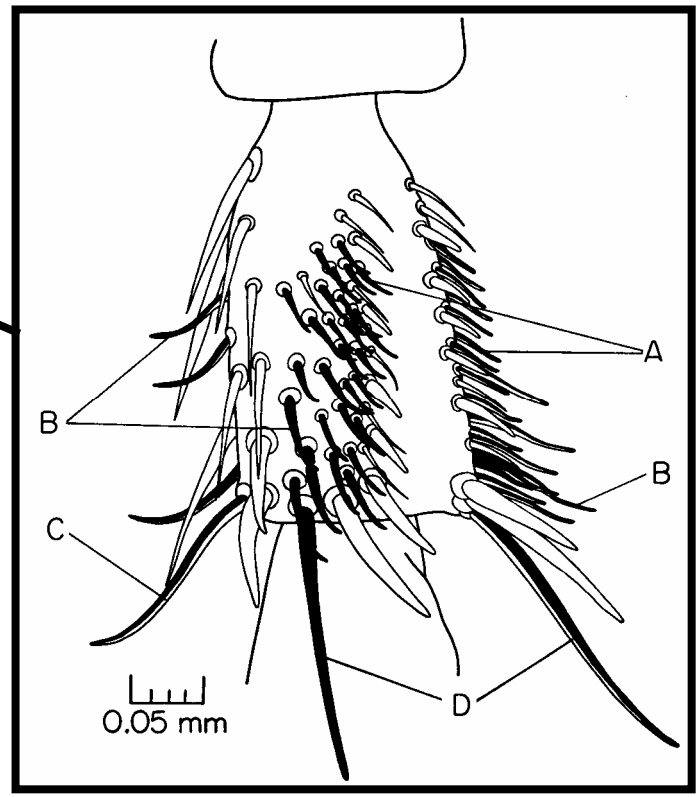
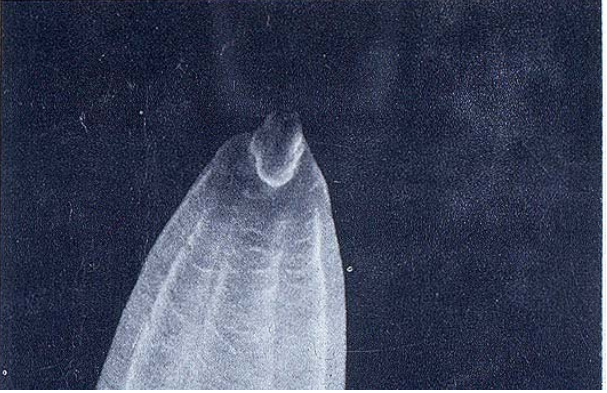
Dipteran alimentary tract

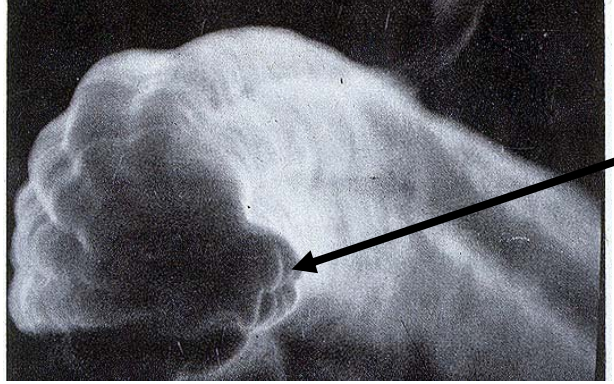


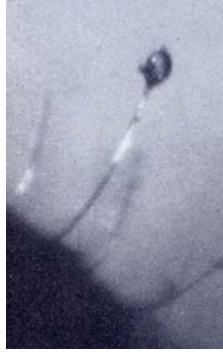
TARSAL CHEMOSENSILLA





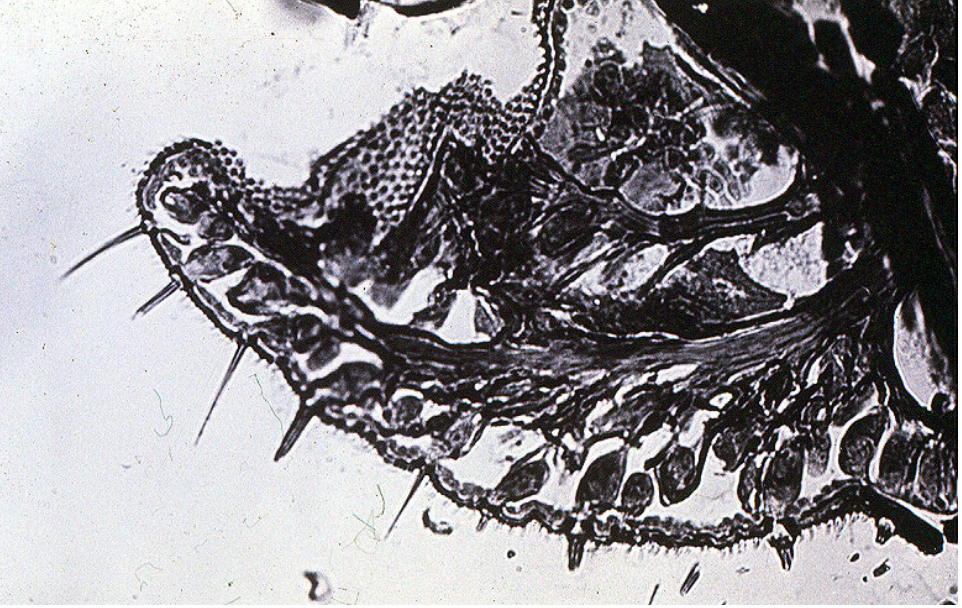




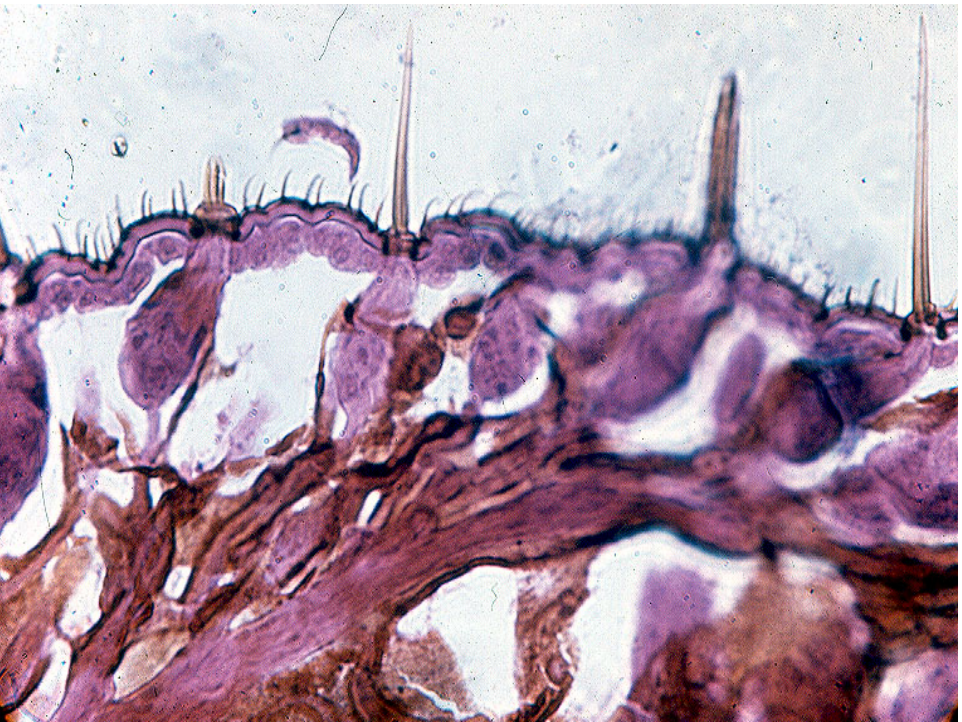


LABELLAR CHEMOSENSILLA

Fig. 39. Details of the structure of a labellar chemosensory hair. The glial cells, tracheoles, and pigmented syncitium are omitted.

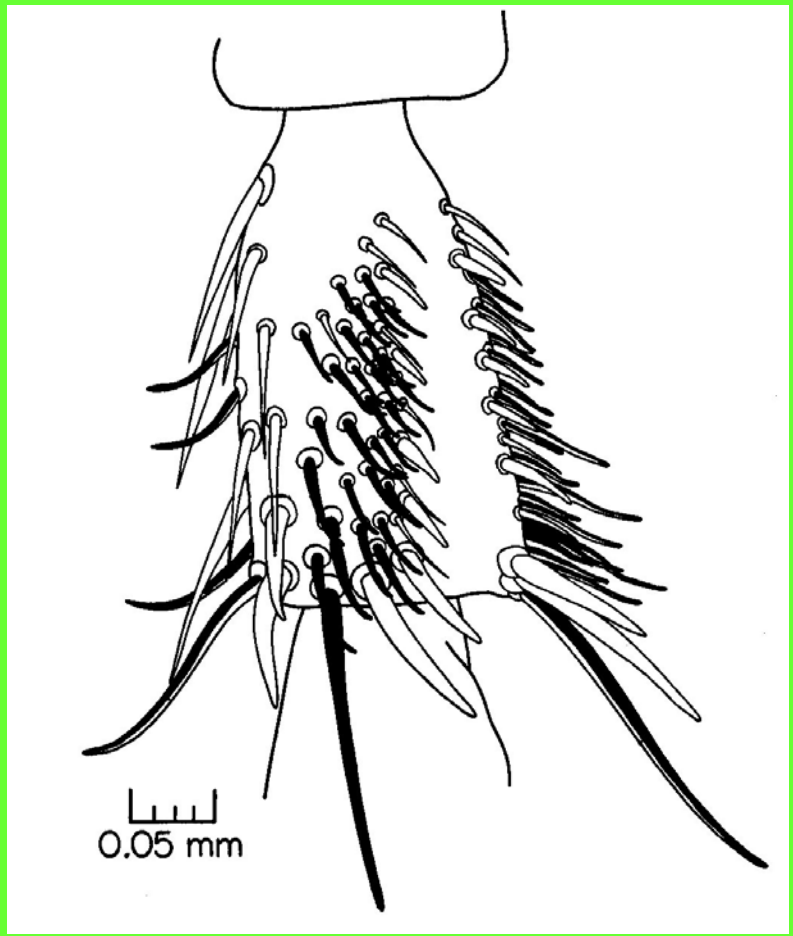


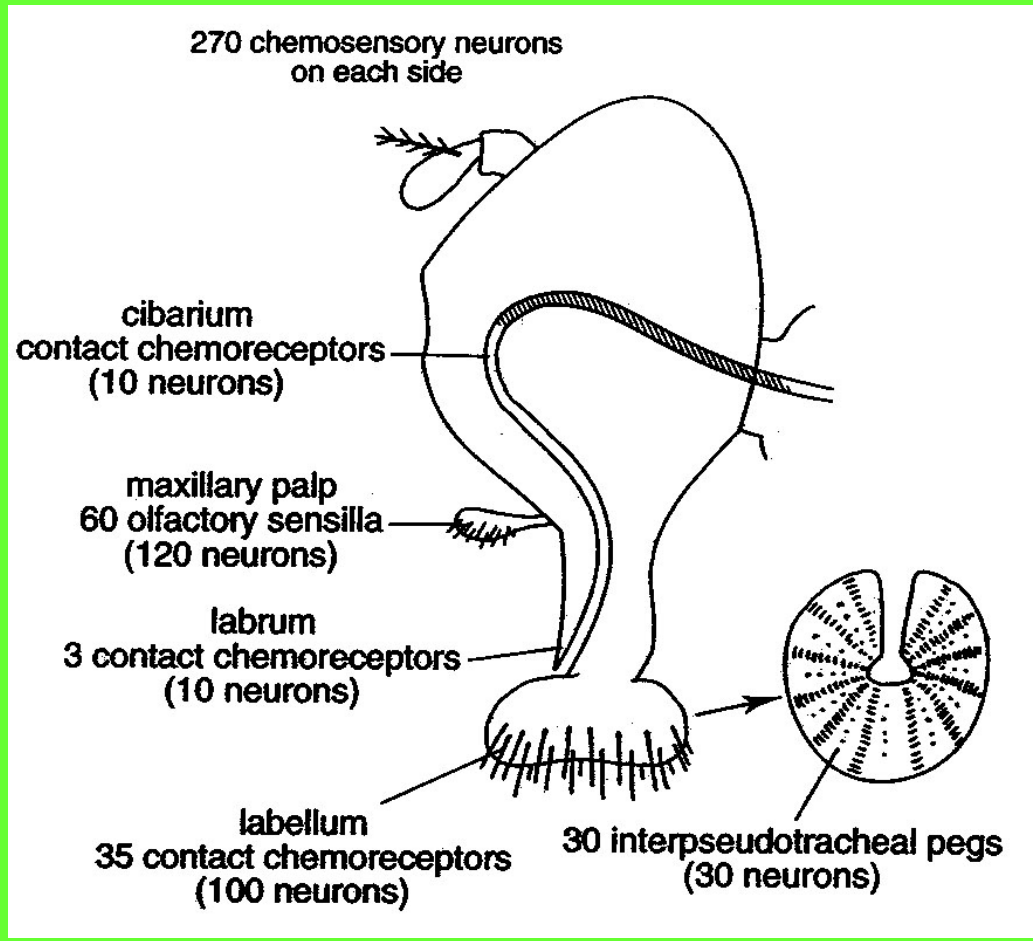
Chemosensilla using histological sections and SEM with freeze fracture (below) showing the socket housing the 5 bipolar neurons and the nerve from that chemosensilla joining to the larger labellar nerves(one on each side).

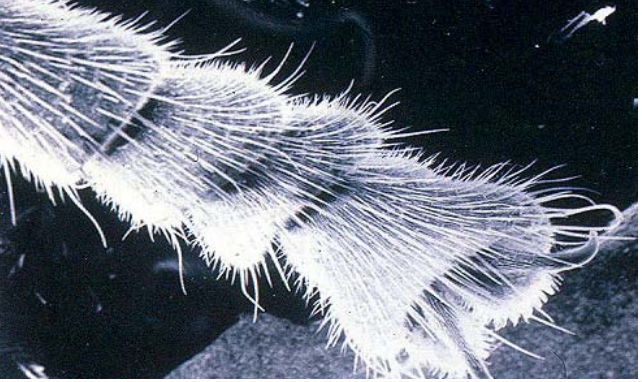


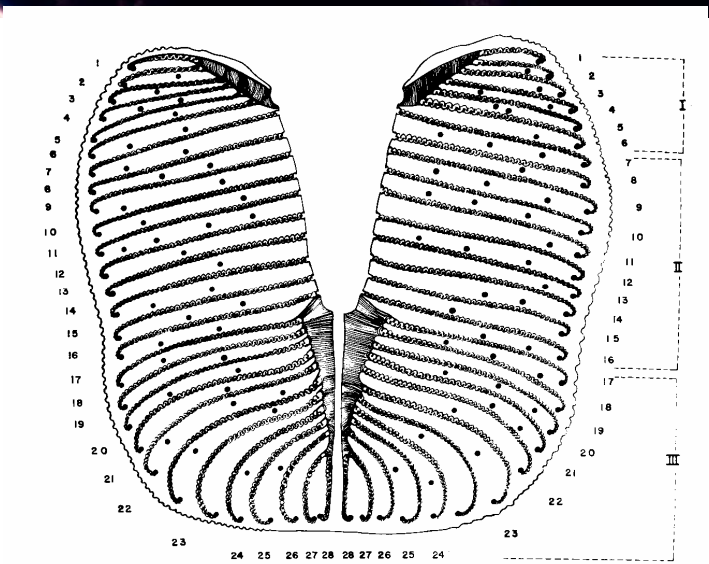
Contact chemoreceptors

- located on: tarsi, labellar lobes, interpsuedotracheal papillae (pegs), cibarium









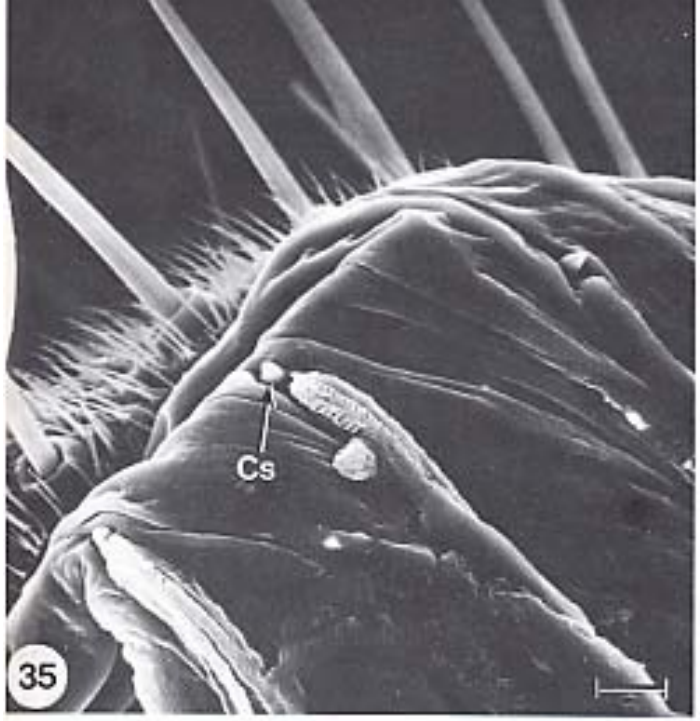
Steps involved in contact with food

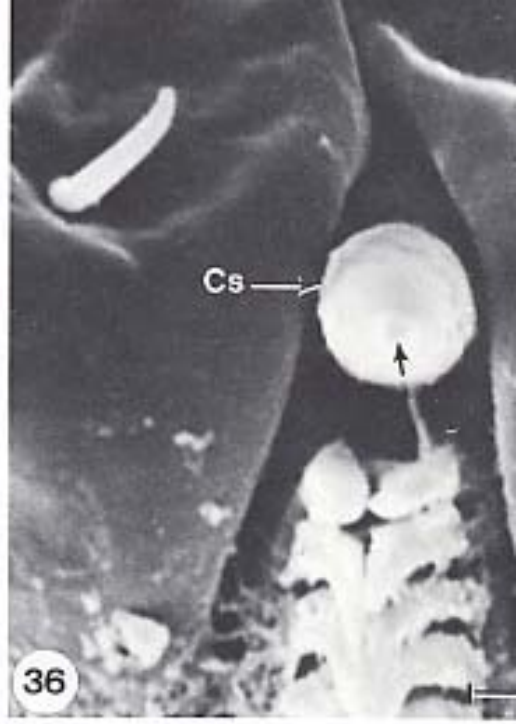
1. Contacts tarsal chemosensillum
2. Proboscis extension
3. Contacts labellar chemosensillum
4. Contacts interpseudotracheal or pseudotracheal chemosensillum
5. Cibarial pump activated
6. Contacts cibarial receptors

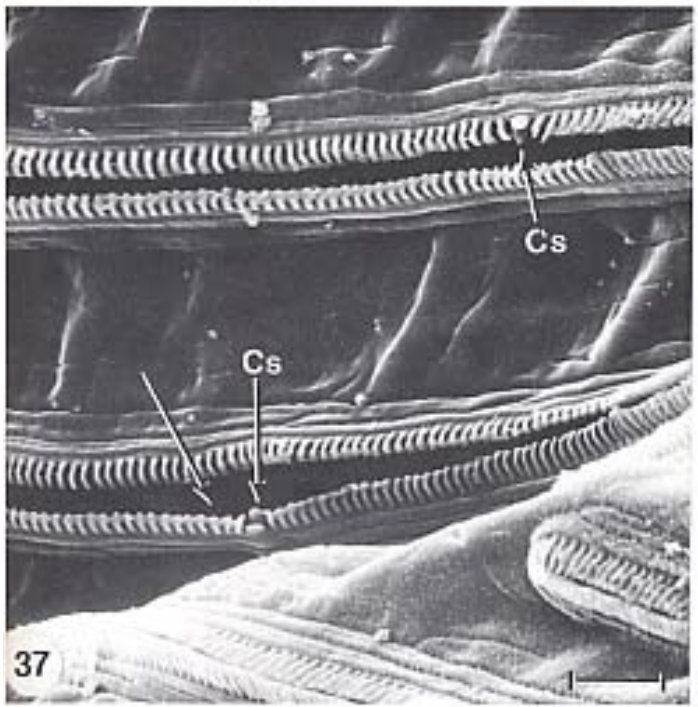
Threshold levels for these sensilla

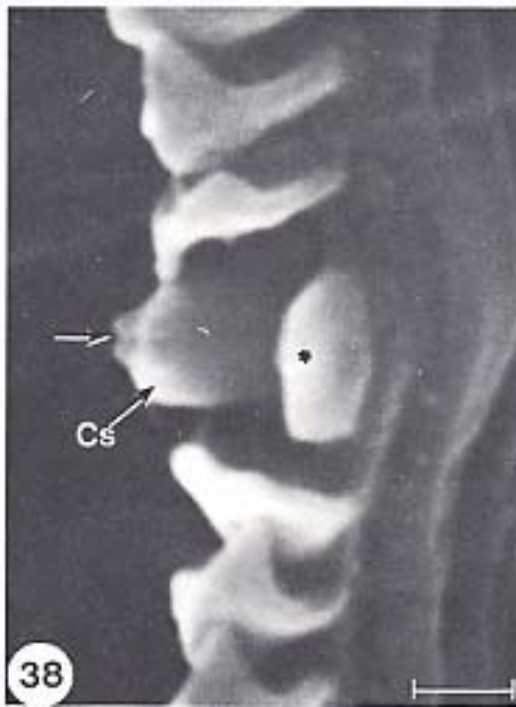
1. Tarsal chemosensilla have the highest and then it decreases in a decending order

Photos to the right are from *Tabanus nigrovittaus.* Note in fig. 35 the long labellar chemosensilla and the small, peg-like chemosensillum at the end of the pseudotrachea. Fig. 36 shows the pore in the peg-like chemosensillum while figs.. 37-38 show the pseudotracheal groove (arrow in fig. 37) that directs the liquid to the mouth opening. Also note the pseudotracheal chemosensilla (Cs) and in fig. 38 the pore in the sensillum.

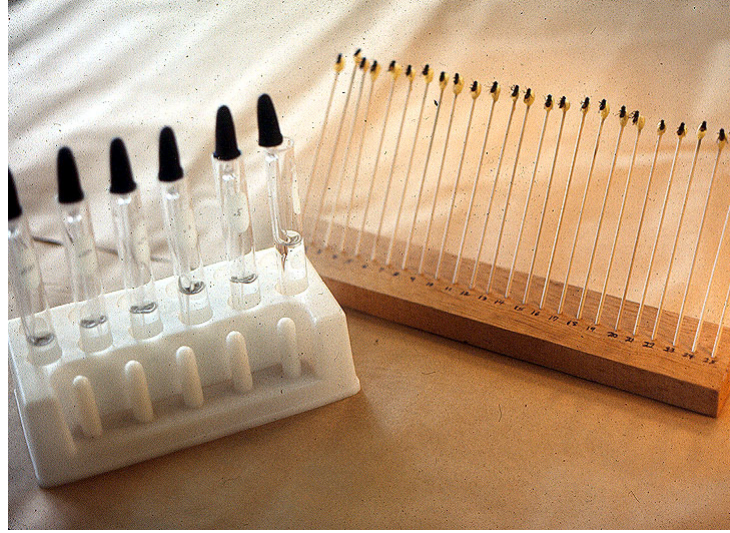




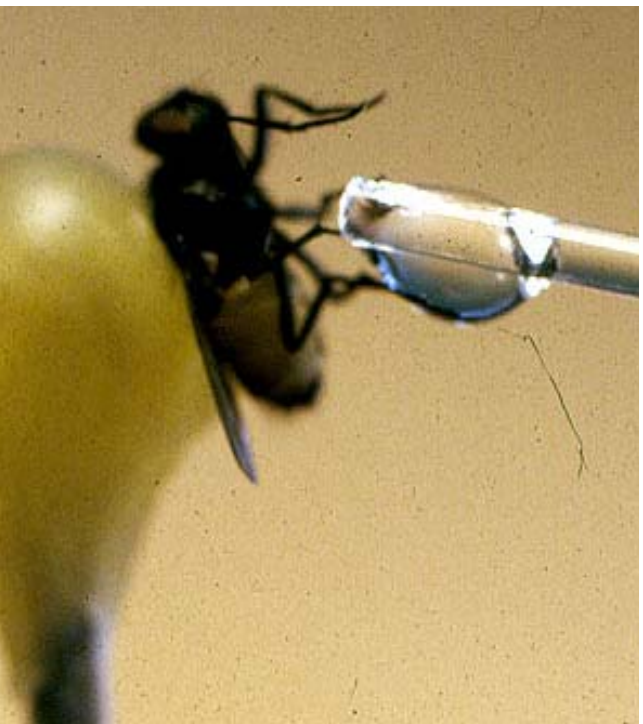




Determining the mean tarsal acceptance threshold (MTAT). Different conc. of sugars are made up and the flies are put on sticks with an adaptation period. First the flies are tested on water. **WHY?** Then they are tested on the lowest conc. until a positive response occurs, which is measured by proboscis extension (middle photo)

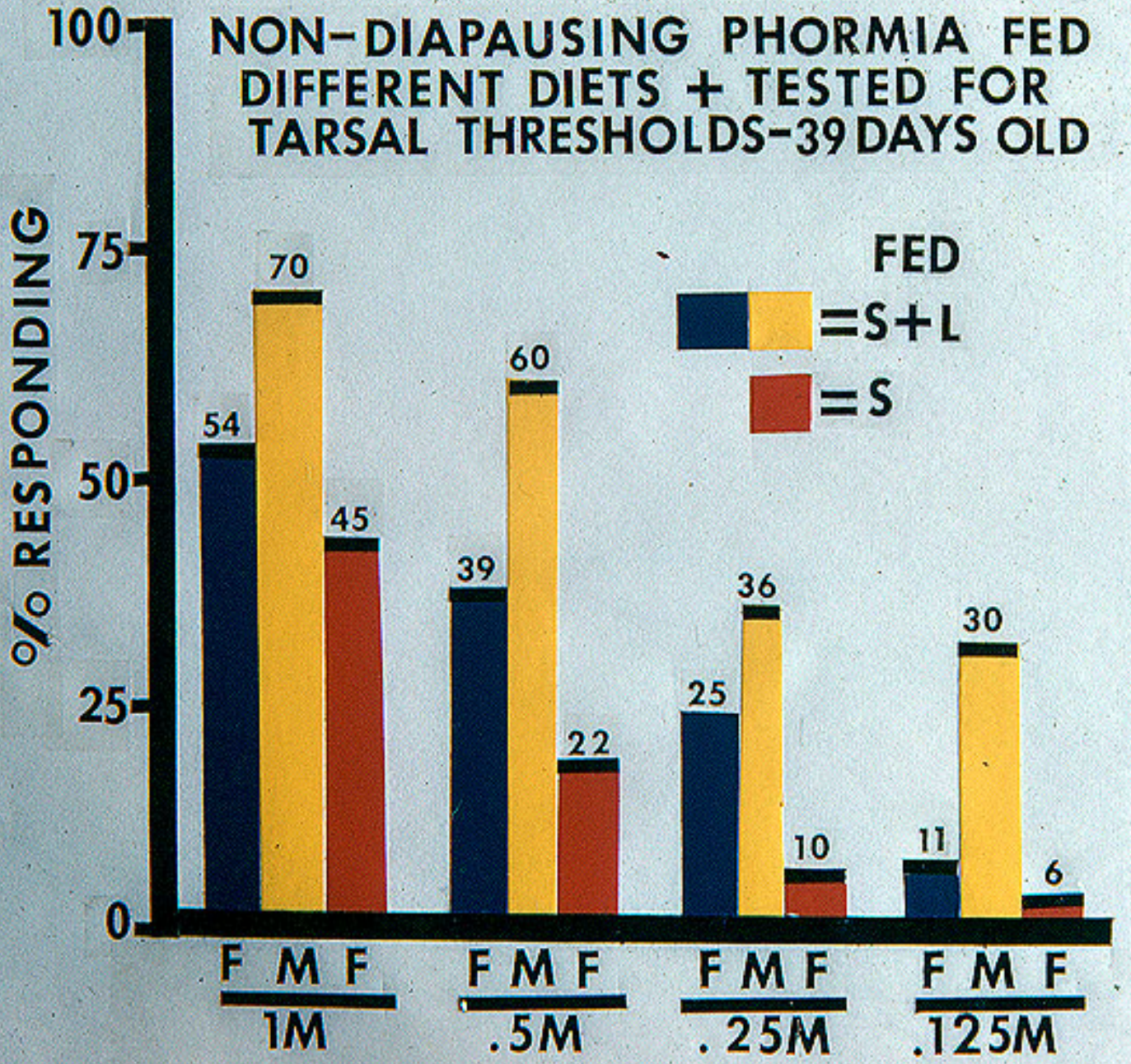


Touch tarsi to solution



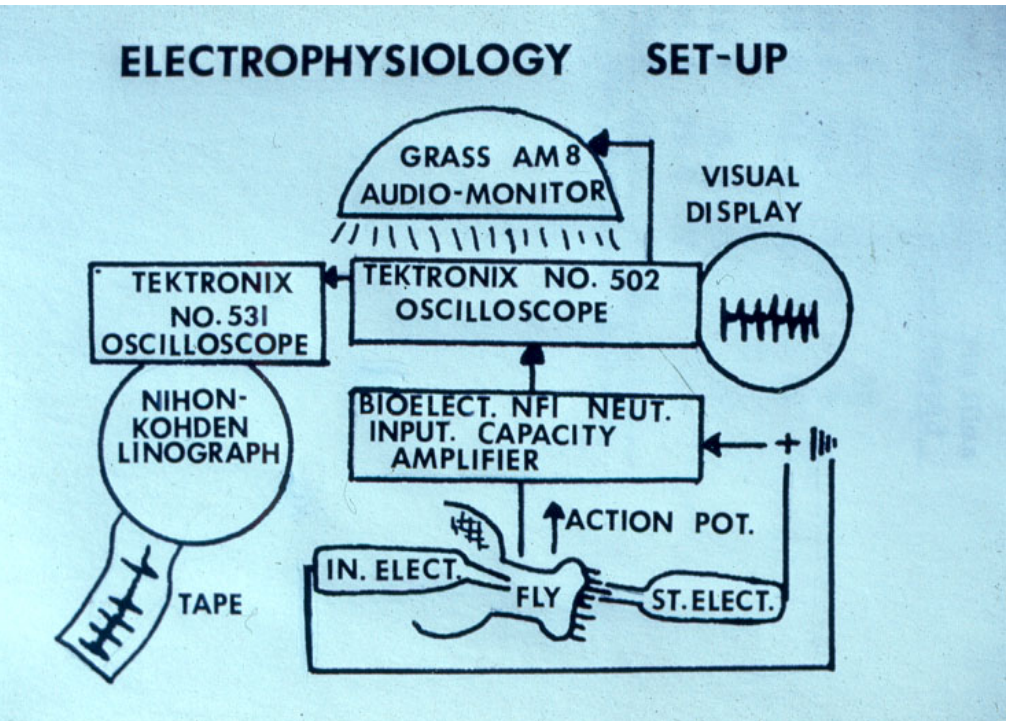
Positive response

Negative response

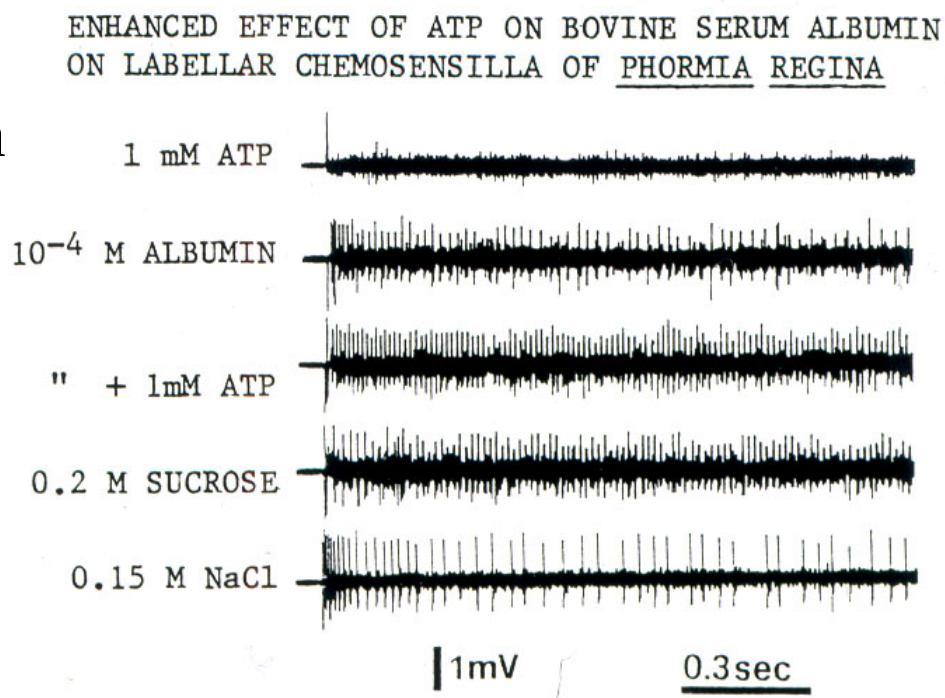

NON-DIAPAUSING PHORMIA FED DIFFERENT DIETS + TESTED FOR TARSAL THRESHOLDS-39 DAYS OLD
% RESPONDING
100
75
50
25
0
FED
=S+L
=S
54
70
45
39
60
22
25
36
10
11
30
6
F M F
1M
F M F
.5M
F M F
.25M
F M F
.125M

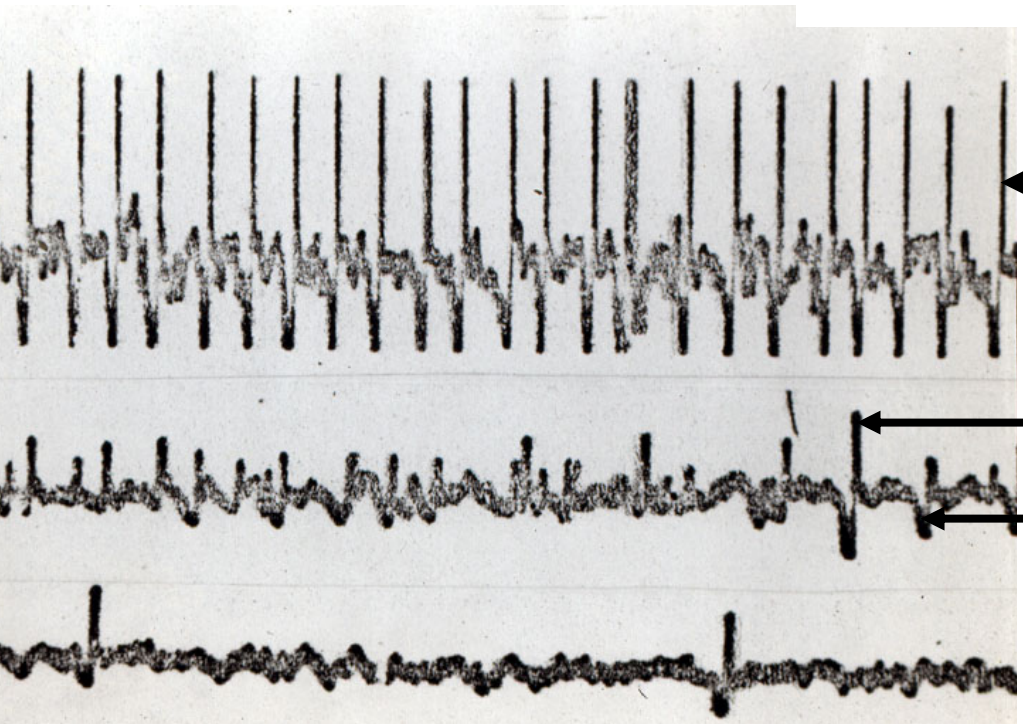
Electrophysiological thresholds and recordings

By starting with very a very dilute solution and gradually increasing the concentration. The point at which action potentials are produced is the electrophysiological threshold concentration.



From each of the 5 bipolar neurons in each chemosensillum one gets a different action potential from each that can be measured by the height of the action potential. The old technique was to hand count these but now computers can do the calculations.





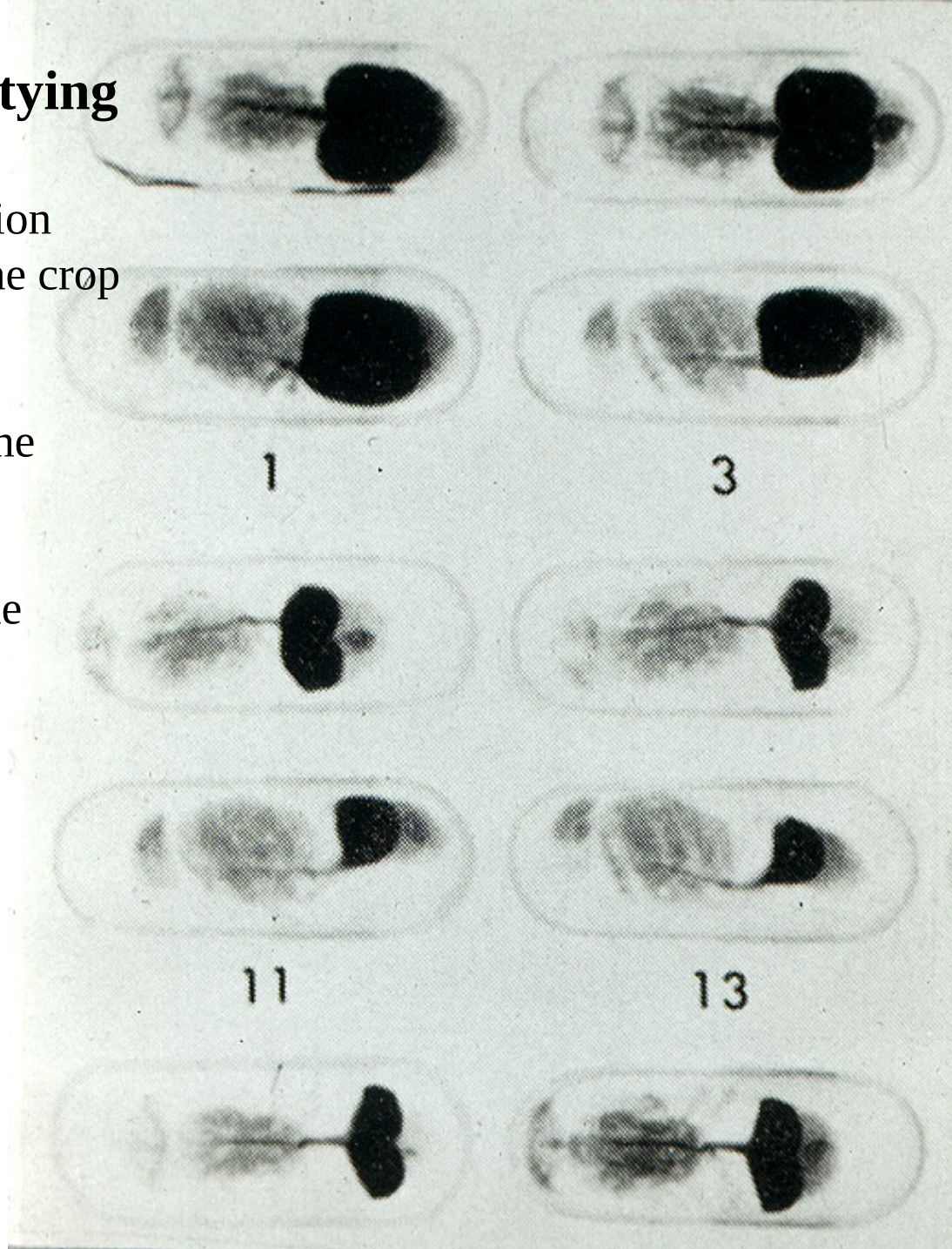
Salt-like action potential or spike

Sugar-like action potential

Water action potential

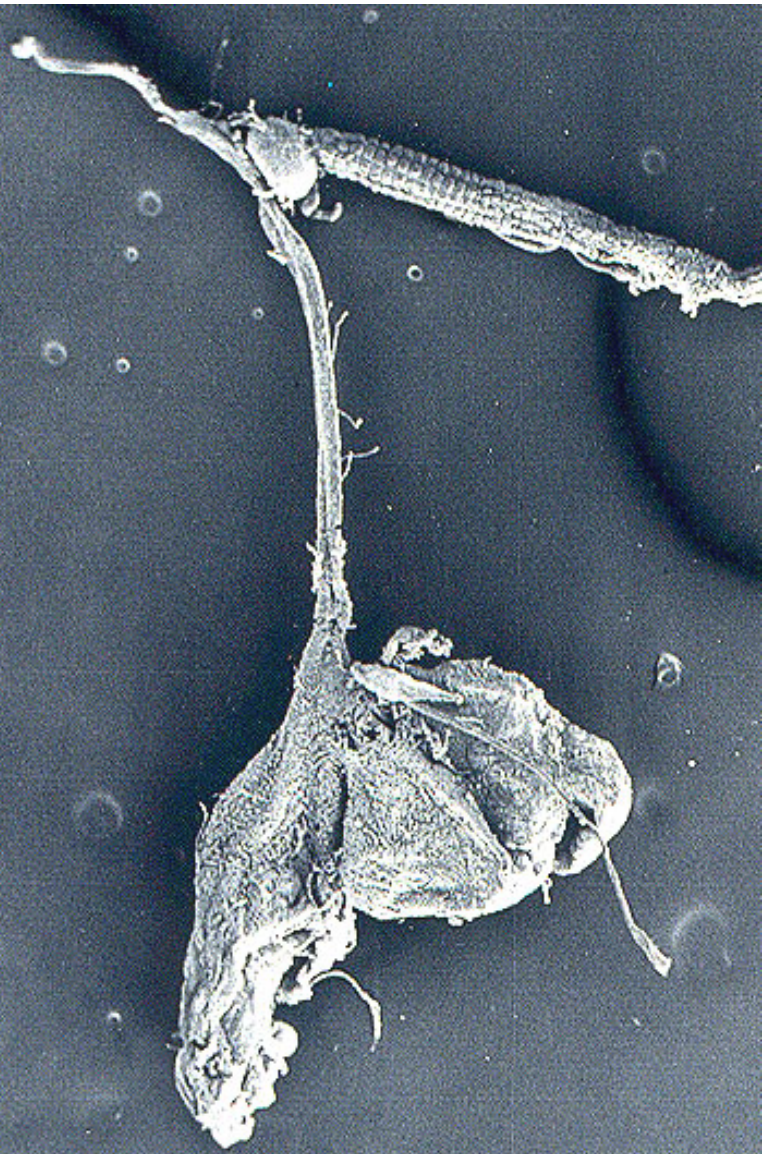
Factors influencing crop emptying

1. Concentration of the imbibed solution
   a. More concentration the slower the crop empties, vice versa
2. Hemolymph sugar concentration
   a. The higher the concentration is the slower the crop empties
3. Fly activity
   a. The higher the activity the fly, the faster the crop empties



Diversion of different solutions in the digestive system of *P. regina*

a. Water to midgut
b. Protein to midgut then to crop
c. Sugar to crop and from there the midgut



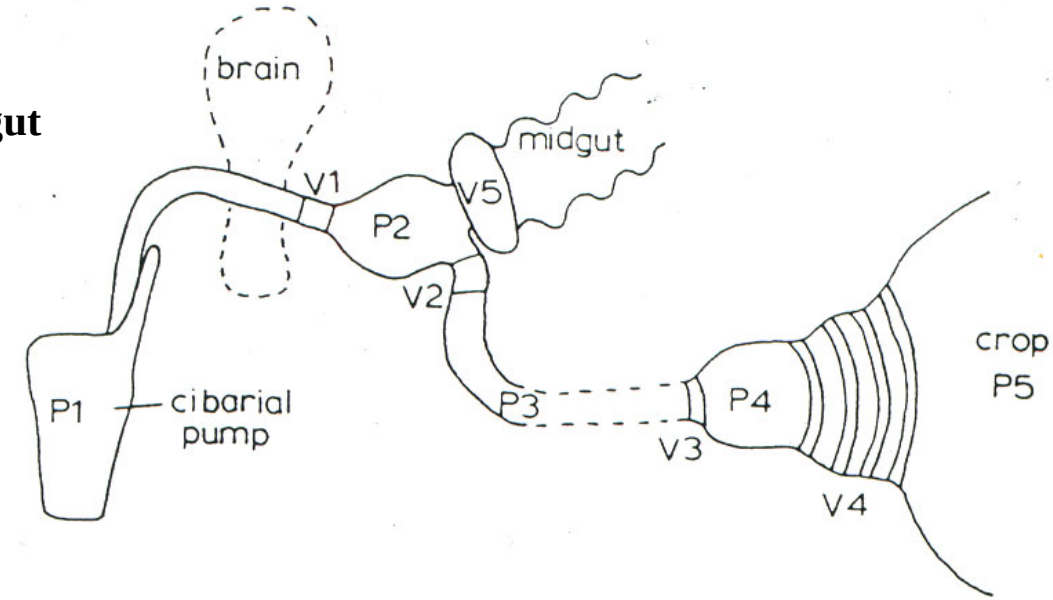
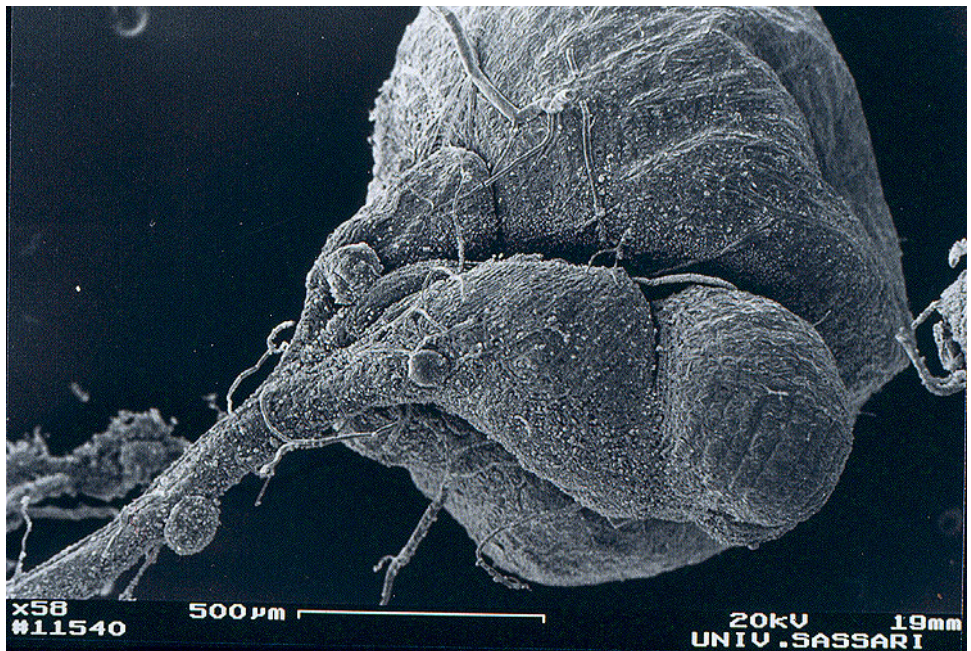


FIG. 1. The anatomy of the foregut of *P. regina* (from Thomson 1975*b*).

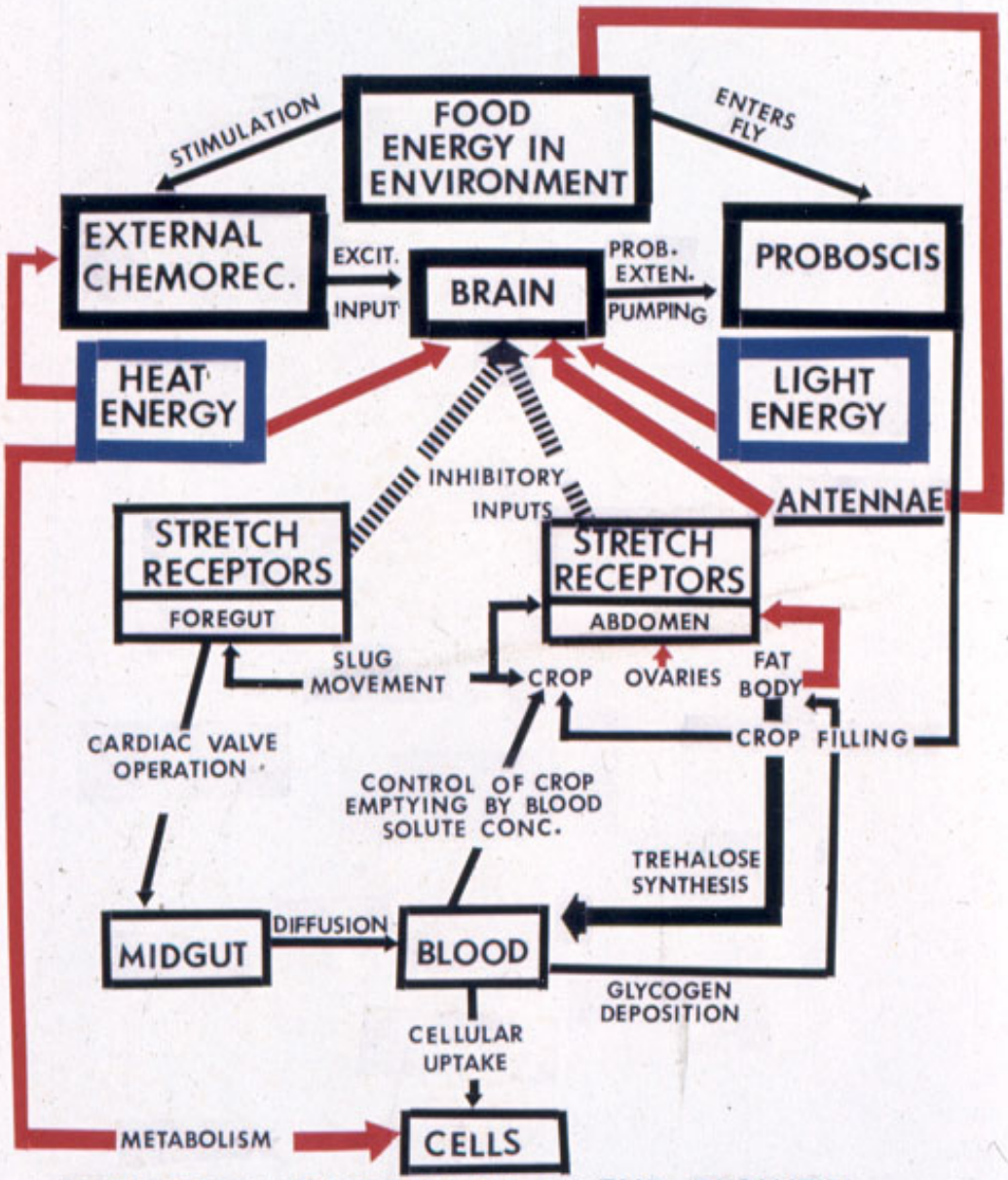


Early generalized scheme of food in *Phormia regina*

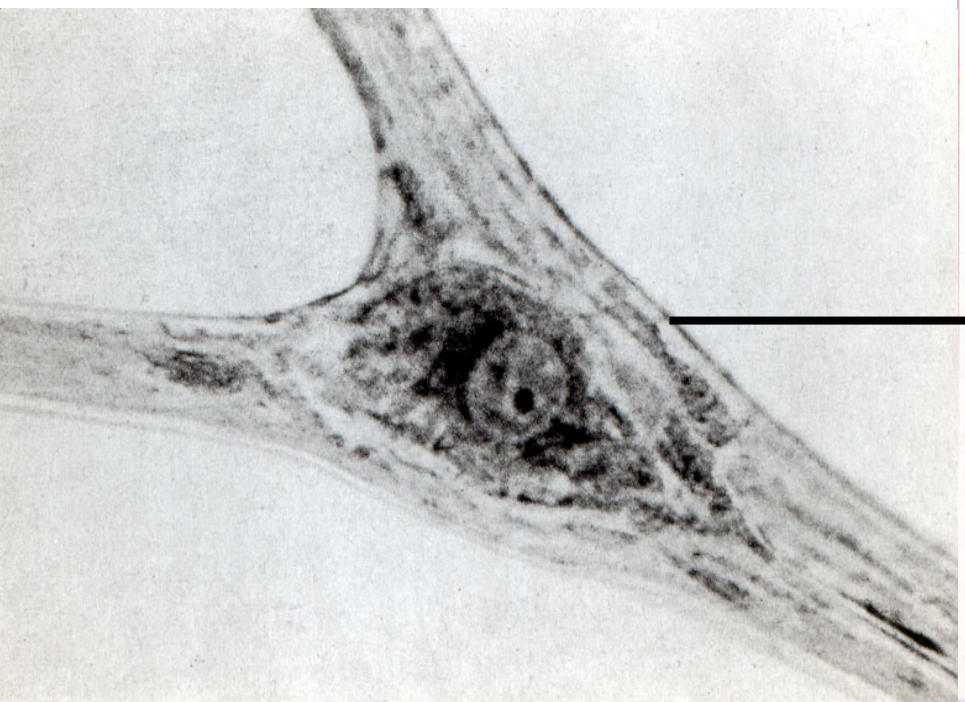
1. Chemosensory inputs are provided by the peripheral chemosensilla located on the tarsi and labellum.

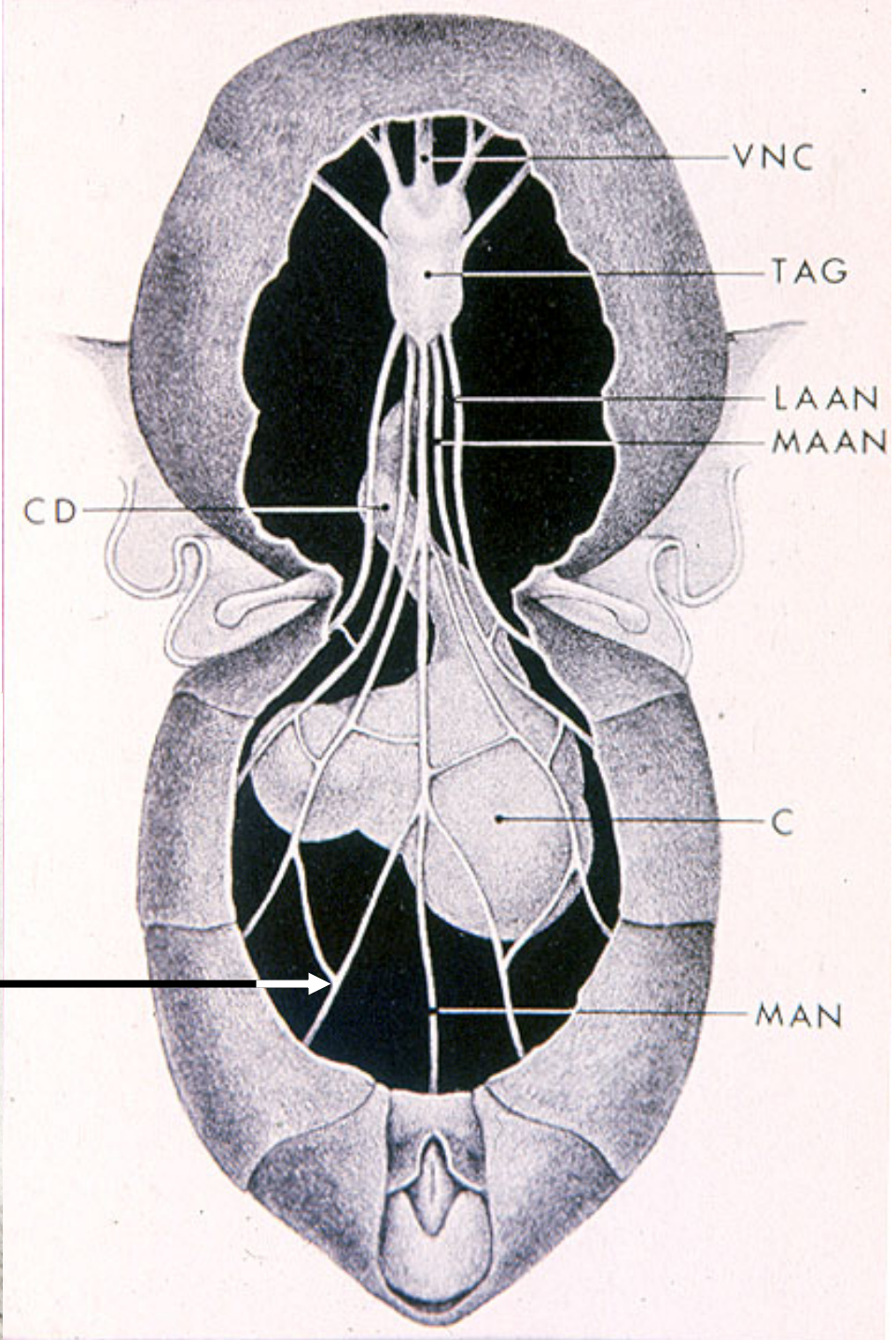
2. Feedback from a full crop and crop slugs of liquid into the esophagous send negative inputs via stretch receptors that elevates the peripheral thresholds, thus cessation of feeding.

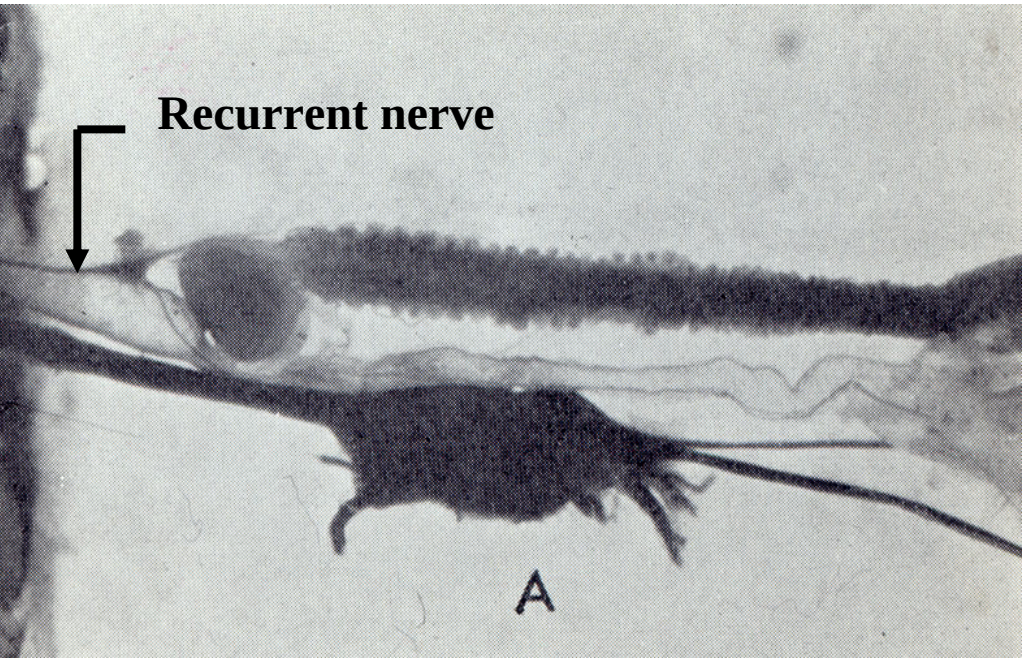
3. As blood sugars are used up in the hemolymph, the crop empties and its effect decreases and feeding resumes



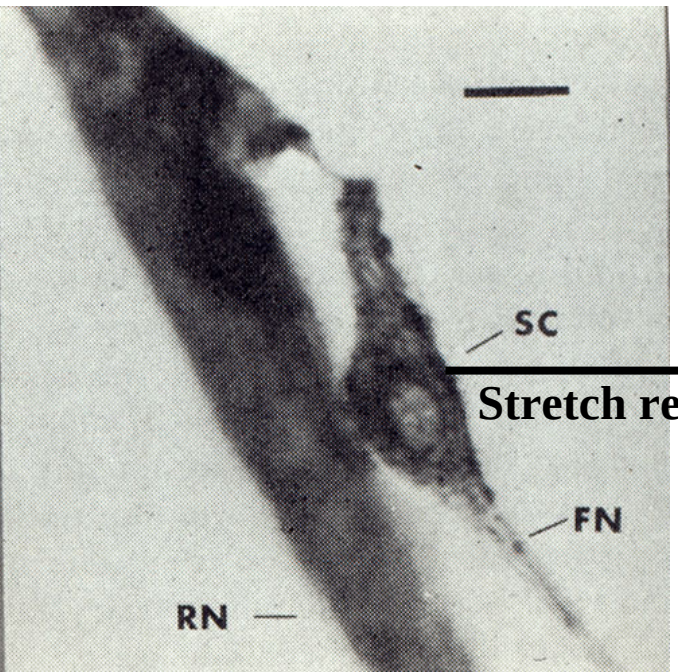
Ventral cutaway showing the crop (C), ventral nerve cord (VNC), thoracico-abdominal ganglion (TAG), crop duct (CD) and below one of the stretch receptors that is found at the junction of the stretch receptors and is involved in sending negative input or feedback to the CNS, which then causes elevation of the tarsal accepance threshold.



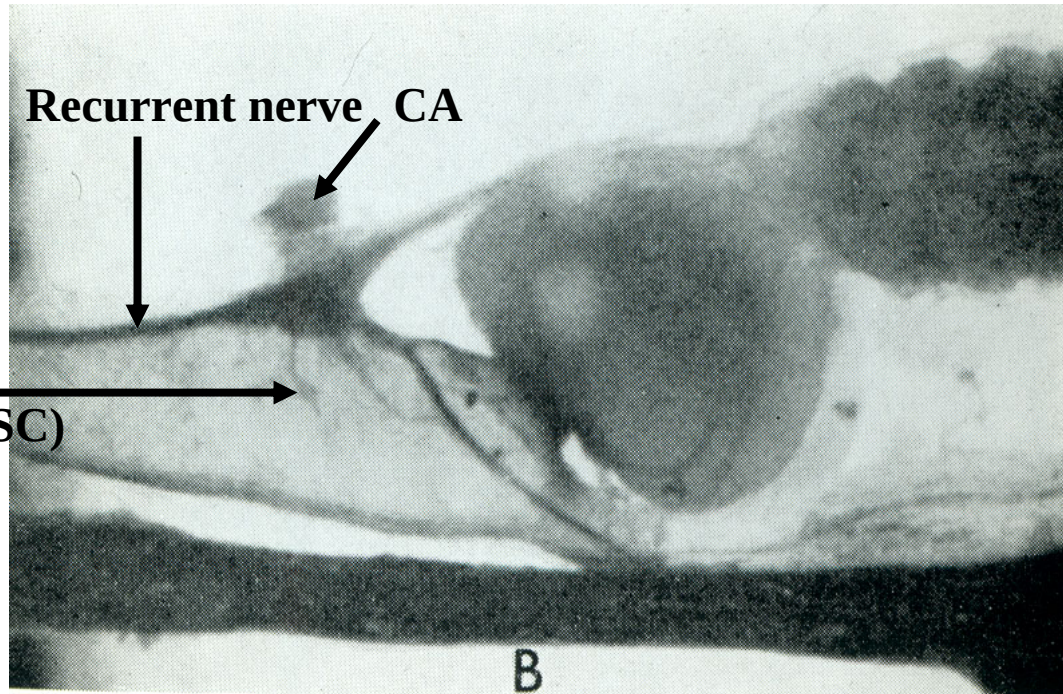


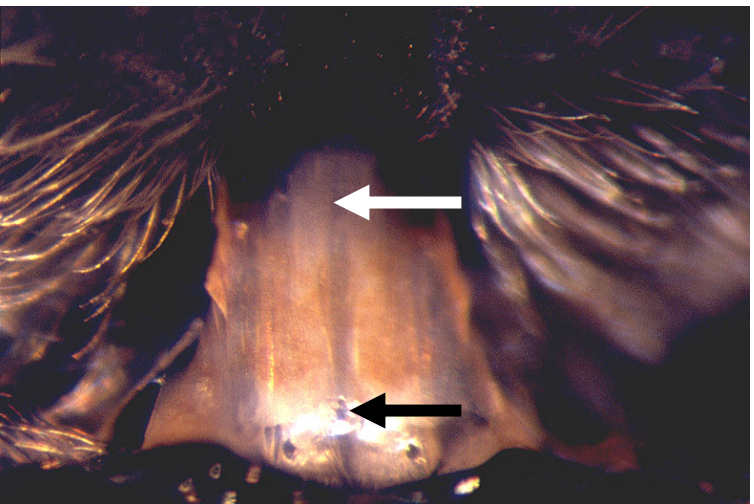


Food in the crop is constantly being moved forward into the esophagous where it causes a stretch in that area. This is monitored by the Stretch receptor cell (SC)
in the region. This sends negative input or feedback to the CNS via the recurrent nerve that also causes elevation of the tarsal acceptance threshold. This provides the fly with information about crop activity and probably something about food being diverted and moved into the midgut

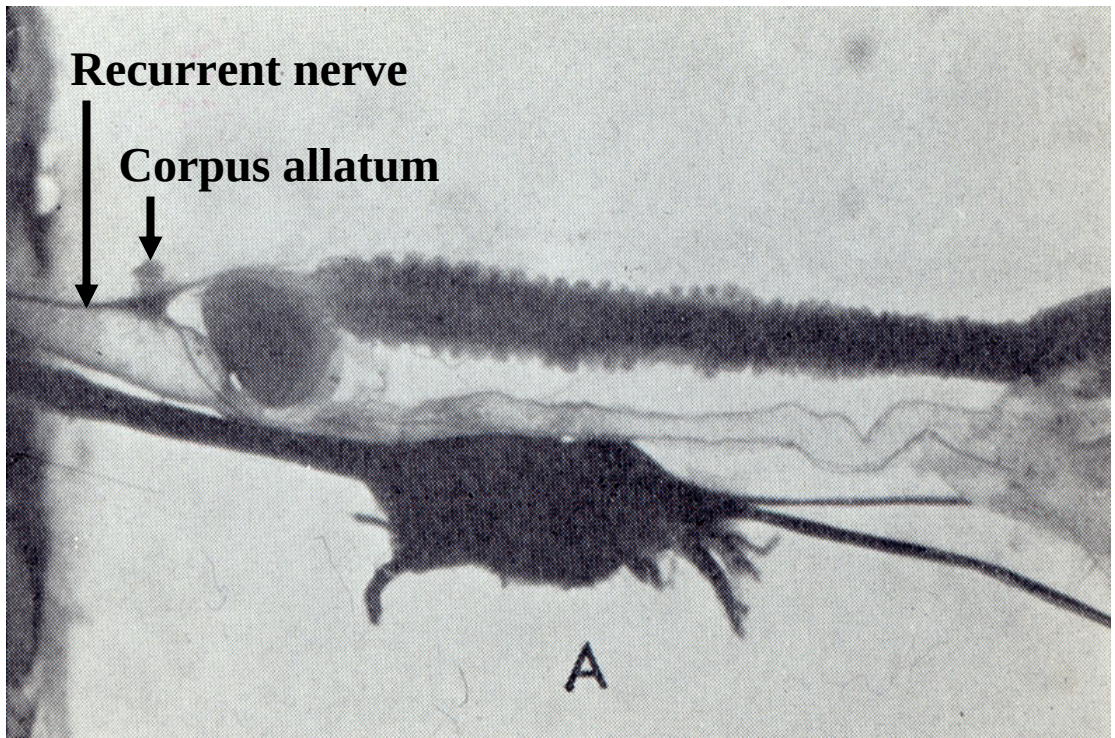


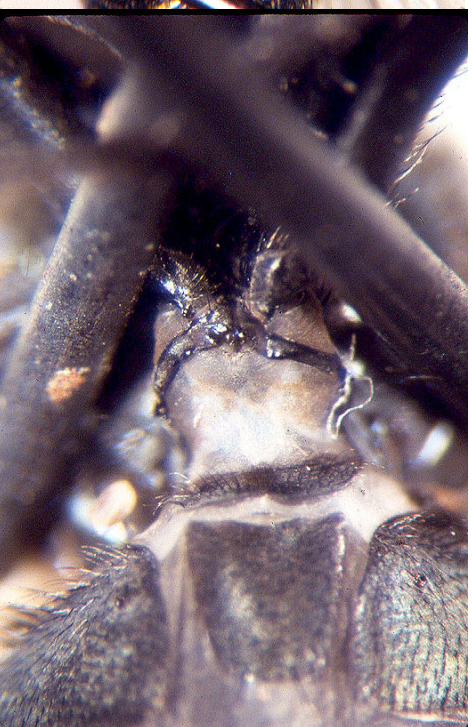
Stretch receptor (SC)



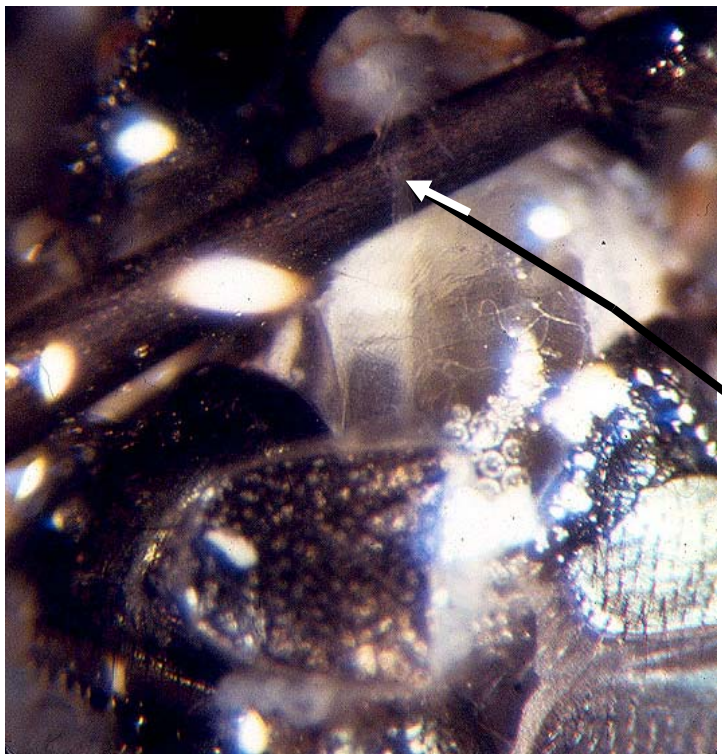


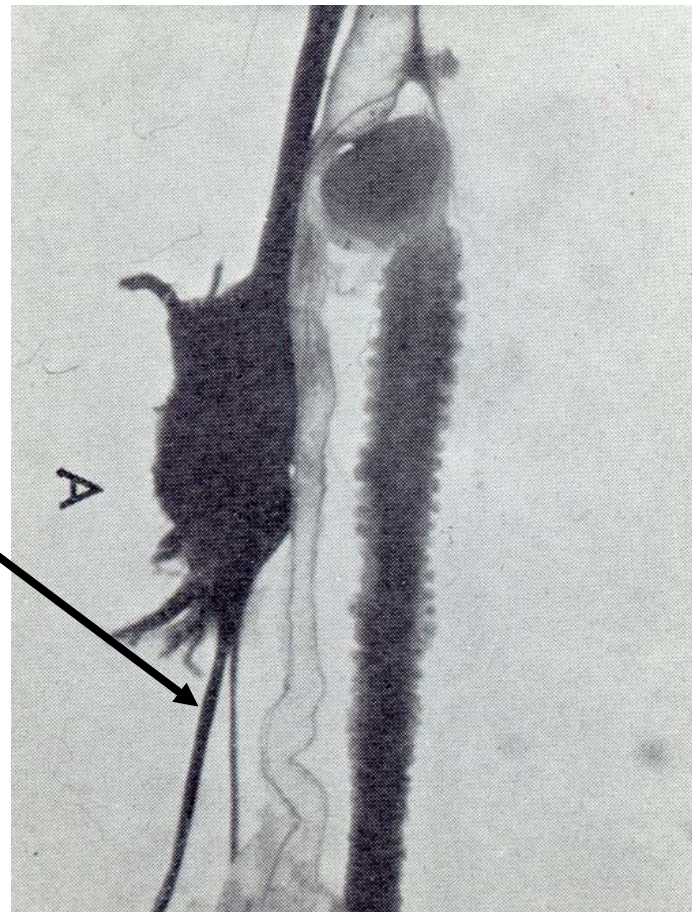
Sectioning the recurrent nerve, thus removing negative feedback from the foregut stretch receptor. To do this one fastens the fly dorsal surface up (top). Using pins one then pulls the head forward so as to stretch the neck area (middle). If you look at the photo below you can see that most anterior to the head area is the recurrent nerve that is in front of the CA (white arrow). The CA can be used as a marker for locating the recurrent nerve which will be below and between the two large tracheal trunks seen in the bottom photo on the left.





In order to section the ventral nerve cord one pins the fly ventral side up (left photo). Using pins one carefully tears away the arthrodial membrane between the thorax and abdomen (bottom left photo). On top of the crop duct, one must look for a translucent ventral nerve cord (see at the end of white arrow). Using a minuten pin pull the nerve and it will break. Lay it on the abdominal sclerite and this will let you see that you severed it. The photo below on the right shows the ventral nerve cord that will be cut.

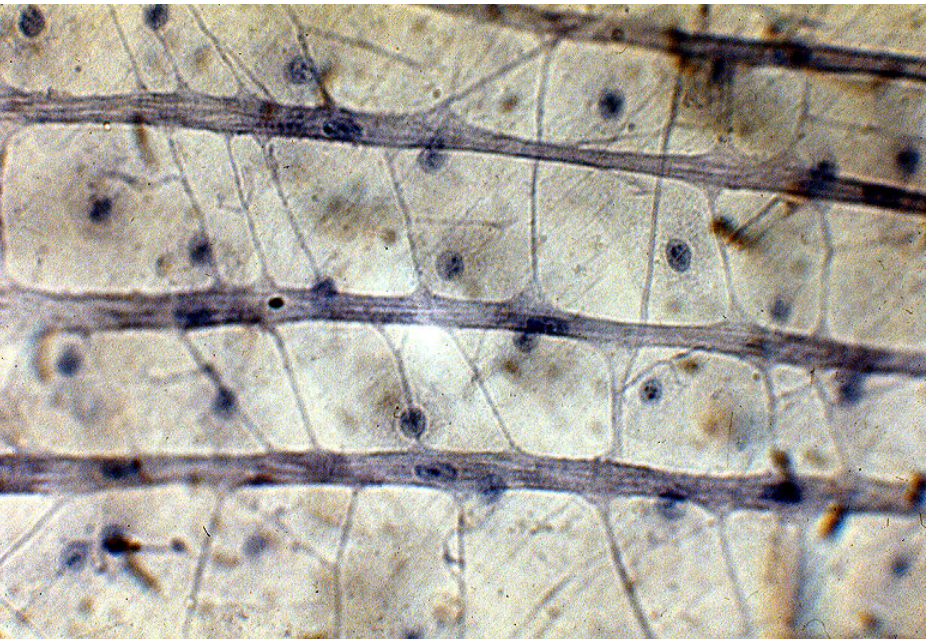




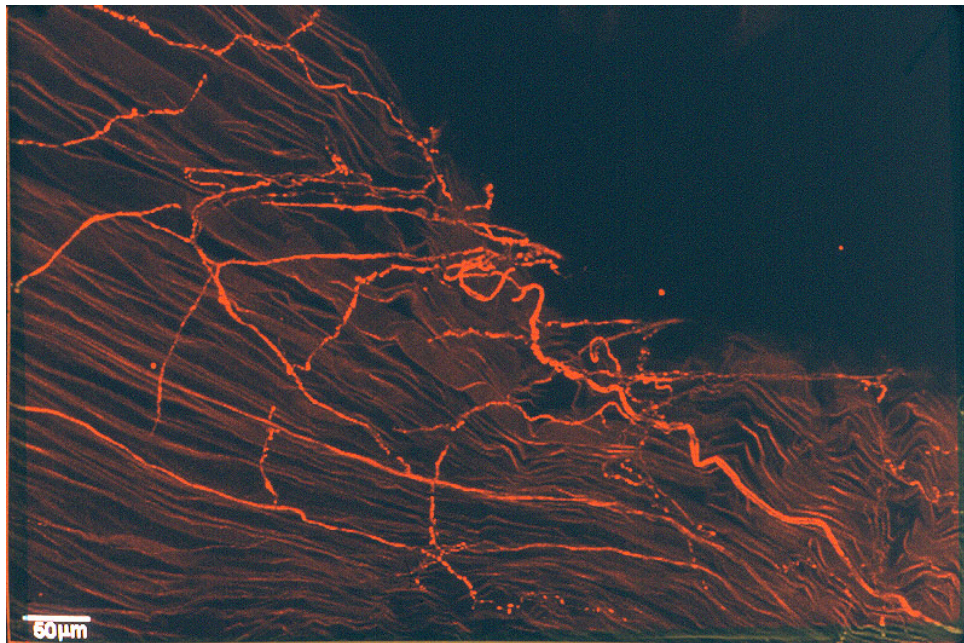
CONTROL OVER CROP MOTILITY

Recent studies have shown that the crop of adult *Drosophila*, *Phormia* and *Musca* is covered by a network of nerves that is positive to the antibody for the peptide dromyosuppressin (DMS). When applied to the crop, this peptide is released and shuts muscle activity down. It is suggested that this is important when the fly is trying to fill the crop. It is counterproductive to try to fill something if it is also being pushed out.

Muscles of P. *regina* crop



Fluorescence showing presence of DMS



Food enters the esophagous (see black arrow) and reaches a point where an internal decision is made: either send the food to the crop (cr) or put it into the midgut (mg). In order to do this the fly has various sphincters and pumps that help divert food and get it into the right part of the digestive tract. We know very little about how these sphincters work.

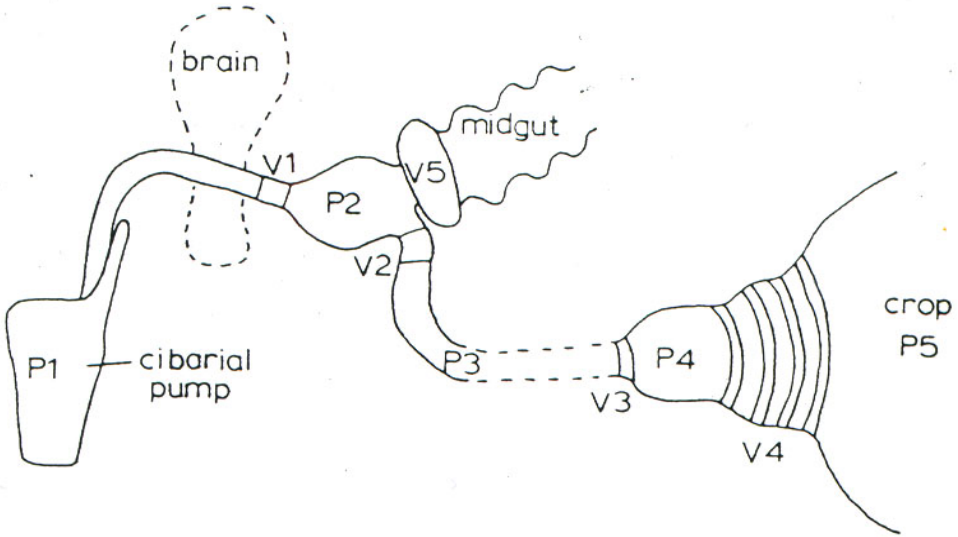
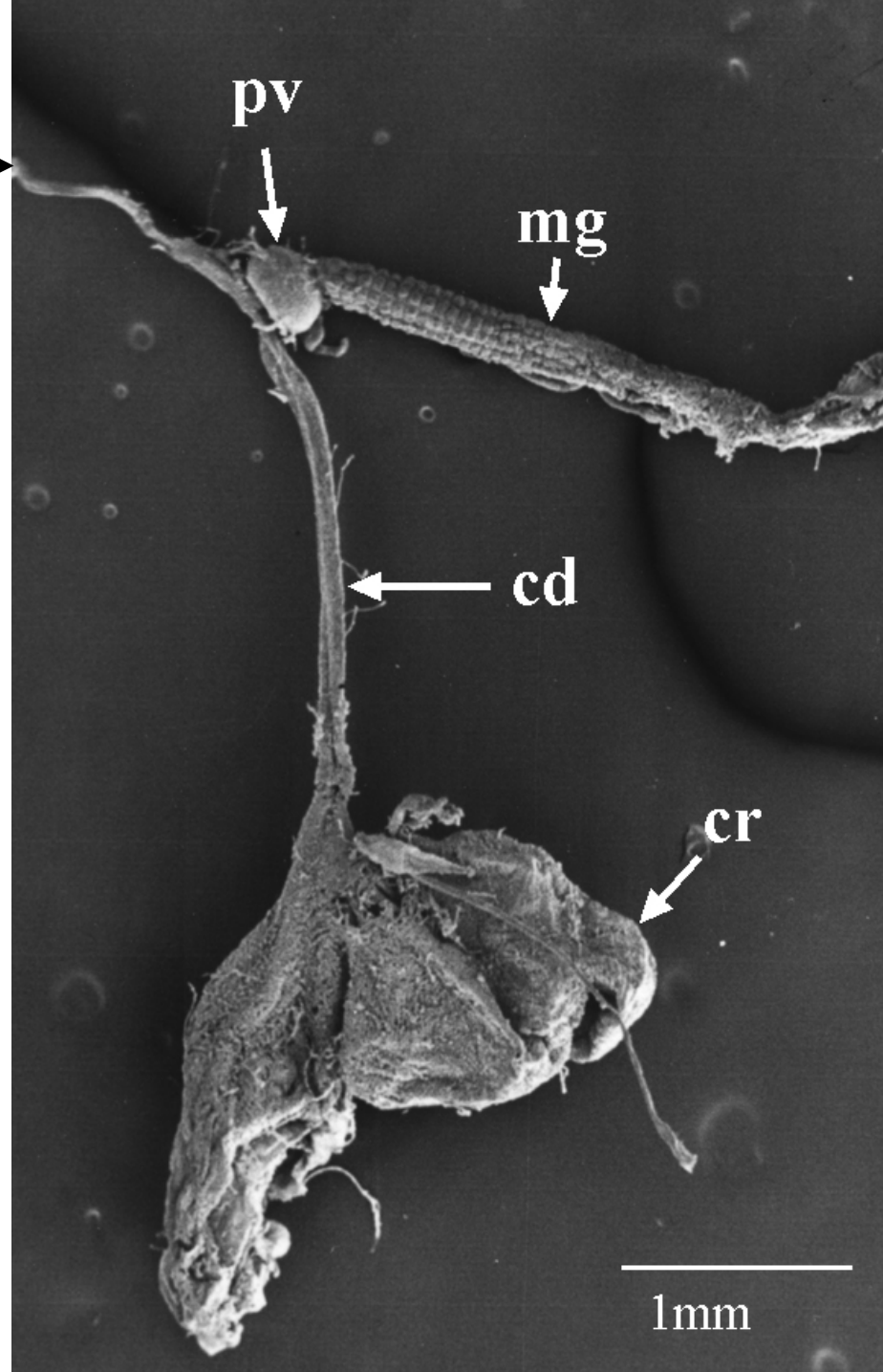


FIG. 1. The anatomy of the foregut of *P. regina* (from Thomson 1975*b*).



DMS and crop contractions

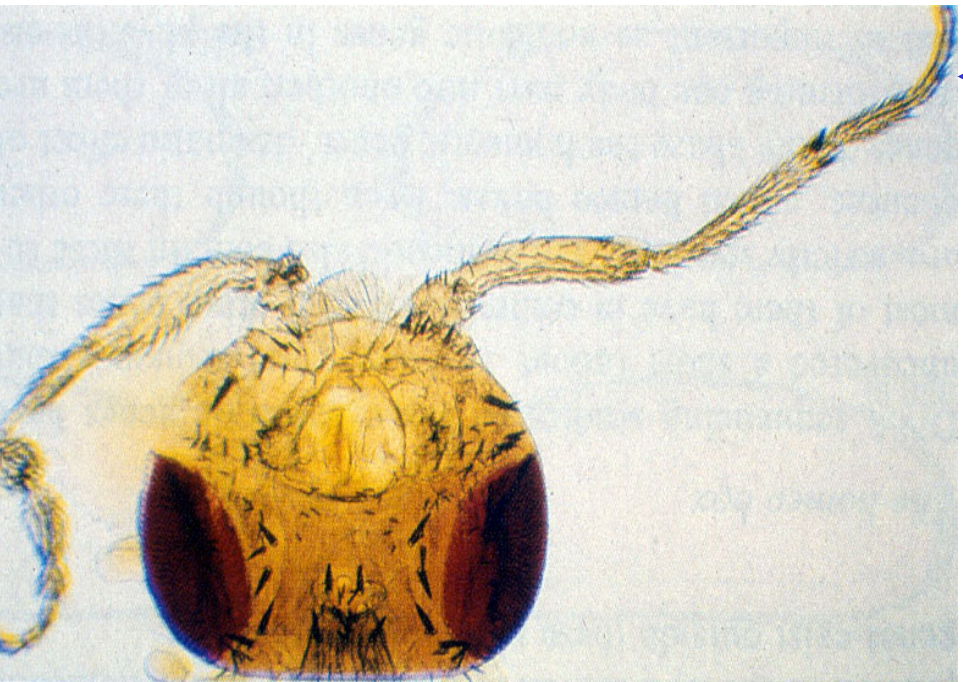
Application of 10-6 M DMS reduced crop contractions by 95% (from 46 to 2 contr./min)

Homeotic mutant appendage known as *Antennapedia* (*Antp*^{73b})

Just finished studying how feeding is controlled in flies. What might be an interesting question to ask here?

Will touching the tarsi of the mesothoracic leg on the antenna elicit a proboscis extension?

Gustatory stimulation of a homeotic mutant appendage, *Antennapedia*, in *Drosophila melanogaster*. R. Stocker. Jour comp. Physiol. A. 115: 351-361.



Mesothoracic leg instead of antenna

The pathway works and touching the tarsi to a stimulating solution elicits proboscis extension.

LONG-TERM INTAKE IN NON-DIAPAUSING P. REGINA

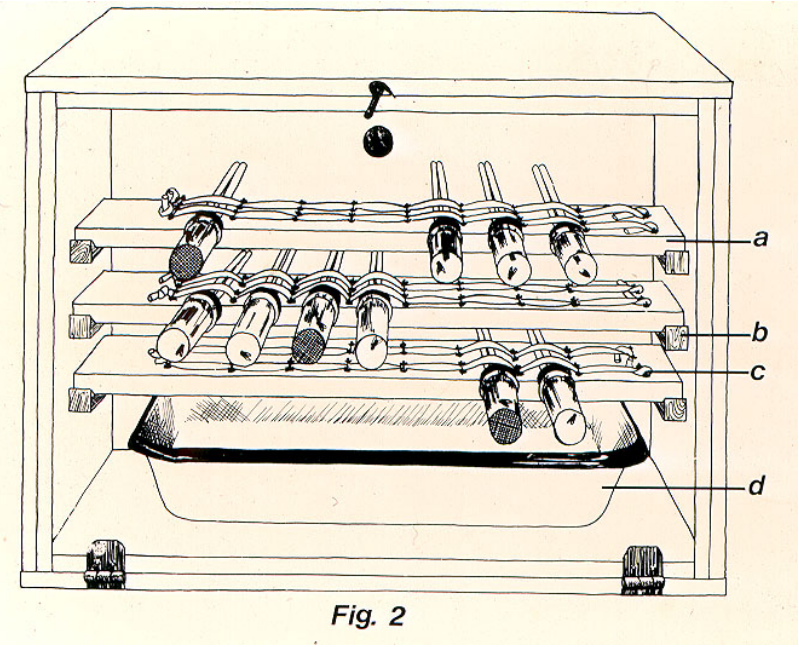


Fig. 2

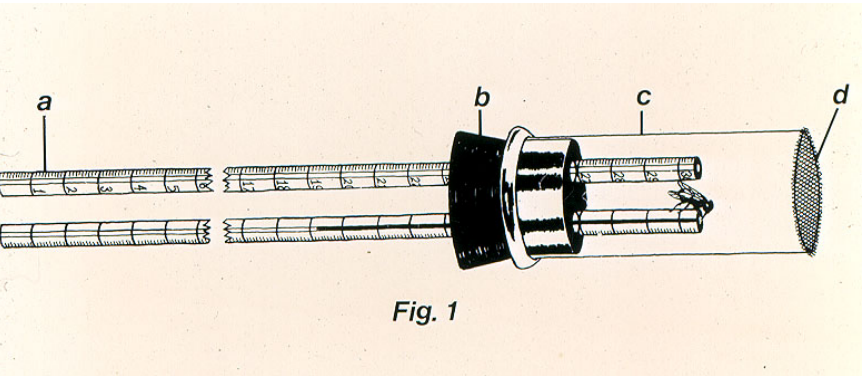
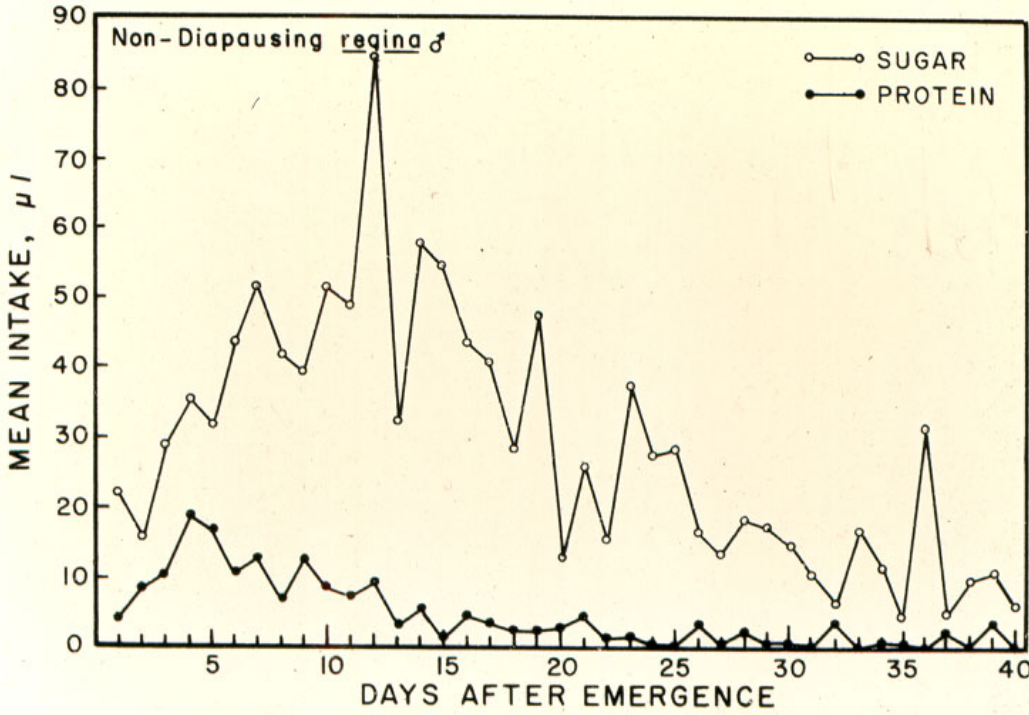
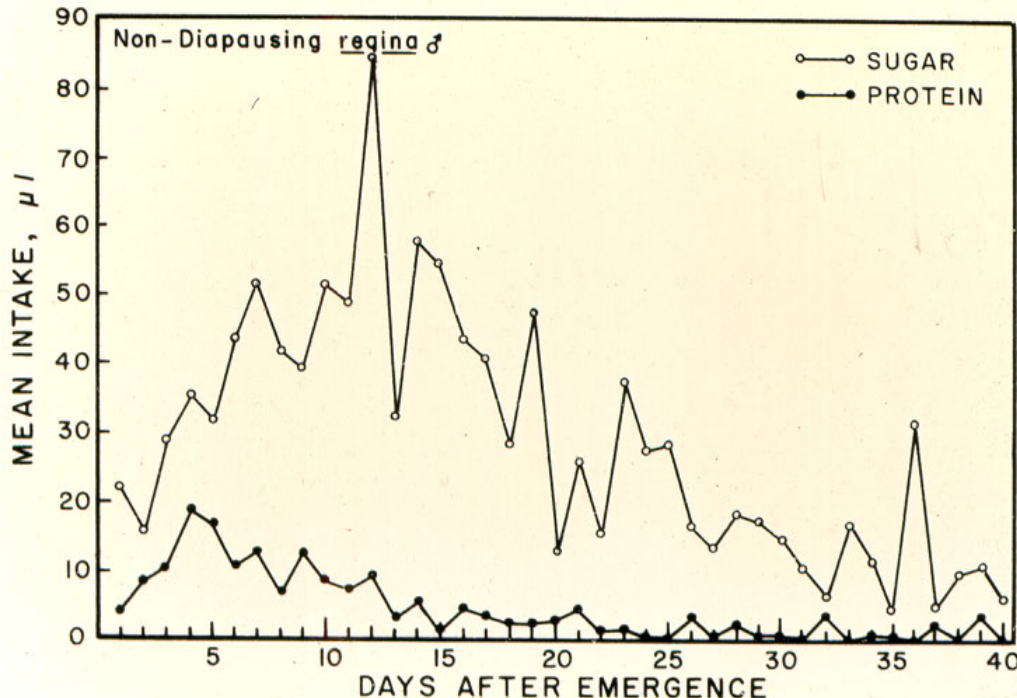
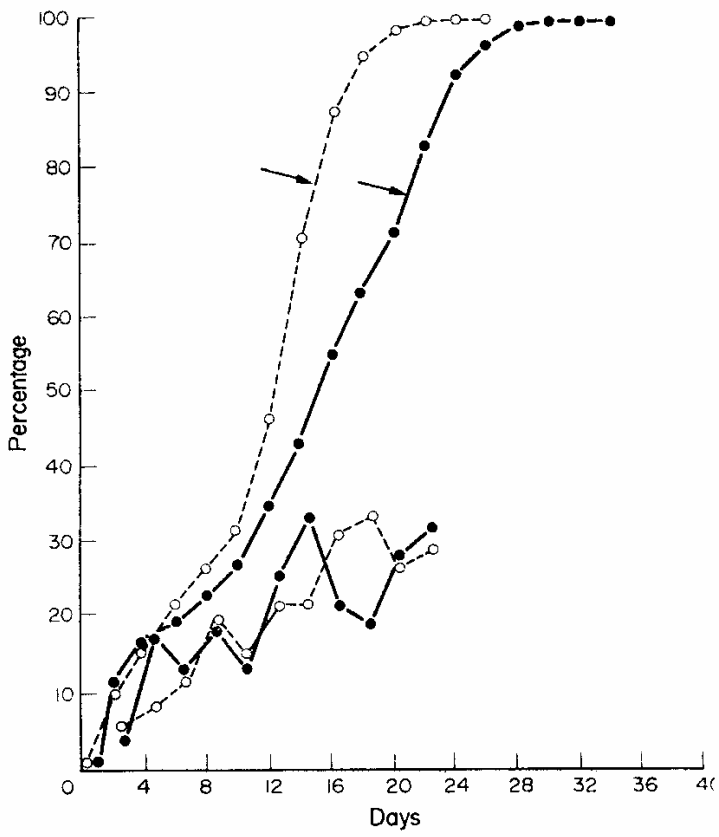


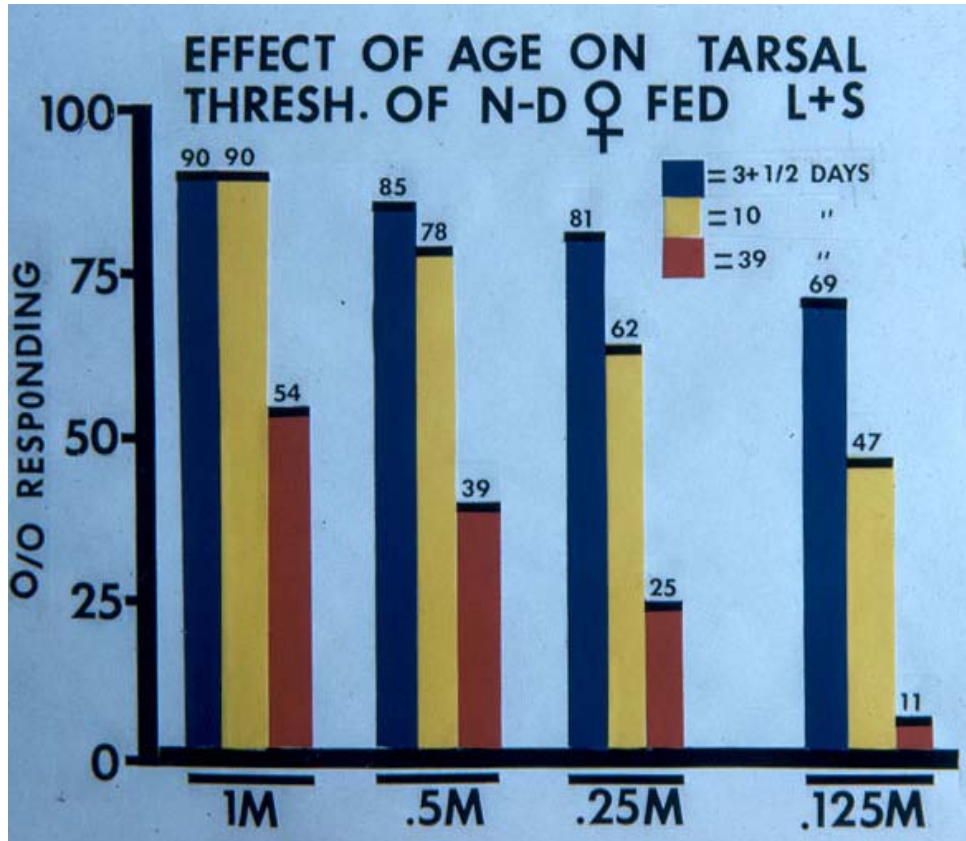
Fig. 1





Effect of age on % inoperativity of labellar chemosensilla and on the tarsal acceptance threshold in *Phormia regina*. The graph below/to the left shows the survivorship curves for both males (open circles) and females (closed circles). Note males live longer than females. Below one can see that as the flies age, the % of operative labellar chemosensilla increases with age to about 30%. Behavioral measurements of the effect of age/diet on tarsal acceptance threshold is shown in the right graph.





PHARMACOLOGICALLY INDUCED HYPERPHAGIA

Hyperphagia is when a fly eats considerably more than it should when fed sucrose

The fly below shows hyperphagia that was induced by cutting the ventral nerve cord

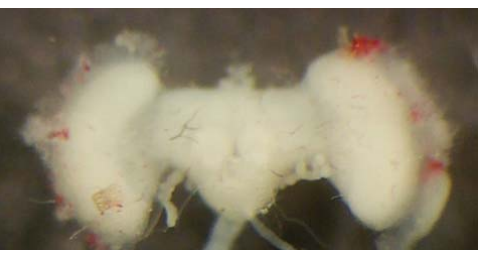
The fly on the left and below was injected with saline and failed to become hyperphagic while the fly below and to the right was injected with saline and clonidine (an octopamine agonist)

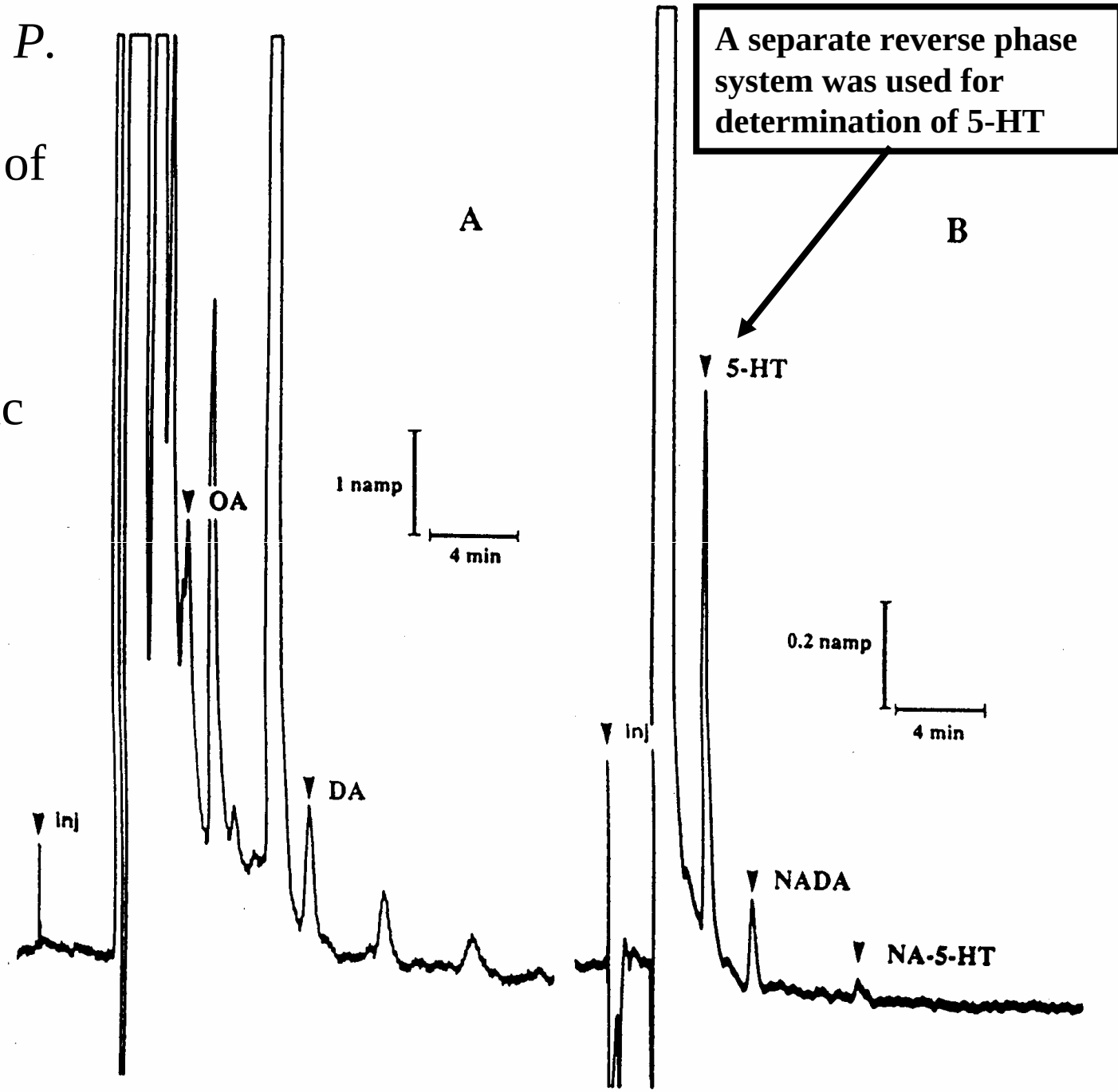
Murdock and student's research on biogenic amines and feeding control in *Phormia regina*

1. Long, T.F. and L.L. Murdock. 1983. Stimulation of blowfly feeding behavior by octopaminergic drugs. Proc. Natl. Acad. Sci. 80: 4159-4163.

Chromatograms of *P. regina's* brain. Microliter portions of the supernatants of pooled-brain homogenates were assayed for biogenic amine content by HPLC-EC.

Phormia's brain





1. **Behavioral measure of the MTAT**

a. Flies injected with chemicals that were octopamine-like were much **more responsive** than the saline controls

Table 1. Effects of octopaminergic agents on MAT to aqueous sucrose by 3-day-old adult blowflies in a typical experiment

Drug (dose per fly)	MAT, mM*
Saline (1 μl)	13.0
DCDM (10 μg)	<0.25†
Clonidine (20 μg)	0.5†
Pargyline (10 μg)	<0.5†
DL-Octopamine (75 μg)	3.0‡

* At least 100 flies were used for each estimate.
† $P < 0.001$ for difference from control by χ^2 test (6).
‡ $P < 0.02$.

2. **Behavioral measure of consumption**

a. Flies injected with chemicals that were octopamine-like **consumed more** sucrose and water compared to the slaine controls

OCTOPAMINE SOMEHOW POSITIVELY MODULATES MTAT AND CONSUMPTION

Table 2. Consumption of water and 1 M sucrose during a 30-min period by control and drug-treated adult blowflies

Drug (dose per fly)	Weight increase (mg) after 30-min consumption of	
	1 M sucrose	Water
Saline (1 μl)	13.4 ± 4.6	1.5 ± 2.1
DCDM (10 μg)	49.5 ± 12.2*	12.8 ± 9.6*
Clonidine (20 μg)	41.3 ± 10.5*	11.4 ± 9.3*
Pargyline (10 μg)	47.4 ± 11.4*	14.3 ± 10.3*

Presentation of the 1 M sucrose began 45 min after injection. Values represent the mean (±SD) weight of 1 M sucrose imbibed per fly by a group of 100 flies.
* Significantly greater than control (saline injection) consumption, $P < 0.001$, Student's t test.

The tarsal MAT is affected by d-amphetamine injection while the the labellar MAT is not (see results from Murdock's group)

Table 1. Effects of D-amphetamine on blowfly responsiveness to labellar or tarsal stimulation with aqueous sucrose. Each point is the average of three separate experiments. Values followed by the same letter in a given column are not significantly different (p>0.01) from each other.

	Labellar M.A.T.		Tarsal M.A.T.	
Saline control	9.9 mM	a	41.8 mM	a
D(+)-Amphetamine (10 ug / fly)	8.4 mM	a	1217 mM	b
Untreated control	8.9 mM	a	25.2 mM	a

IF OCTOPAMINE SOMEHOW POSITIVELY MODULATES MTAT AND CONSUMPTION, WHAT MIGHT BE THE NEXT STEP IN AN EXPERIMENTAL APPROACH TO UNDERSTANDING WHAT IS GOING ON?

Find a way to remove the octopamine from the insect

This can be done using amphetamine, which causes the depletion of the brain biogenic amines.

Brookhart, G.L., R.S. Edgecomb and L.L. Murdock. 1987. Amphetamine and reserpine deplete brain biogenic amines and alter blow fly feeding behavior. Jour. Neurochemistry 48: 1307-1315.

1. Injected flies with amphetamine

2. Time in minutes is after infection

3. Notice that the levels of the 3 biogenic amines in the brain tissue rapidly drops but then recovers

4. Notice that as the 3 biogenic amines drop in the brain that the MTAT correspondingly increases|

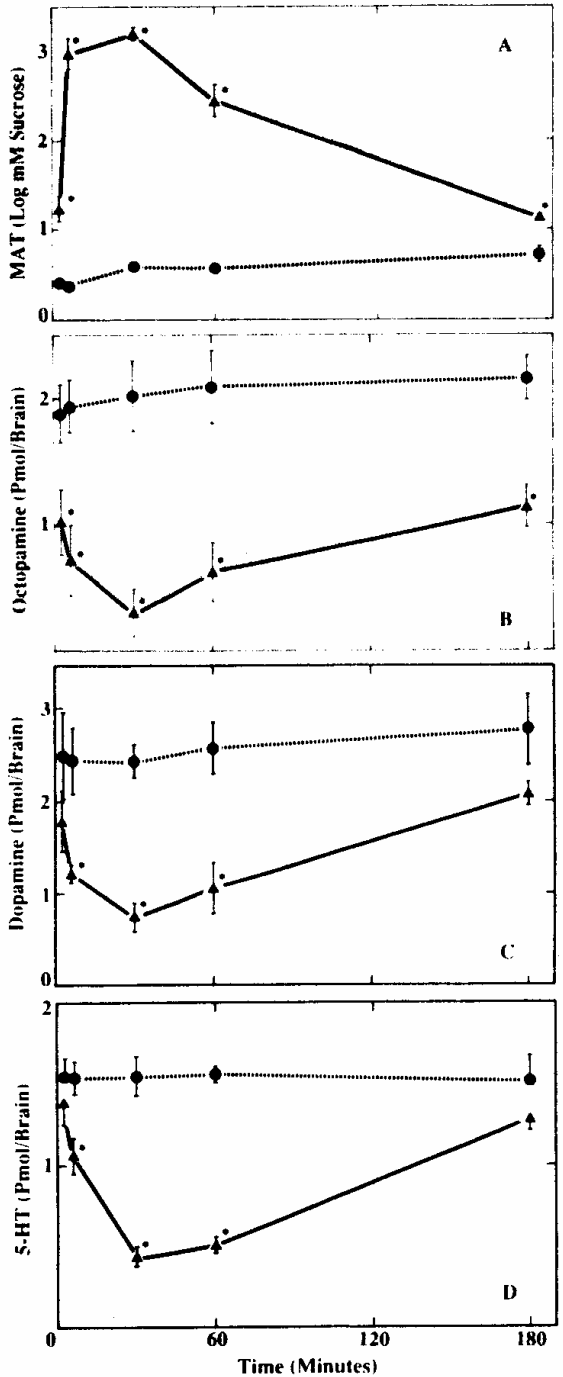
Thus, removing octopamine from the brain removes its positive effect on MTAT

MTAT →

Octopamine →

Dopamine →

Serotonin →



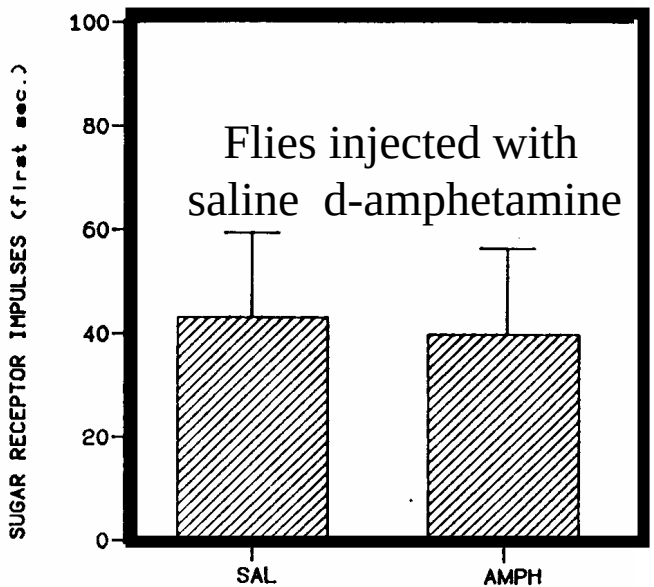
WHERE ARE THESE CHANGES INDUCED BY EITHER OCTOPAMINE INJECTION (CAUSING DECREASED MTAT) OR d-AMPHETAMINE (CAUSING INCREASED MTAT) TAKE PLACE?

CENTRAL OR PERIPHERAL AND HOW TO TEST IT???

By testing the electrical activity at the tarsal level using electrophysiologcal techniques.

No significant alteration in the tarsal electrophysiological response to sugar when injected with d-amphetamine

Edgecomb, et al. 1987
J. exp. Biol. 127: 79-94



CONCLUDING REMARKS

1. Feeding behavior is a complex behavior that is highly regulated by the nervous, as well as by various chemicals that can modulate the system.

2. Octopamine negatively modulates or inhibits the electrical, negative input signals, coming from the stretch receptors in the nerve net covering the crop and the stretch receptor in the esophagous. These are probably post-synaptic events.

3. Electrical input in the form of action potentials from the various chemosensilla provide positive input to the central control to feed.

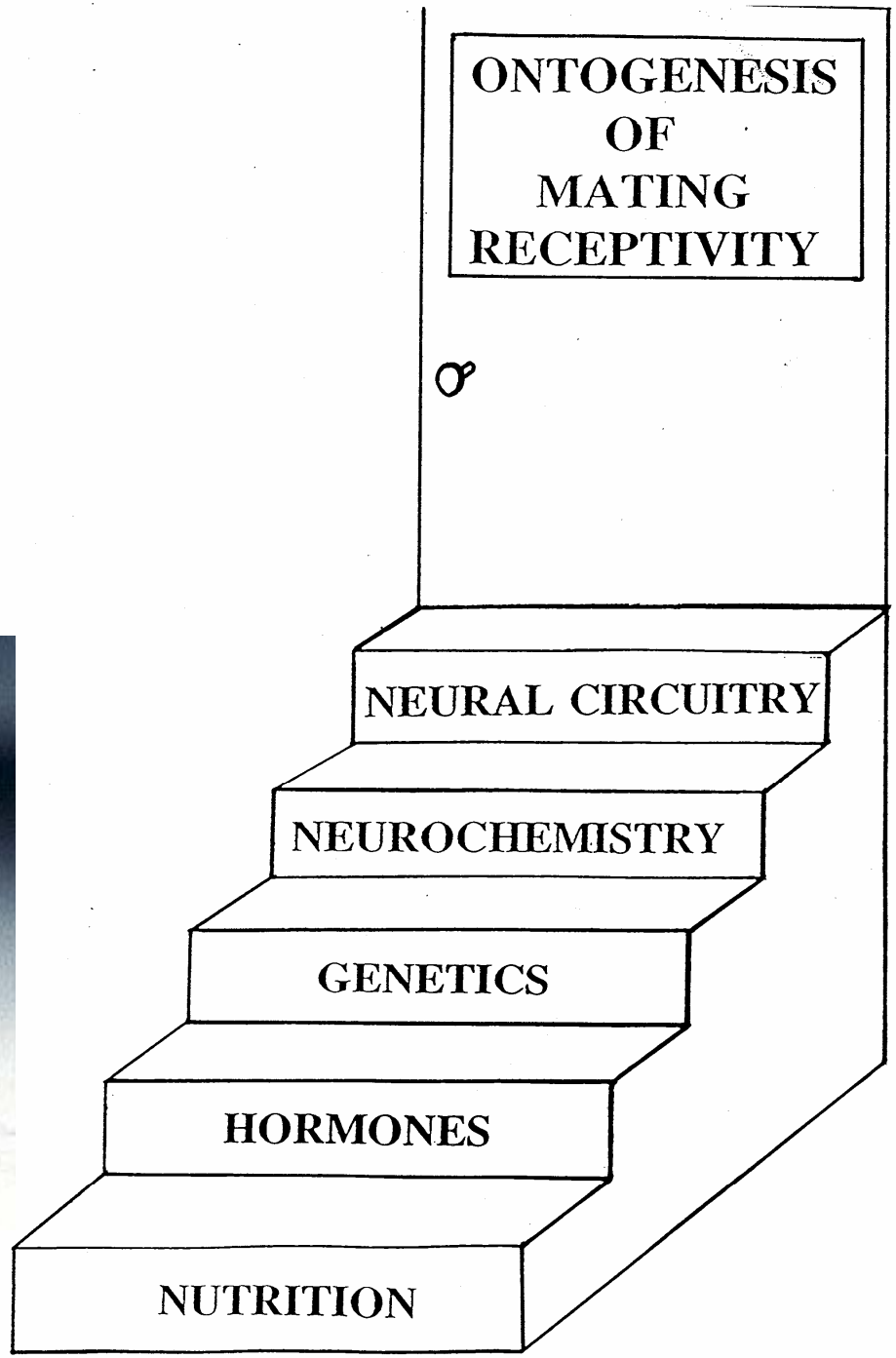
4. Events controlling crop motility are not fully understood, as are the various pumps and sphincters involved in moving food from one site to the next.

CONTROL OF SEXUAL BEHAVIOR

A. Nutrient control

B. Hormonal control

C. Role of biogenic amines



EFFECT OF MALE DIET ON LEVEL OF MATING ACTIVITY

1. Complete absence of male mating behavior in
   a. Prey-deprived male *Scatophaga stercoraria*….Foster, 1967
   b. Nonblood-fed *Stomoxys calcitrans*……..Meola et al. 1977
   c. Non-protein fed *Protophormia terrae-novae*…Parker, 1968
   e. Non-protein fed *Lucilia cuprina*……Bartell et al., 1969

2. Number of oriented mounts (OM) by sugar-fed *Protophormia terrae-novae* was 5 OM/15 min. compared to 162 OM/15 min for protein-fed males.

Mating studies of male *P. regina*

Table 1. Effect of male diet and duration sexes were together on percentage of females inseminated

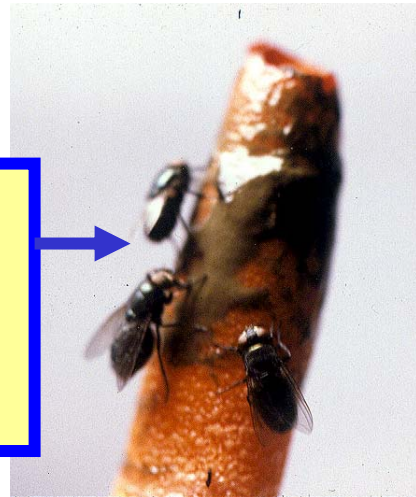
Male diet	Duration ♂ fed diet	Time sexes together, h	Controls nonprotein-fed ♂		Protein-fed ♂	
			n	%	*n*	%
Beef liver[a]	3 d	1.5	114	10.5	107	83.2
Beef liver[a]	3 d	24.0	118	70.3	112	75.9
Chicken feces[b]	3 d	1.5	110	13.6	109	79.8
Gleba[a]	1.5 h	1.5	49	8.2	66	7.6
Gleba[a]	3 d	1.5	116	12.9	110	58.2

Females were always fed liver and then placed with males fed the different diets.
[a] Replicated three times.
[b] Replicated two times.

a. Non-protein fed male *P. regina* inseminate few females compared to protein fed males
b. When kept with females for 24 h the number of females mated increased from 1.5 to 24.0 %.

Why the difference?

Gleba is the slimy matrix where the spores are found in various fungi



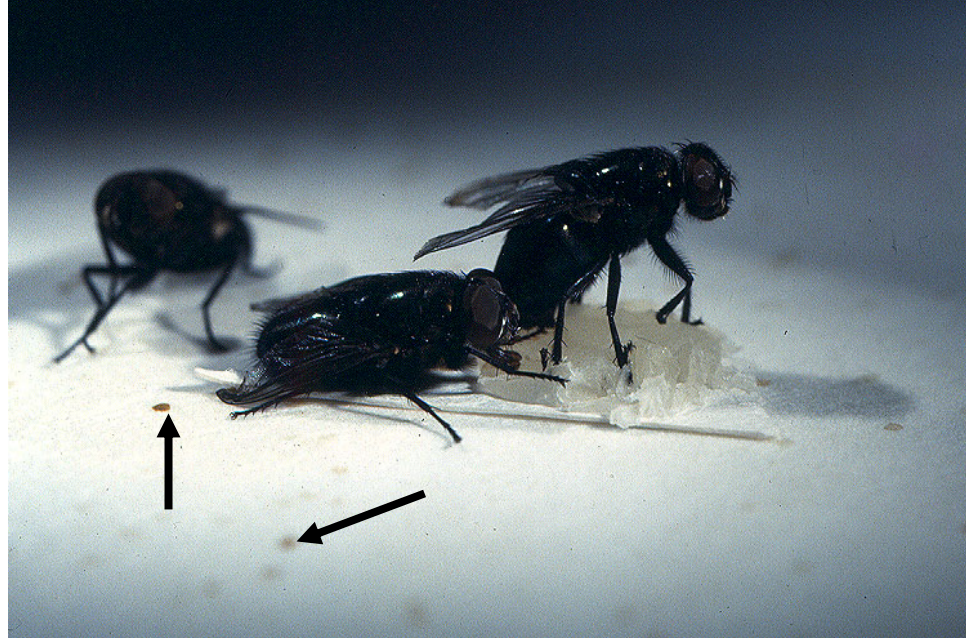
Protein deprived male *P. regina* when paired with liver-fed females for:

a. 1.5 h successfully inseminated 10.5% of the females

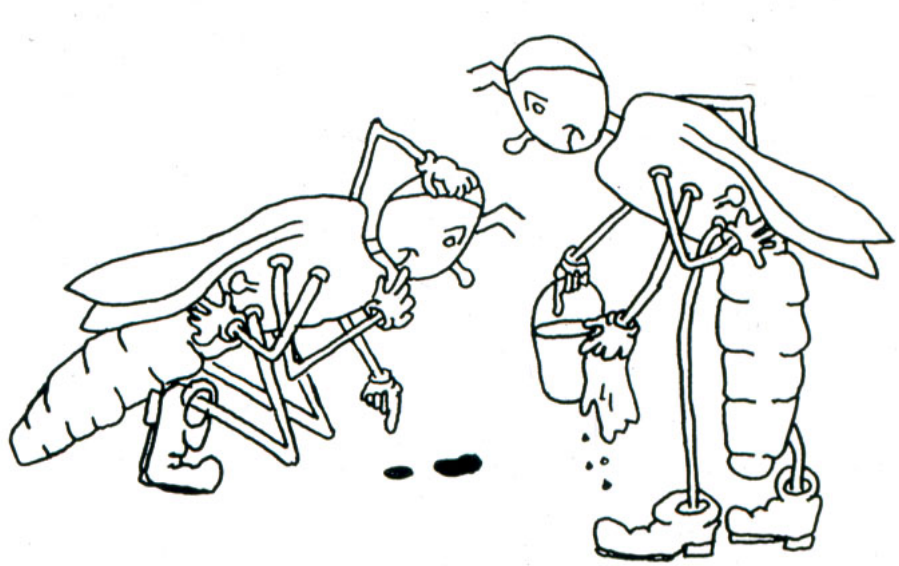
but

b. 24 h successfully inseminated 70.3% of the females

Males were shown to be feeding on vomit and fecal spots from the liver-fed females, thus getting the protein they needed from these these two sources



Notice dark fecal spots containing protein



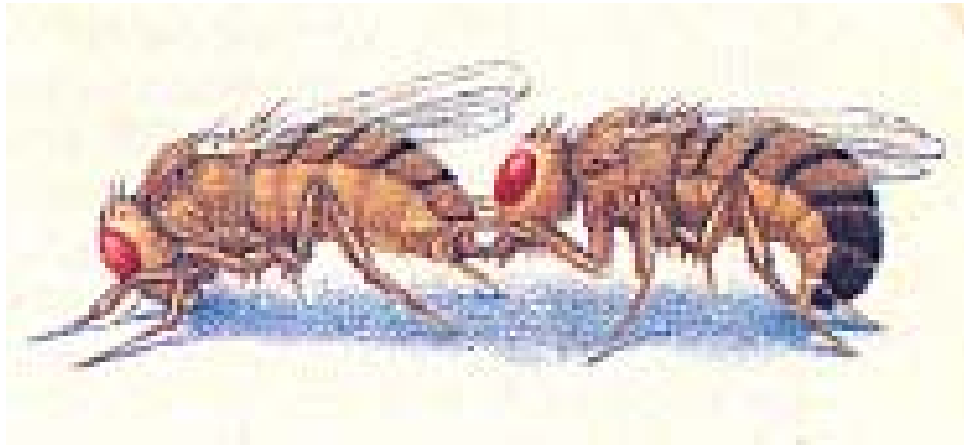
FLY FECES-A SOURCE OF PROTEIN

How do various behaviors start?

Protein starved male *P. regina* performs an extremely unusual behavior when paired with liver-fed females. The male feeds on anal secretions from the anus (proven using fluorescent label in liver juice given to the female. Stoffolano and his students proposed that the normal "licking" component of the female *Drosophila's* anus area is a carry-over when males acquired their protein from the female.

Stoffolano et al. 1995. Ann. Ent. Soc. Amer. 88: 240-246.

Licking component of normal mating behavior in *Drosophila*



Licking component of abnormal feeding behavior in protein deprived *P. regina* males to obtain protein from liver fed females

Factors affecting mating in *Phormia regina*

1. Males need a protein meal as do females (see tables)
2. Is failure to mate due to the absence of a pheromone in the sugar versus the protein fed flies?

EFFECT OF MALE DIET ON PERCENTAGE OF SEXUALLY MATURE, VIRGIN FEMALES INSEMINATED

DAYS EXPOSED	LF-MALES	NLF-MALES
1	53.6	1.8
2	65.4	7.1
3	81.8	24.1

3 replicates; 52 flies smallest sample; flies 4 days old at beginning of experiment

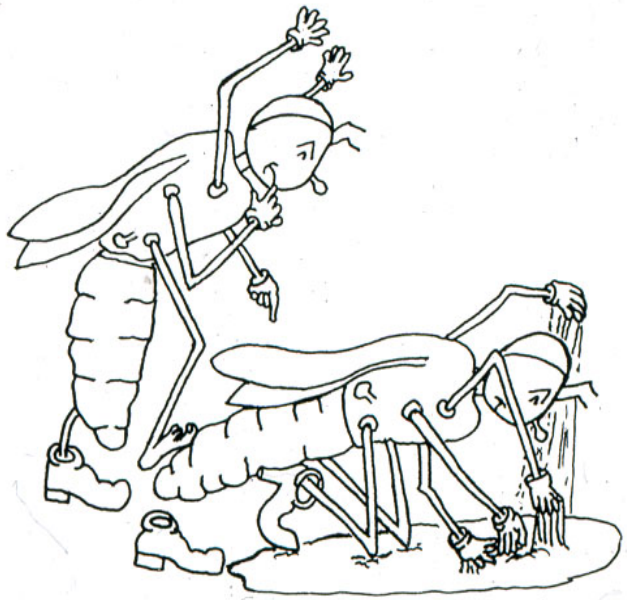
RELATIONSHIP BETWEEN SEXUAL RECEPTIVITY AND STAGE OF FOLLICLE DEVELOPMENT

Stage of follicle	% Insemination
1 to 3	11
4	24
5	52
6	68
7	77
8	76
9	84
10	84

WHY WERE MALES NOT MATING ?

To rule out the effect of diet or age on males not mating females were examined for the presence or absence of a pheromone

A. MATING STUDIES CONDUCTED WITH FROZEN DECOYS



SENSING CONTACT PHEROMONE

Mating studies in *Phormia regina* using frozen decoys

TABLE 2. Effect of the female decoy's previous diet on copulatory attempts and other parameters by sexually aggressive males*

		a.m.				p.m.			
		No. of			s	No. of			s
Dietary treatment		C.C.	M.S.	C.A.	T.M.	C.C.	M.S.	C.A.	T.M.
Protein-fed decoy	Tot.	256	102	20	45:40	217	75	17	46:03
	Avg.	8.53	3.40	0.67	1:31	7.23	2.50	.57	1:32
Sugar-fed decoy	Tot.	198	77	18	33:27	248	75	17	33:25
	Avg.	6.60	2.57	0.60	1:07	8.29	2.50	.57	1:07

*Values shown represent totals and averages for all three days of testing. n = 30 females for each diet group and session tested; replicated 3 × . C.C., casual contacts; M.S., mounting strikes; C.A., copulatory attempts; T.M., time mounted; protein-fed = liver-fed; sugar-fed = sucrose-fed.

Males made as many copulatory attempts regardless of the female's diet

Stoffolano et al. 1997. Cuticular hydrocarbons and their role in copulatory behavior in *Phormia regina* (Meigen). J. Insect Physiol. 43: 1065-1076.

Mating studies in *Phormia regina* using frozen decoys

TABLE 3. Effect of hexane washed, protein-fed female decoys on copulatory attempts and other parameters by sexually aggressive males*

		a.m.				p.m.			
		No. of			s	No. of			s
Treatment		C.C.	M.S.	C.A.	T.M.	C.C.	M.S.	C.A.	T.M.
Unwashed decoy	Tot.	239	103	23	50:37	278	93	29	59:22
	Avg.	7.97	3.43	.77	1:41	9.27	3.10	.97	1:59
Washed decoy†	Tot.	454	131	0	1:36	459	13	1	3:23
	Avg.	15.13	4.37	0	:03	15.30	4.40	.03	:07

*Values shown represent totals and averages for all three days of testing. $n = 30$ females for each treatment group tested per session; replicated 3 × . C.C., casual contacts; M.S., mounting strikes; C.A., copulatory attempts; T.M., time mounted.
†Females washed in hexane to remove cuticular hydrocarbons. A 30 min drying period prior to testing was provided.

Washing the protein-fed female decoy with hexane had a drastic effect on both copulatory attempts and time mounted. Something was removed that is essential for these two components for male mating behavior.

Mating studies in *Phormia regina* using frozen decoys

TABLE 4. Effect of the sex of the protein-fed decoy on copulatory attempts and other parameters by sexually aggressive males*

		a.m.				p.m.			
		No. of			s	No. of			s
Sex of decoy		C.C.	M.S.	C.A.	T.M.	C.C.	M.S.	C.A.	T.M.
Female	Tot.	238	151	26	61:04	287	112	20	55:25
	Avg.	7.93	5.03	0.87	2:02	9.57	3.73	0.67	1:51
Male	Tot.	348	156	23	36:38	283	107	24	44:24
	Avg.	11.60	5.20	0.77	1:13	9.43	3.57	0.80	1:29

*Replicated 3 × ; n = 30 decoys tested for each sex per testing session.
All decoys were obtained from protein-fed flies.
Values shown represent totals and averages for all three days of testing.

Males did not differentiate between sex of the decoy for copulatory attempts made but spent less time mounted on their own sex

Mating studies in *Phormia regina* using frozen decoys

TABLE 5. Effect of male palpectomy and antennectomy on copulatory behavior and other parameters when paired with an unwashed female decoy*.

Treatment		# Casual contacts	# Mounting strikes	# Copulatory attempts	Time (s) mounted
Unoperated	Tot.	53	38	7	11:27
	Avg.	5.3	3.8	0.7	1:09
Operated	Tot.	38	54	11	17:38
	Avg.	3.8	5.4	1.1	1:46

* = Experiments conducted during the a.m. and tested only once.
Values shown represent totals and averages for each testing period. n = 10 males for each treatment group per session.

No significant difference due to removal of male's palps and/or antenna. The response was tarsal contact with the decoy

Mating studies in *Phormia regina* using frozen decoys

TABLE 6. Effect of adding the cuticular hydrocarbons from protein-fed flies to the hexane-washed, protein-fed decoys.

Treatment		# Casual contacts	# Mounting strikes	# Copulatory attempts	Time (s) mounted
A. Cuticular hydrocarbon depleted female decoy (control)	Tot.	369	81	0	1:29
	Avg.	12.30	2.70	0	:03
Cuticular hydrocarbon reapplied decoy*	Tot.	388	116	8	20:48
	Avg.	12.93	3.86	0.27	:42
B. Cuticular hydrocarbon depleted male decoy (control)	Tot.	548	67	0	1:11
	Avg.	18.27	2.23	0	:02
Cuticular hydrocarbon reapplied decoy†	Tot.	368	116	20	38:31
	Avg.	12.27	3.87	0.67	1:17

*Cuticular hydrocarbons were obtained from protein-fed females and applied to the treated female decoy at four female equivalents/decoy.
†Cuticular hydrocarbons were obtained from protein-fed males and applied to the treated male decoys at four male equivalents per decoy.
All tests were conducted during the p.m. and replicated 3 × . $n = 30$ decoys tested per treatment. Values shown represent totals and averages for all three days of testing.

Males did not attempt to mate with decoys washing in hexane (cuticular hydrocarbon removed). By reapplying it to washed decoys, however, males attempted to copulate and remain mounted to the decoy

Dave Carlson’s sex pheromone of screw worm

Videos http://cmave.usda.ufl.edu/researchunits/screwwormvideo.html

CONCLUSIONS ON: Mating in *Phormia regina* using frozen decoys

1. Males respond to a cuticular hydrocarbon on the female that leads to copulatory attempts and mounted staying time

2. Both males and females possess the same cuticular hydrocarbon, which can explain some of the homosexual behavior

3. The presence of the cuticular hydrocarbon is independent of diet. Thus, any failure of a protein-fed male failing to mate with a protein deprived females is not due to lack of a sex pheromone

4. Studies also showed that when house fly decoys were used, male *Phormia regina* did not show copulatory attempts or stay mounted on the decoy. Thus, the contact pheromone is species specific.

5. Using gas chromatography-mass spectrometry the cuticular hydrocarbon was characterized as a complex mixture of saturated n-, monomethyl- and dimethylalkanes from 23 to 33 total carbons.

1. Both male and female *Phormia regina* require protein in their diet for normal expression of mating behavior and/or receptivity on the females part

2. In nature males go feces to get their protein and meet females also at the feces getting their protein. If she has previously fed on protein and has started egg development she will mate but if this is her first protein meal, she will not mate. Once mated, ARG fluid from the male renders the female unreceptive to other matings and she now seeks out a place to lay eggs. Males generally don't frequent the site of oviposition, which is a dead carcass.

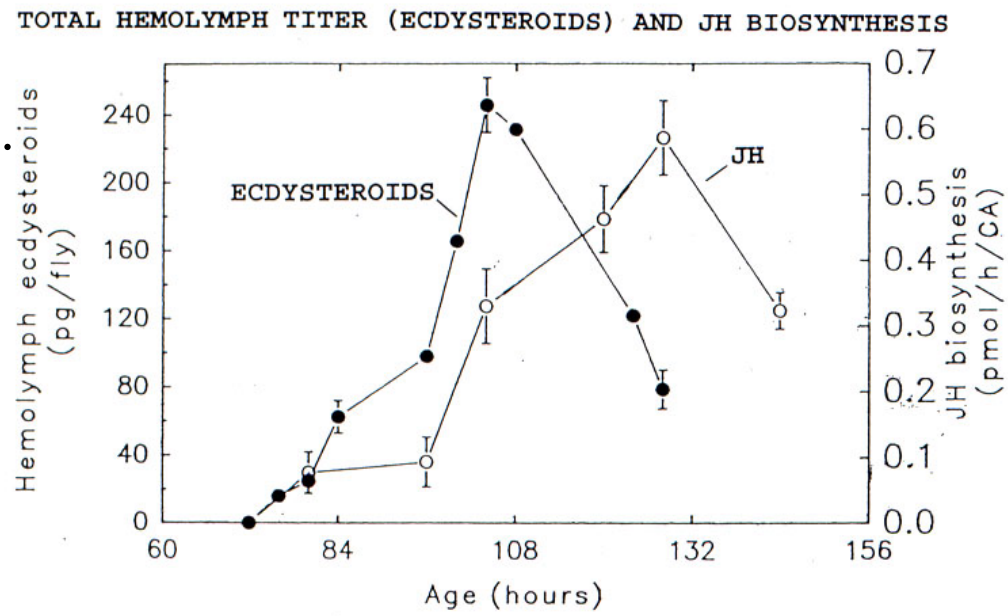
3. Regardless of age, diet, or sex the cuticular hydrocarbon contact pheromone is present.

Studies on Various Diptera that Implicate the Importance of Diet, the Corpus Allatum (CA) or Juvenile Hormone (JH) on Mating Behavior.

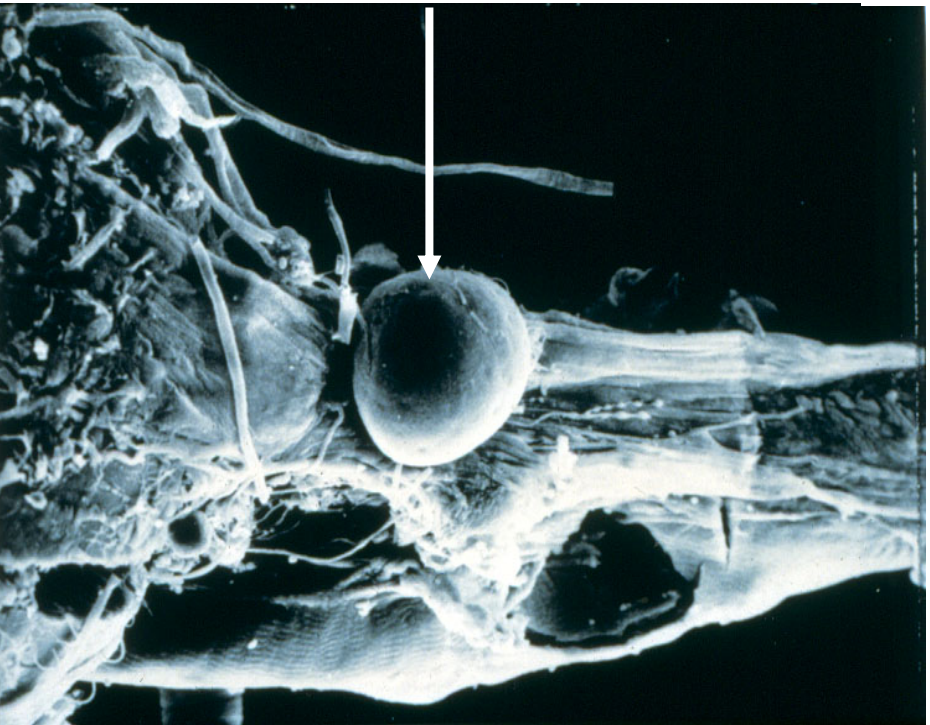
REFERENCE	DIET OR CA SPECIES + SEX	INVOLVEMENT
Adams & Hintz, 1969	♀ *M. domestica*	C.A.
Adams & Nelson, 1990	*M. domestica*	diet
Anderson, 1966	*S. calcitrans*	diet
Anderson, 1978	*S. calcitrans*	diet
Barton Browne, 1958	*L. cuprina*	diet
Barton Browne et al., 1973	*L. cuprina*	diet
Chaudhury et al., 1973	*M. autumnalis*	diet
DeClerck & DeLoof, 1983	*S. bullata*	diet
Foster, 1967	♂ *S. stercoraria*	diet
Foster, 1976	♂ *G. morsitans & austeni*	diet
Gillot & Langley, 1981	*G. Morsitans*	J.H.
Gwadz, 1972	♀ *A. aegypti*	J.H.
Gwadz et al., 1971	♀ *A. aegypti*	J.H
Lea, 1968	♀ *M. domestica*	C.A.
Lea, 1972	*M. domestica*	C.A.
Manning, 1966	♀ *D. melanogaster*	C.A.
Manning, 1967	♀ *D. melanogaster*	C.A.
Meola et al., 1977	*S. calcitrans*	diet
Odhiambo, 1968	*Glossina sp.*	diet
Sanderson & Charnley, 1983	*C. vicina*	diet
Spielman et al., 1969	♀ *A. aegypti*	
→ Stoffolano, 1974a	*P. regina*	diet
Strangways-Dixon, 1961	*C. erythrocephala*	diet
Tobin, 1979	*P. regina*	diet
Trabalon et al., 1984	*C. vomitoria*	J.H.
Trabalon et al., 1984	*C. vomitoria*	J.H.
Tyndale-Biscoe, 1971	*M. vetustssima*	diet
Webber, 1958	*L. cuprina*	diet

What about the effect of the CA + JH on mating behavior in *Phormia regina*?

Remember the previous studies on JH titers following the protein meal in ***Phormia*** (see graph to the right).



A protein meal is essential for JH production/release acting via the midgut hormone that acts on the brain neurosecretory cells to produce allatotropin, that stimulates the CA to release JH



Effect of allatectomies on male mating

Table 5
Effect of CA^- and JHA treatment[a] on the % of male inseminating females **of P. regina.**

Treatment	N[b]	% Inseminating
CA^-	6/25	24.0 ± 1.6
Sham-CA^-	18/25	72.0 ± 4.8
CA^- + JHA	15/23	65.2 ± 5.1
CA^- + Acetone	7/22	31.8 ± 5.1
Unoperated control	19/25	76.0 ± 1.6

[a] Methoprene 10 µg was topically applied at 12 h after the onset of liver meal to CA^- males. Acetone (2 µl) was topically applied to solvent control (CA^- + Acetone) flies.

[b] No. of inseminating males/No. of males tested.

Yin, Qin + Stoffolano (1999). J.I.P. 45: 815-822.

Effect of allatectomies and ovariectomies on female mating

Table 6
Effect of CA^-, OV^- and JHA treatment[a] on sexual receptivity (i.e., being inseminated) of female *P. regina*

Treatment	N[b]	% Inseminated
CA^-	11/30	36.7 ± 5.8
Sham-CA^-	18/23	78.2 ± 6.2
CA^- + JHA	15/21	71.4 ± 14.3
CA^- + Acetone	12/29	41.4 ± 2.5
OV^-	5/23	21.7 ± 6.2
Sham-OV^-	21/28	75.0 ± 5.0
Unoperated control	15/19	78.9 ± 6.9

[a] Methoprene 10 μg was topically applied at 12 h after the onset of liver meal to CA^- females. Acetone (2 μl) was topically applied to solvent-control flies.

[b] No. of females being inseminated/No. of females tested.

Female Sexual Receptivity Is Defective in Juvenile Hormone-Deficient Mutants of the *apterous* Gene of *Drosophila melanogaster*

John Ringo,[1,2] Ruth Werczberger,[3] Michal Altaratz,[3] and Daniel Segal[3]

Received 28 Aug. 1990—Final 22 Mar. 1991

Behavior Genetics 21 (no. 5, 1991)

CONCLUSIONS TO DATE ON *PHORMIA* MATING

1. Protein is essential in the diet for normal expression of mating behavior
2. Juvenile hormone is also essential in both sexes for normal mating and possibly ecdysteroids in female

Studies on Various Diptera that Implicate the Importance of Diet, the Corpus Allatum (CA) or Juvenile Hormone (JH) on Mating Behavior.

REFERENCE	SPECIES + SEX	DIET OR CA INVOLVEMENT
Adams & Hintz, 1969	♀ *M. domestica*	C.A.
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Anderson, 1978	*S. calcitrans*	diet
Barton Browne, 1958	*L. cuprina*	diet
Barton Browne et al., 1973	*L. cuprina*	diet
Chaudhury et al., 1973	*M. autumnalis*	diet
DeClerck & DeLoof, 1983	*S. bullata*	diet
Foster, 1967	♂ *S. stercoraria*	diet
Foster, 1976	♂ *G. morsitans & austeni*	diet
Gillot & Langley, 1981	*G. Morsitans*	J.H.
Gwadz, 1972	♀ *A. aegypti*	J.H.
Gwadz et al., 1971	♀ *A. aegypti*	J.H
Lea, 1968	♀ *M. domestica*	C.A.
Lea, 1972	*M. domestica*	C.A.
Manning, 1966	♀ *D. melanogaster*	C.A.
Manning, 1967	♀ *D. melanogaster*	C.A.
Meola et al., 1977	*S. calcitrans*	diet
Odhiambo, 1968	*Glossina sp.*	diet
Sanderson & Charnley, 1983	*C. vicina*	diet
Spielman et al., 1969	♀ *A. aegypti*	
Stoffolano, 1974a	*P. regina*	diet
Strangways-Dixon, 1961	*C. erythrocephala*	diet
Tobin, 1979	*P. regina*	diet
Trabalon et al., 1984	*C. vomitoria*	J.H.
Trabalon et al., 1984	*C. vomitoria*	J.H.
Tyndale-Biscoe, 1971	*M. vetuststssima*	diet
Webber, 1958	*L. cuprina*	diet

What about the role of biogenic amines on mating behavior since Murdock et. al. have shown an effect on feeding?

What about the effect of the CA + JH on mating behavior in *Phormia regina*?

REMEMBER! Sugar-fed flies fail to mate in *P. regina*

Can injecting various biogenic amines into sugar-fed flies cause them to mate?

Table I. The Effect of Biogenic Amines and Specific Agonists on Female Insemination in Sugar-Fed *Phormia regina* (152--154 h of Age)[a]

Drug	Dose (μg)	% insemination		% mortality
		Saline-injected	Drug-injected[b]	
Octopamine	30	3.3	3.3	0.0
	75	6.7	56.0**	16.7
Dopamine	30	3.3	6.7	0.0
	50	3.3	22.2*	10.0
Serotonin	30	6.7	6.7	0.0
	50	3.3	12.0	16.7
Clonidine	20	6.7	46.4**	6.7
Naphazoline	15	0.0	24.5**	2.0
Naphazoline + clonidine	5 + 20	3.3	51.9**	10.0

[a] Three replicates of 10 saline-injected and 10 drug-injected females per replicate were performed with the exception of naphazoline, for which five replicates were performed.

[b] Insemination percentages significantly different from the saline-injected females by the chi-square test are indicated by asterisk superscripts (*$P < 0.05$; **$P < 0.001$).

Biogenic amines can induce mating in sugar-fed female *P. regina*

The graph to the right shows that at various hours following injection of clonidine, octopamine agonist, there is an increase in the number of sugar-fed females that now mate.

Evans, Stoffolano, Yin and Meyer. 1997. A pharmaacological and endocrinological study of female insemination in *Phormia regina* (Diptera: Calliphoridae). Jour. Insect Behavior 10: 493-508.

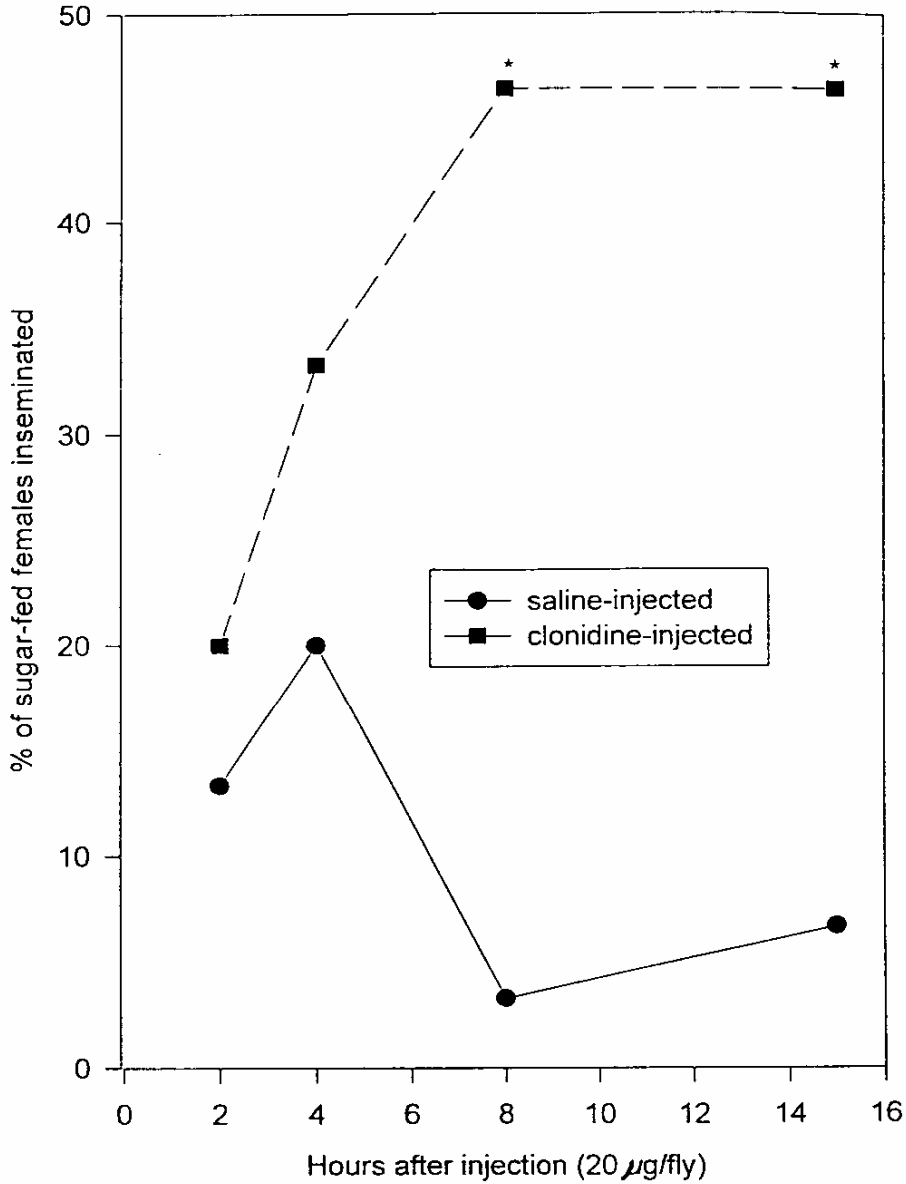


Fig. 1. The mean percentages of sugar-fed, female *Phormia regina* (152–154 h of age) inseminated at various times following injection with clonidine (20 μg) (n = 30 for each point). Squares indicate clonidine-injected females and circles indicate saline-injected females. Insemination percentages significantly different (chi-square tests; $P < 0.001$) from those of the saline-injected females are indicated by an asterisk.

MANIPULATION OF JH ON MATING IN FEMALE *P. REGINA*

Table III. The Effect of Methoprene (JHA) on Female Insemination in Sugar-Fed *Phormia regina* (152–154 h of Age)

Treatment	#/*n*[a]	% insemination*	% mortality[b]
Acetone	0/39	0.0 A	0.0
Methoprene (2 × 5 μg)	32/39	82.0 B	0.0
Methoprene (2 × 10 μg)	30/38	78.9 B	2.6

[a] The number of females inseminated over the total number of females through three trials.
[b] The mortality observed prior to placing the female and males together.
* Percentages within the same column not followed by the same letter indicate significantly different insemination percentages, chi-square tests; $P < 0.001$.

MANIPULATION OF JH ON MATING IN FEMALE *P. REGINA*

Table IV. The Effect of Precocene on Female Insemination Enhanced by Clonidine in Sugar-Fed *Phormia regina* (152–154 h of Age)

Treatment	#/*n*[a]	% insemination*	% mortality[b]
Group 1 (2× precocene)			
Saline + acetone	1/45	2.2 A,C	2.2
Clonidine + acetone	16/45	35.6 B,D	8.9
Saline + precocene	2/39	5.1 A,C	2.6
Clonidine + precocene	18/39	46.2 B,D	10.3
Group 2 (3× precocene)			
Saline + acetone	0/43	0.0 A,C	0.0
Clonidine + acetone	13/43	30.2 B,D	7.0
Saline + precocene	1/45	2.2 A,C	0.0
Clonidine + precocene	15/45	33.3 B,D	6.7

[a]The number of females inseminated over the total number of females through three trials.
[b]The mortality during the 15-h period the female was with the three males.
*Percentages of each group which are not followed by the same letter are significantly different insemination percentages, chi-square tests; $P < 0.001$.

The CA and its hormone, JH are essential for mating

Is the biogenic amine effect (clonidine) on mating upstream or downstream of the JH effect?

Table V. The Effect of Clonidine on Female Insemination in Allatectomized, Sugar-Fed Female *Phormia regina* (152–154 h of Age)

Treatment	#/*n*	% insemination*	% mortality[a]
Ca^- + saline	0/27	0.0 A	13.3
CA^- + clonidine	14/25	56.0 B	16.7
CA + saline	1/31	3.2 A	0.0
CA + clonidine	16/30	53.3 B	0.0

[a]The mortality during the 15-h period the female was with the three males.

*Percentages within the same column not followed by the same letter are significantly different insemination percentages, chi-square test; $P < 0.001$.

Sugar-fed flies without a CA fail to mate but if clonidine is added they do

JH and clonidine together are not additive or synergistic

Clonidine, an octopamine agonist, causes sugar-fed flies to mate.

Can we inhibit mating in liver-fed flies if we deplete the biogenic supply in the brain by injecting the liver-fed flies with d-amphetamine?

This shows that one can effectively reduce mating in liver-fed flies by injecting d-amphetamine even though the flies have started to make JH

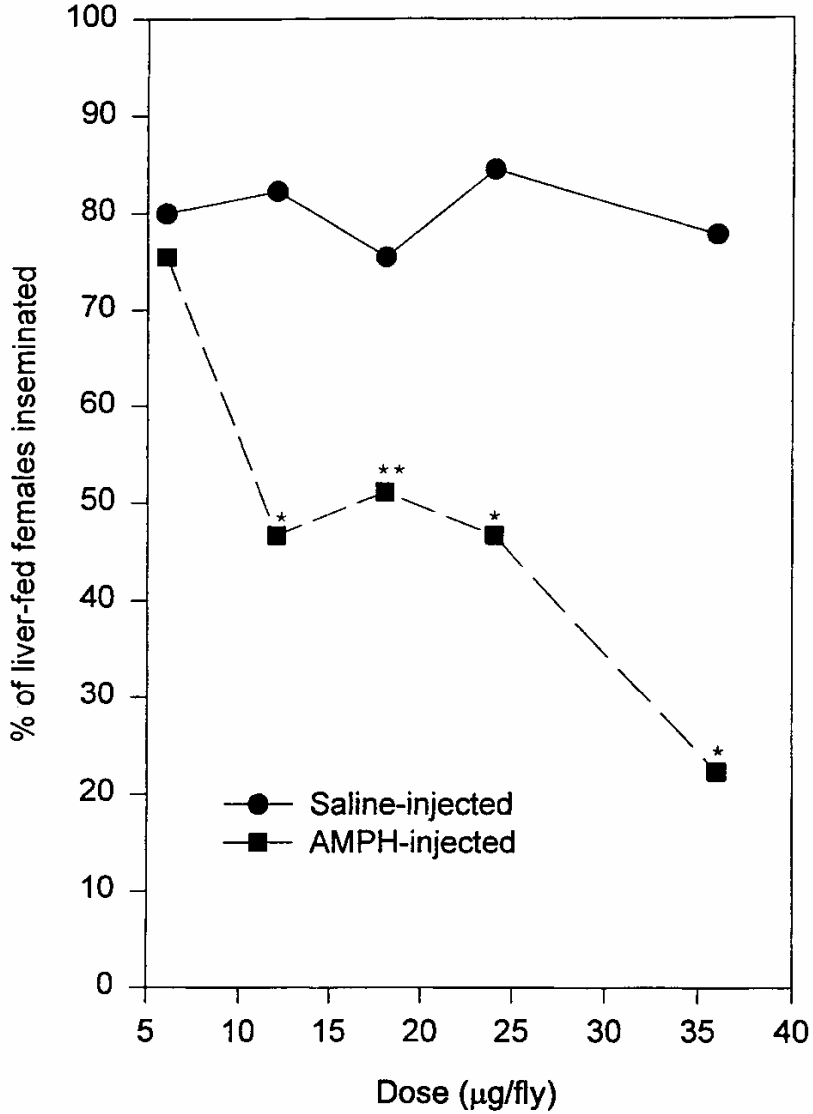


Fig. 1. The effect of various doses of amphetamine on insemination in female *Phormia regina* (80–88 h after onset of liver feeding). One injected female was placed with three normal males from 2–90 min post-injection. Three replicates of fifteen females per replicate were performed for each dose. Means of treated groups significantly different from the controls are indicated by $*P < 0.025$ and $** P < 0.001$ (χ^2-test).

Evans, Stoffolano, Yin and Meyer.
1998, The effects of injection of amphetamine on female insemination in the black blow fly, *Phormia regina* (Diptera: Calliphoridae). Phys. Entomol. 23: 20-24.

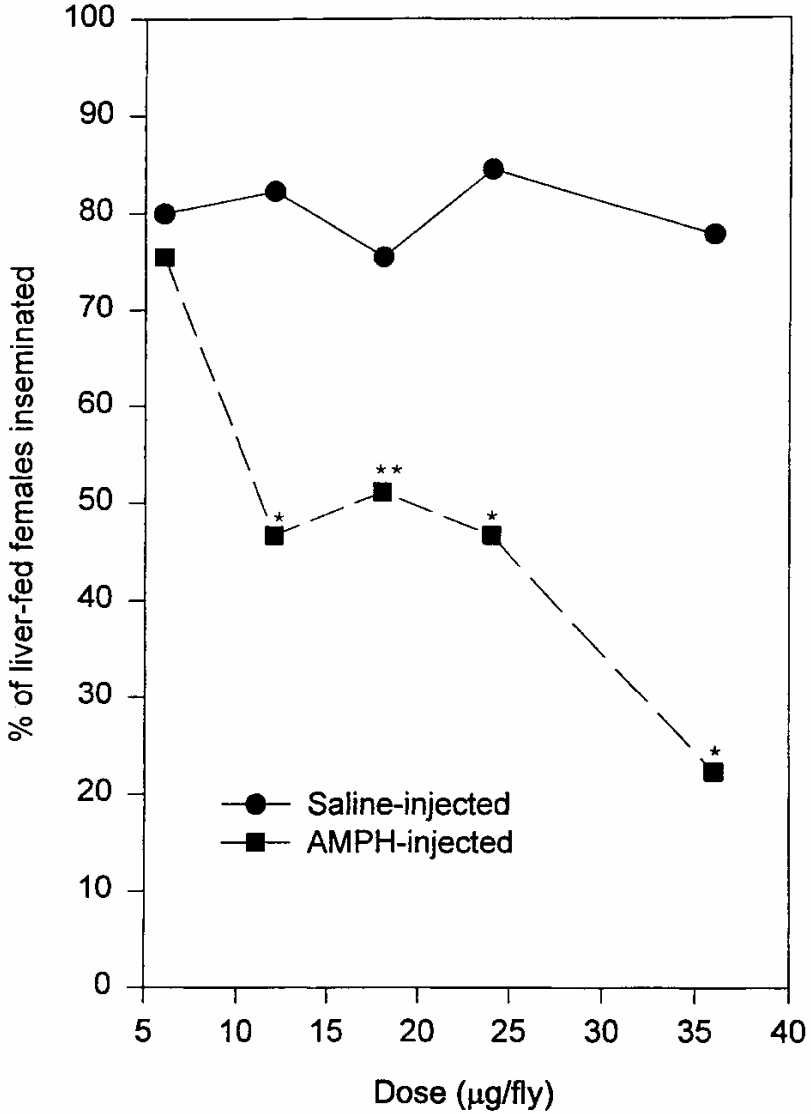
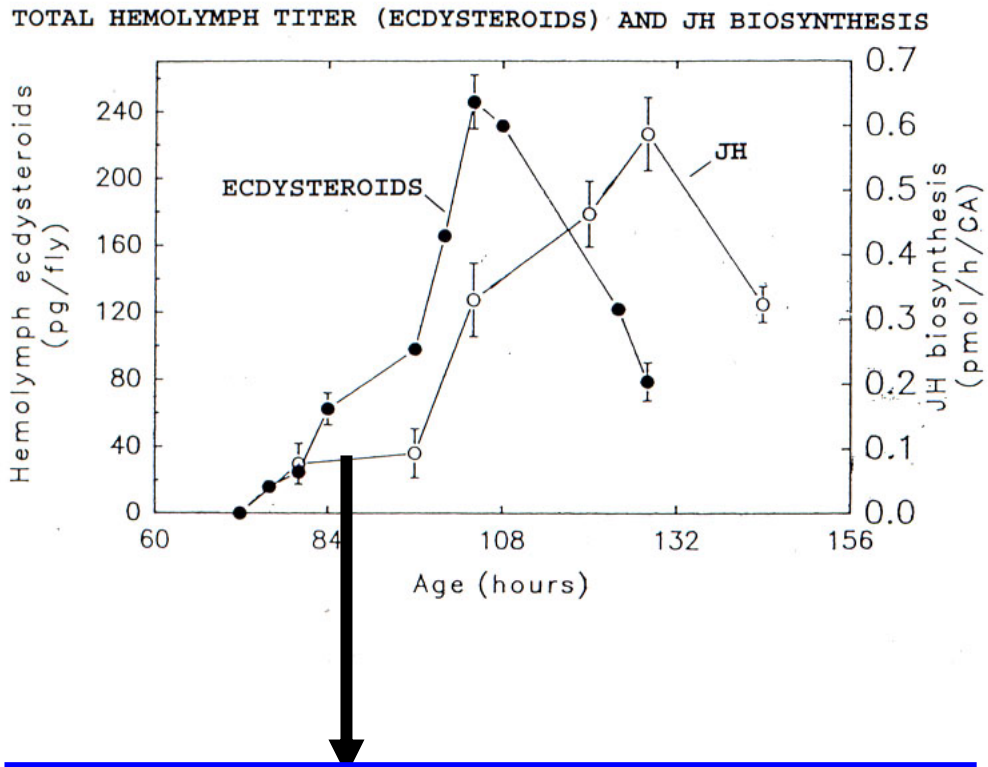
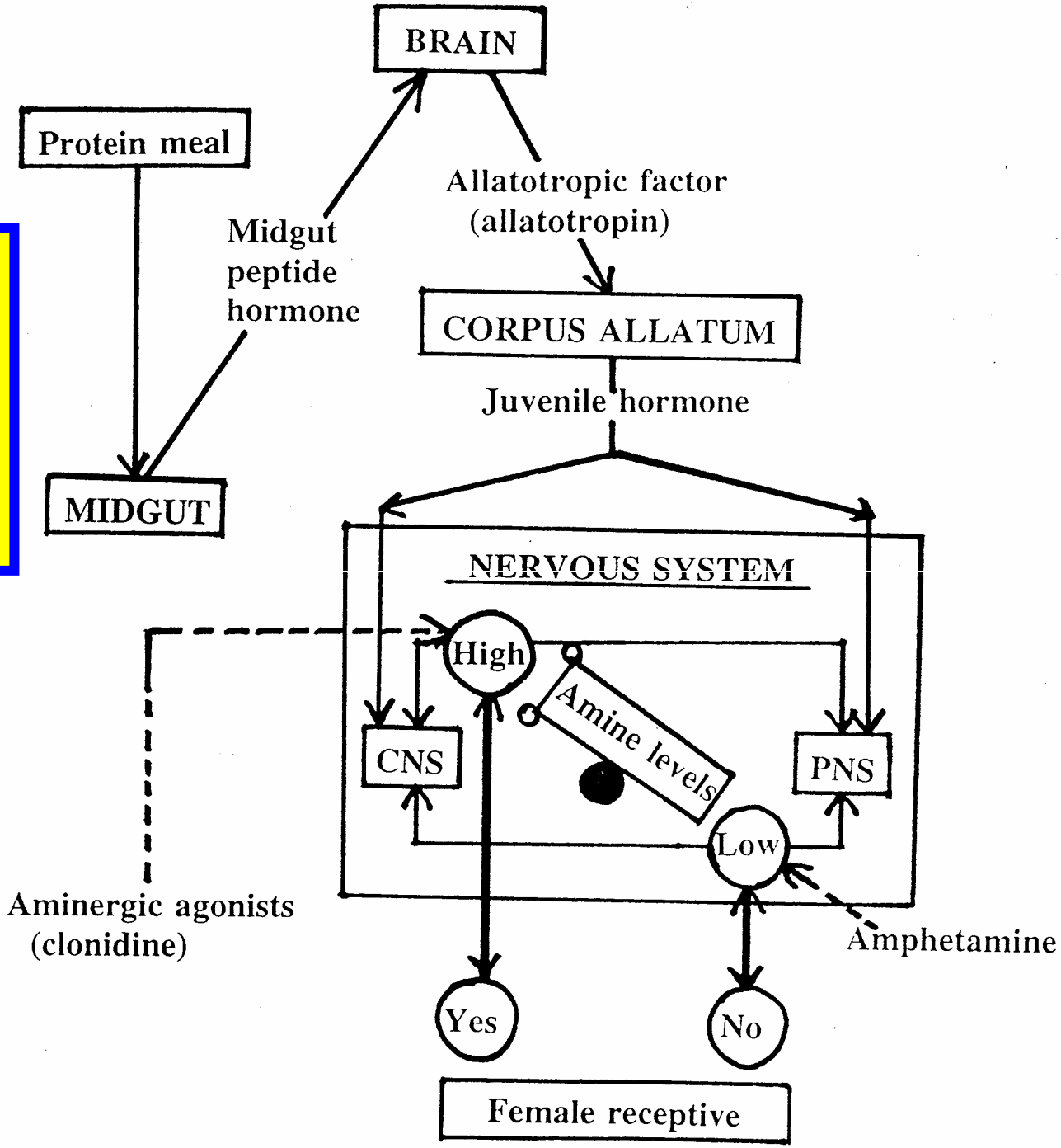


Fig. 1. The effect of various doses of amphetamine on insemination in female *Phormia regina* (80–88 h after onset of liver feeding). One injected female was placed with three normal males from 2–90 min post-injection. Three replicates of fifteen females per replicate were performed for each dose. Means of treated groups significantly different from the controls are indicated by $*P < 0.025$ and $** P < 0.001$ (χ^2-test).

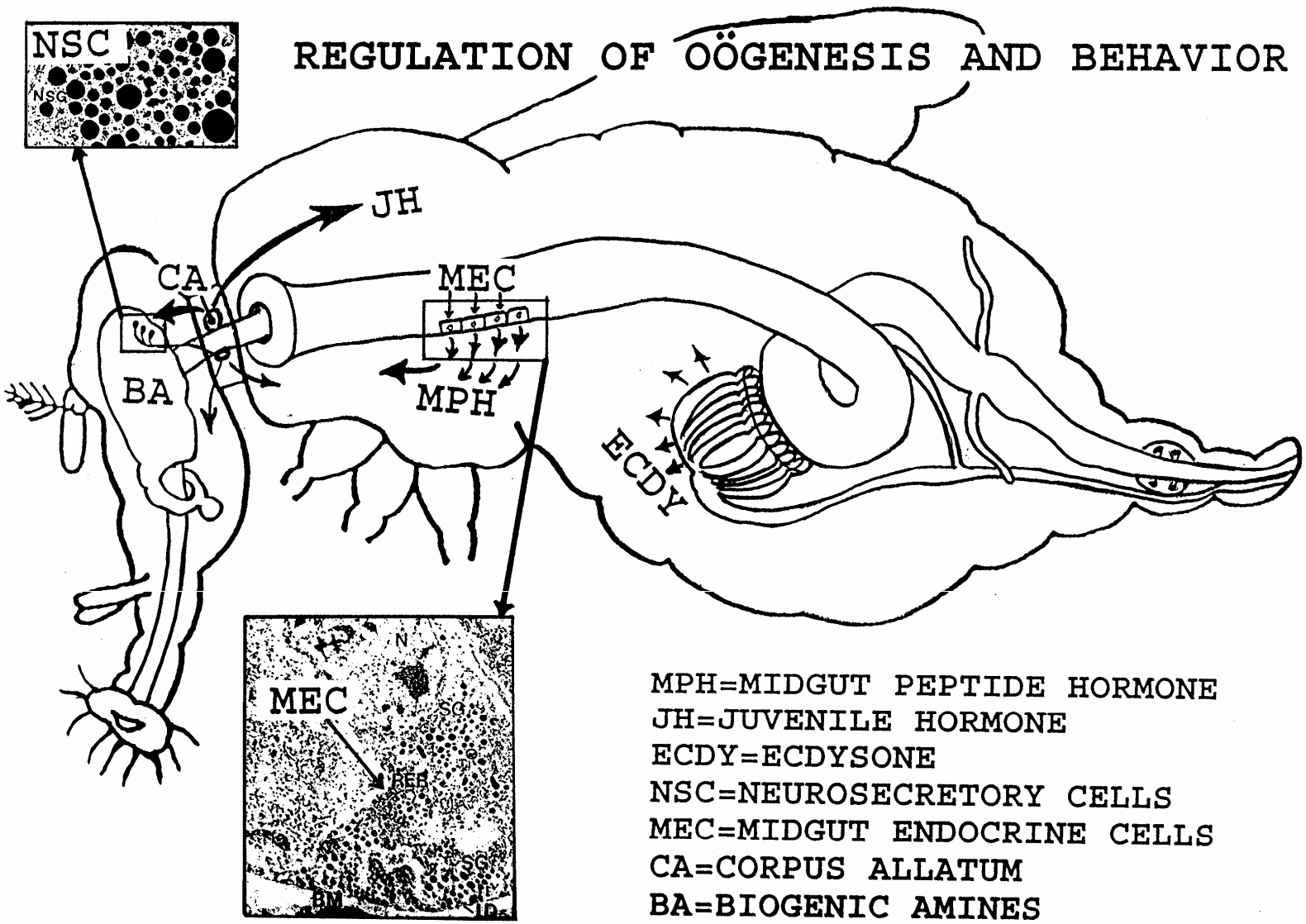


At 80-88 hrs. after liver feeding, note that JH is already present and the d-amphetamine depletion of the biogenic amines is able to drastically reduce mating in these females

Proposed scheme showing the importance of protein, JH and biogenic amine levels on successful mating in *Phormia regina*



It is now evident that oogenesis and mating behavior in *P. regina* are linked by protein, various hormones and biogenic amines



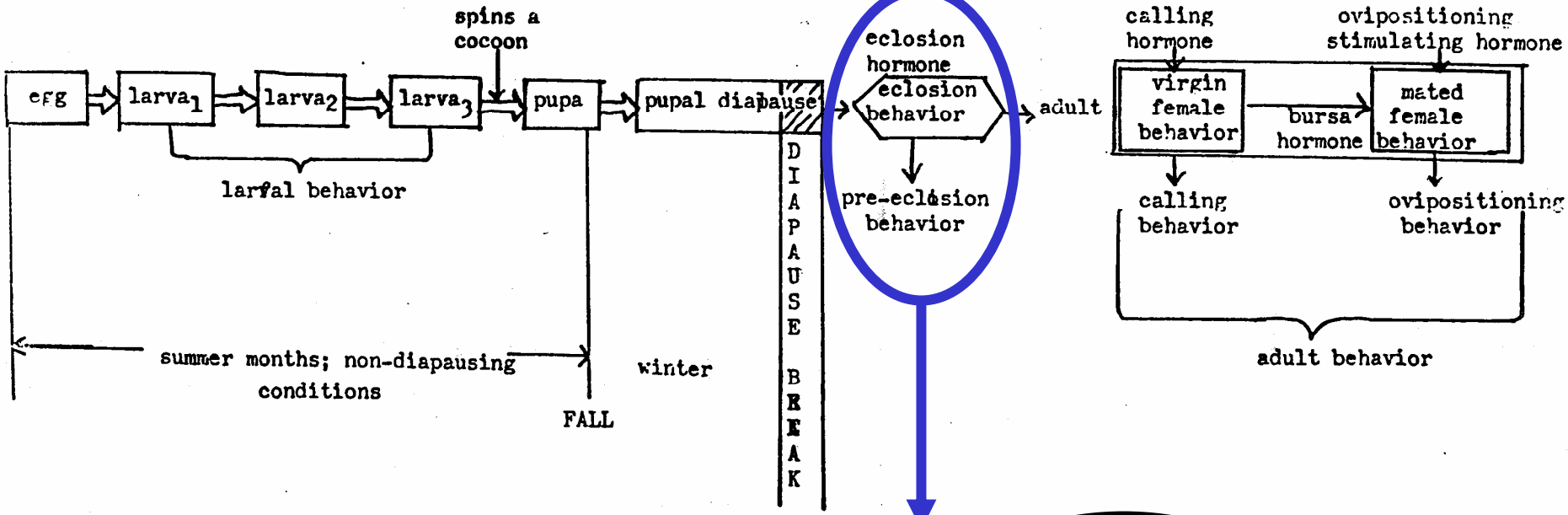
ECLOSION BEHAVIOR-turning on adult behavior

Getting out of the pupal case and cocoon

1. Correct timing
2. Getting out of the pupal case
3. Getting out of the cocoon
4. Switching from pupal to adult behavior

5. **The wings must be expanded or spread**

Hyalophora cecropia-a great model system



Brain (timer) Neurosecretory cells → **Corpora cardiaca ECLOSION HORMONE** → **Preprogrammed Motor program in Abdominal ganglia** → **Program Read out**

Operations performed on pupal brains

Each species of moth has its own eclosion gate as when to eclose.

1. Brain contains the endogenous, circadian clock for eclosion time and is species specific
2. Brain contains the directions for starting and putting into motion all of the events involved in eclosion behavior. This is the neuropeptide called the eclosion hormone

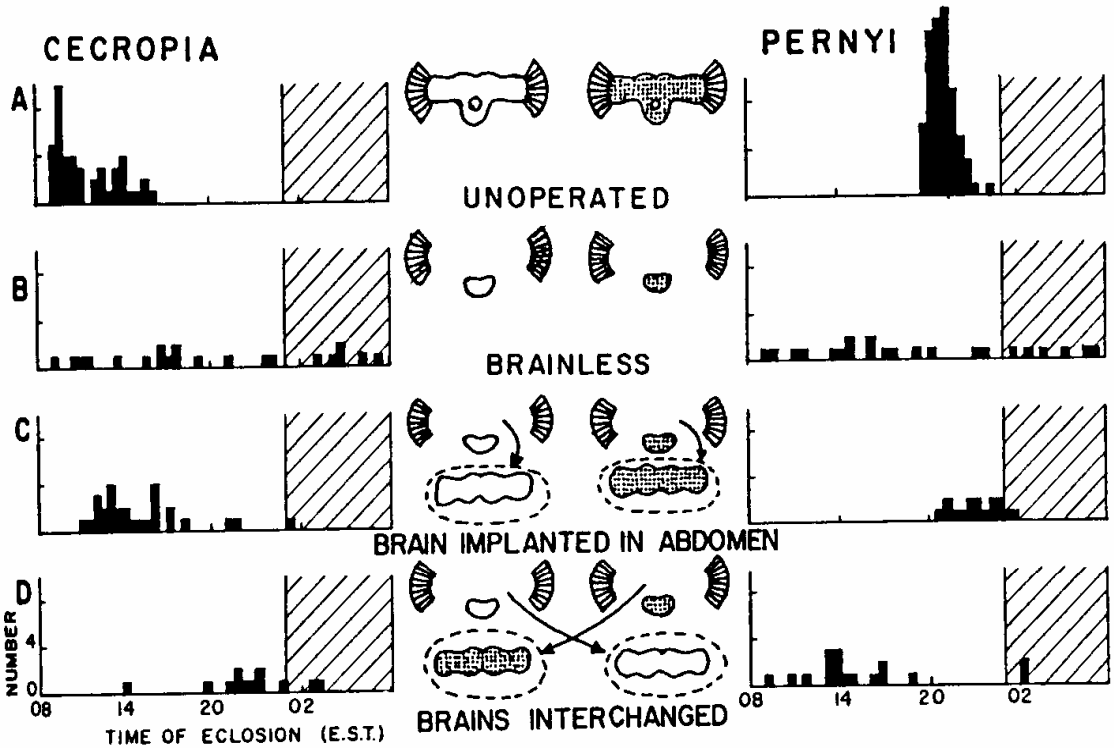
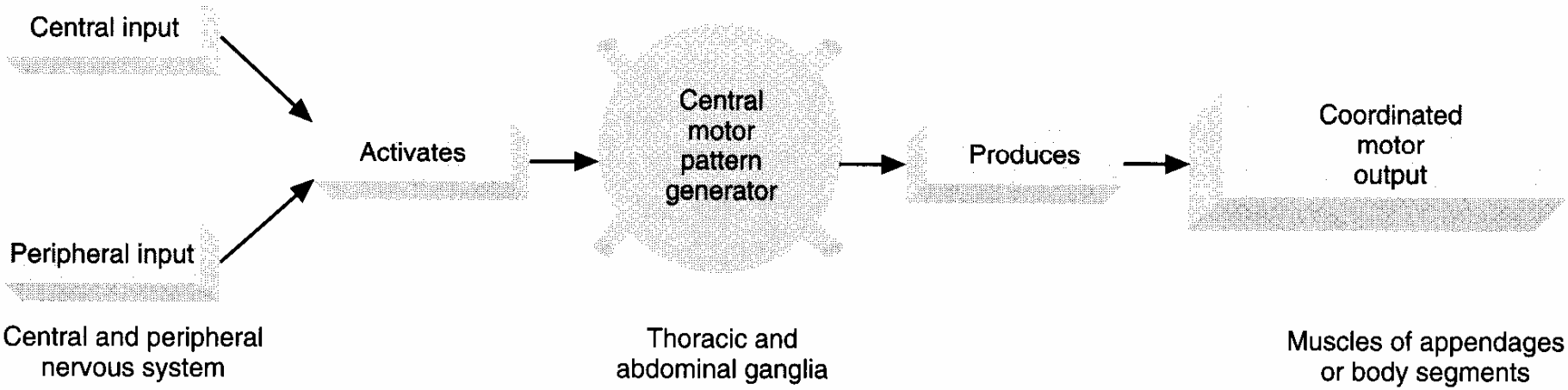
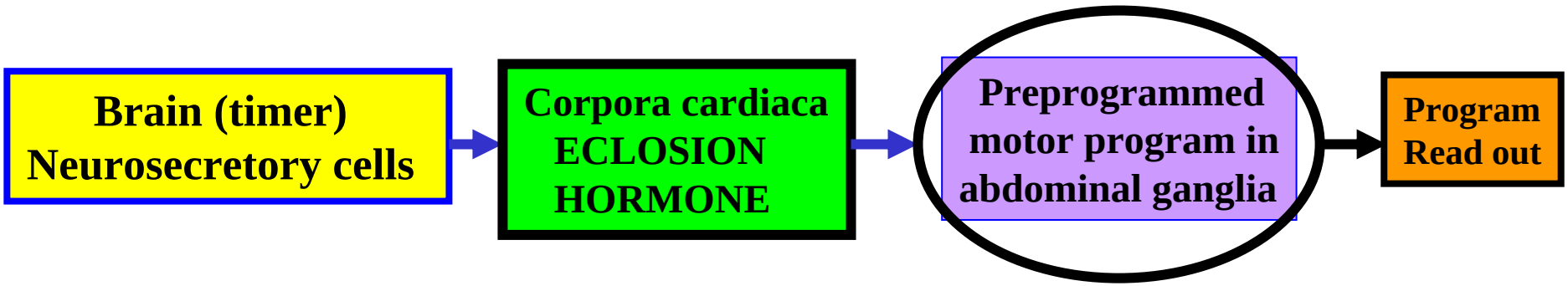
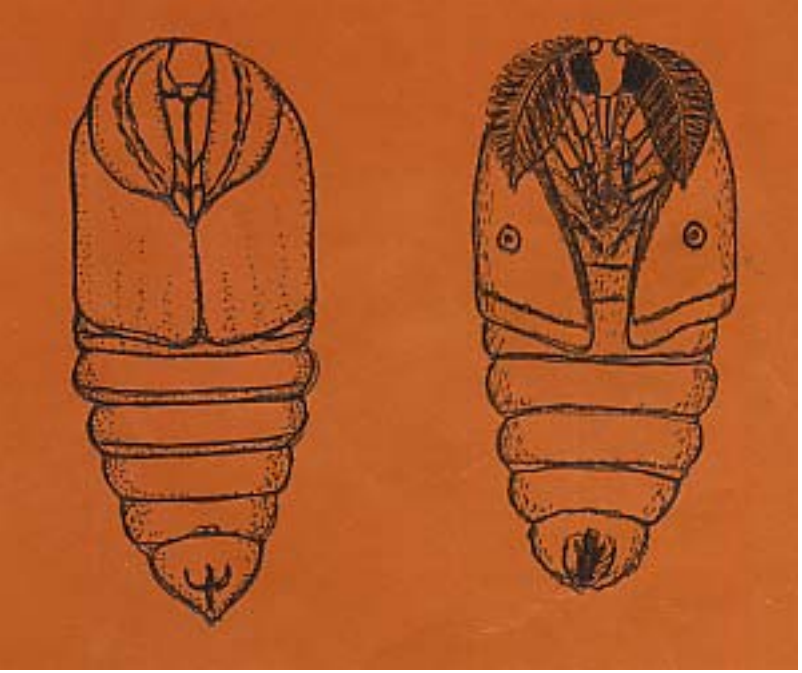


Figure 9.1. Timing of eclosion of *Hyalophora cecropia* and *Antheraea pernyi* under a 17L : 7D photoperiod regimen (daytime is white, nighttime is cross-hatched). (A) Species-specific eclosion times of intact unoperated animals. (B) Brainless animals eclose at random times during the day and night. (C) Brains reimplanted into the abdomens of brainless animals restore the normal eclosion time for each species. (D) Brains interchanged between the two species and implanted into the abdomen cause each to eclose at the time characteristic of the brain donor, although the eclosion behavior remains characteristic of the recipient. (From Truman, 1971b. Reprinted with permission of Pudoc Press.)

Figure 4.23 Schematic model of nervous control and coordination of preprogrammed motor outputs or central motor pattern generators.







Pharate moth inside pupal case (left) and pharate moth outside pupal case (right). Pharate moths left outside and on a table exhibit pupal behavior, only rotating abdomen if touched. When, however, they are 'ready' to emerge, two new types of behaviors (rotatory and peristaltic) emerge.

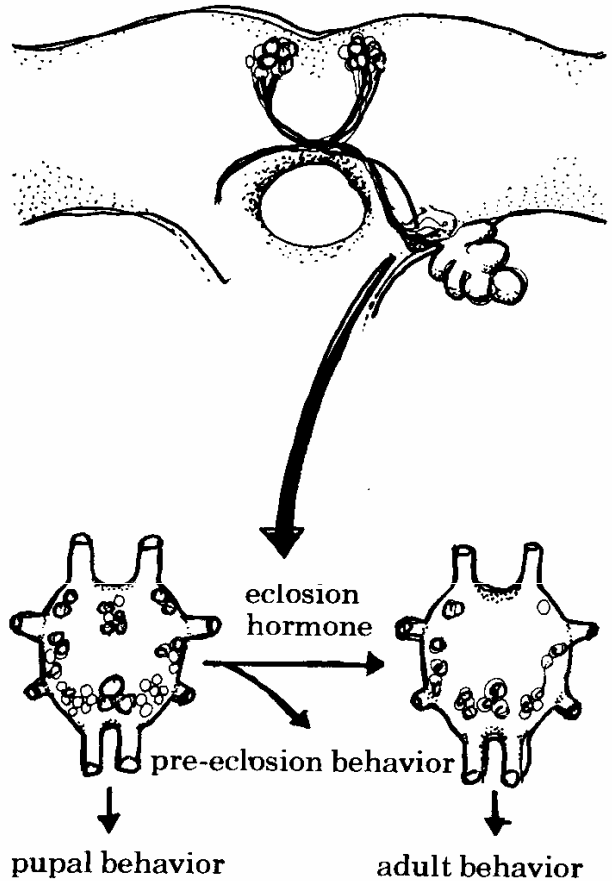


Figure 7. The eclosion hormone, which is produced in the median neurosecretory cells of the brain, is released into the blood from the corpora cardiaca. It then acts on the abdominal ganglia to trigger the pre-eclosion behavior and to turn on adult behavior. The death of the neurons which presumably were responsible for pupal behavior then follows.

Truman, J.W. 1973. How moths 'turn on'" a study of the action of Hormones on the nervous system. Amer. Sci. 61: 700-706.

The program below begins 0.5 hrs after the eclosion hormone is added & lasts for 1.5 hrs.

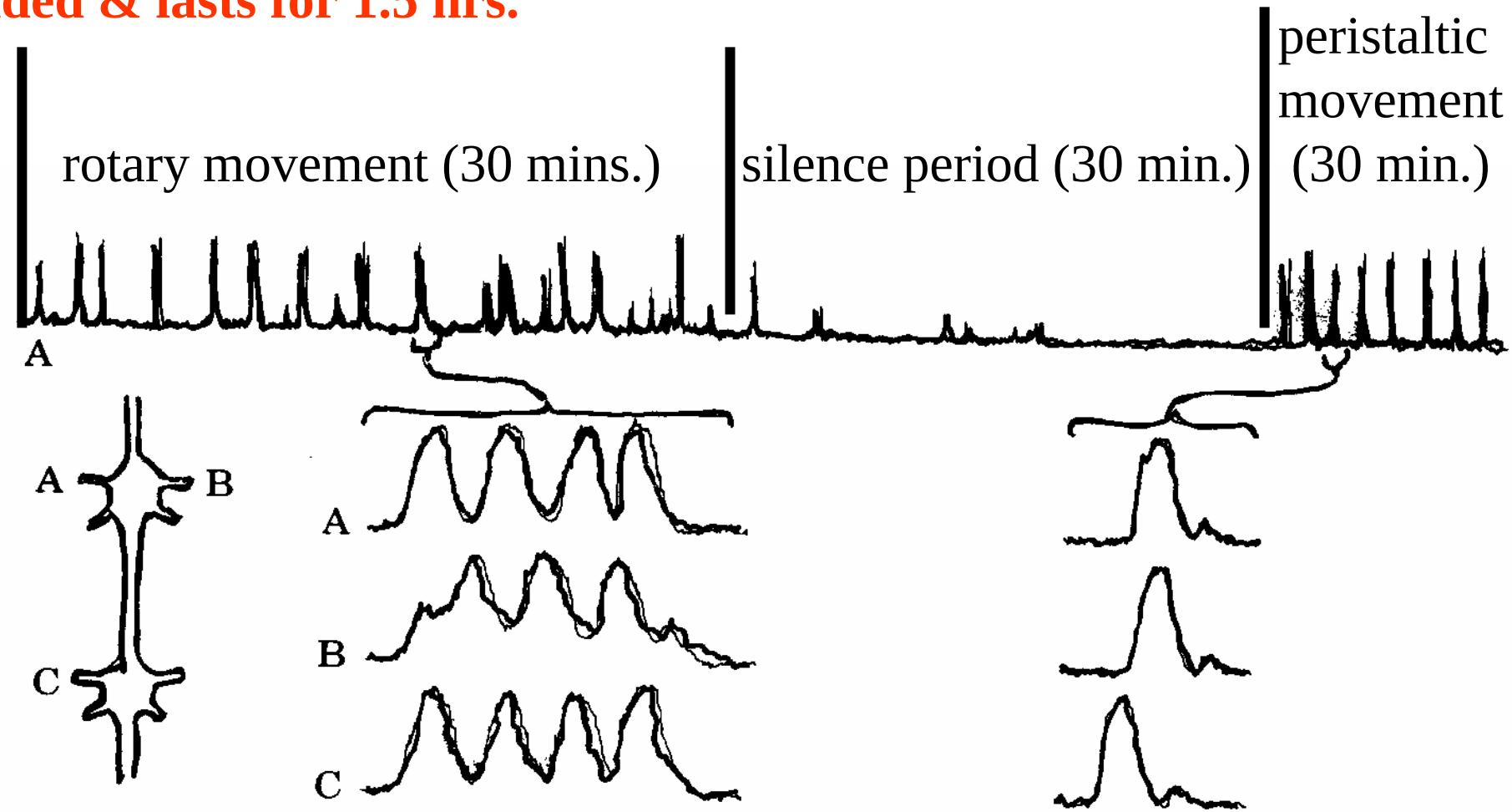
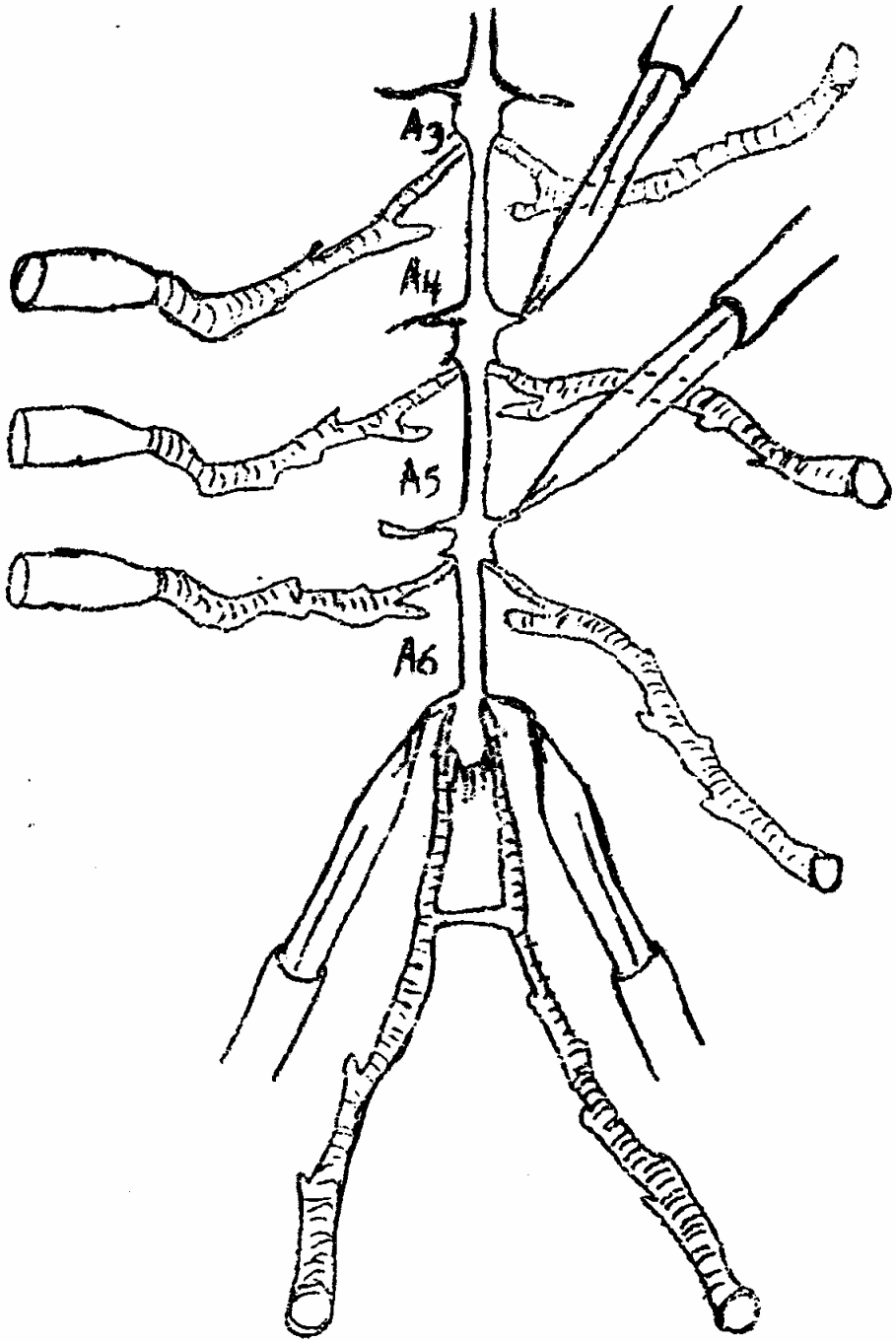


Figure 6. Response of a deafferented Cecropia abdominal nerve cord to the addition of the eclosion hormone. Across the top is a record of the integrated motor activity that begins approximately 0.5 hour after hormone addition and lasts for 1.5 hours; below are higher speed recordings that show the "fine structure" of the motor bursts. Letters indicate the dorsal nerve from which the recording was obtained. (Drawings based on data from ref. *19*, Truman and Sokolove 1972.)

What one thinks they have may not be the whole story????

Initially when Truman did his recordings he found that it was essential to aerate the preparation via a system attached to the trachea going to the abdominal ganglia. In a discussion with Jim at a meeting he said the story was not that simple. In otherwords, there was more to the story than just needing the tracheal system.

A new discovery and a new hormone



EXPTS. BASED ON THE ISOLATED ABDOMINAL GANGLIA

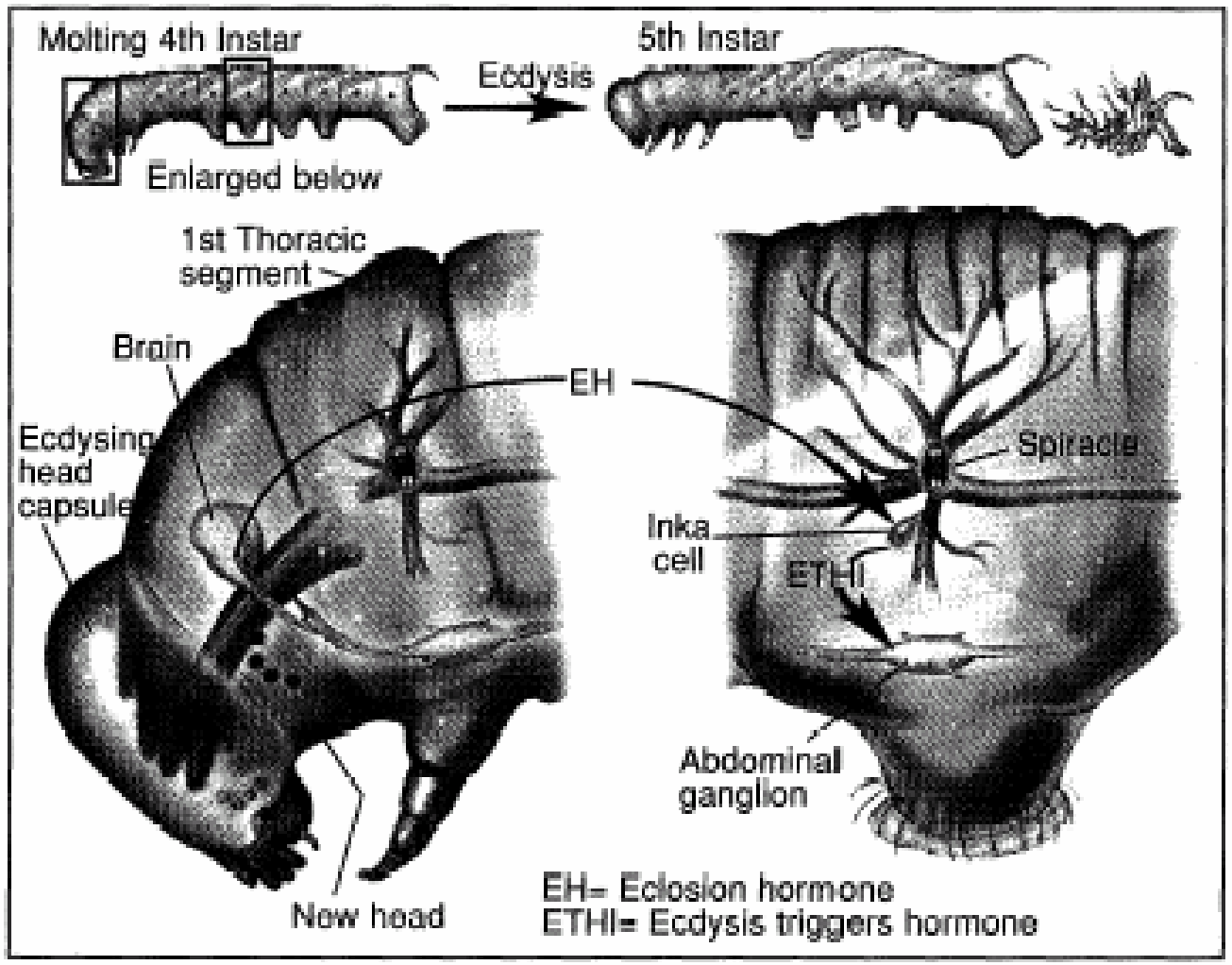
1. **Without the tracheal oxygenation the system doesn't respond to the eclosion hormone treatment**

2. **Without the tracheal oxygenation the system does respond to Mas-ETH by generating both the pre-ecdysis and ecdysis motor programs**

3. **Without the tracheal oxygenation the system can be made to respond to eclosion hormone if the inka cells are placed into the invitro bath**

4. **It appears that the inka cells monitor the changes in during the molt at the tracheal/spiracle level. The lining removal of the trachea too soon will damage the oxygen delivery system, thus death**

Ecdysis control sheds another layer. James Truman. Science 1996. 271: 40-41.



Mechanism of ecdysis control. The pathway for triggering ecdysis behavior originates in the brain but is relayed through the Inka cells, a set of glands in the periphery.

Identification of ecdysis-triggering hormone from an epitracheal endocrine system. Zitnan et al. Jan. 5, 1996. Science 271: 88-91.

Mas-ETH=*Manduca sexa* eclosion triggering hormone

Manduca sexta

A=locatioin of 18 epitracheal glands (EG)
Each is composed of an inka cell and a glandular cell

B=EG attached to large tracheal trunk that is immediately adjacent to spiracle

C=EG 3 hr before ecdysis showing inka cells (arrow), white and opaque

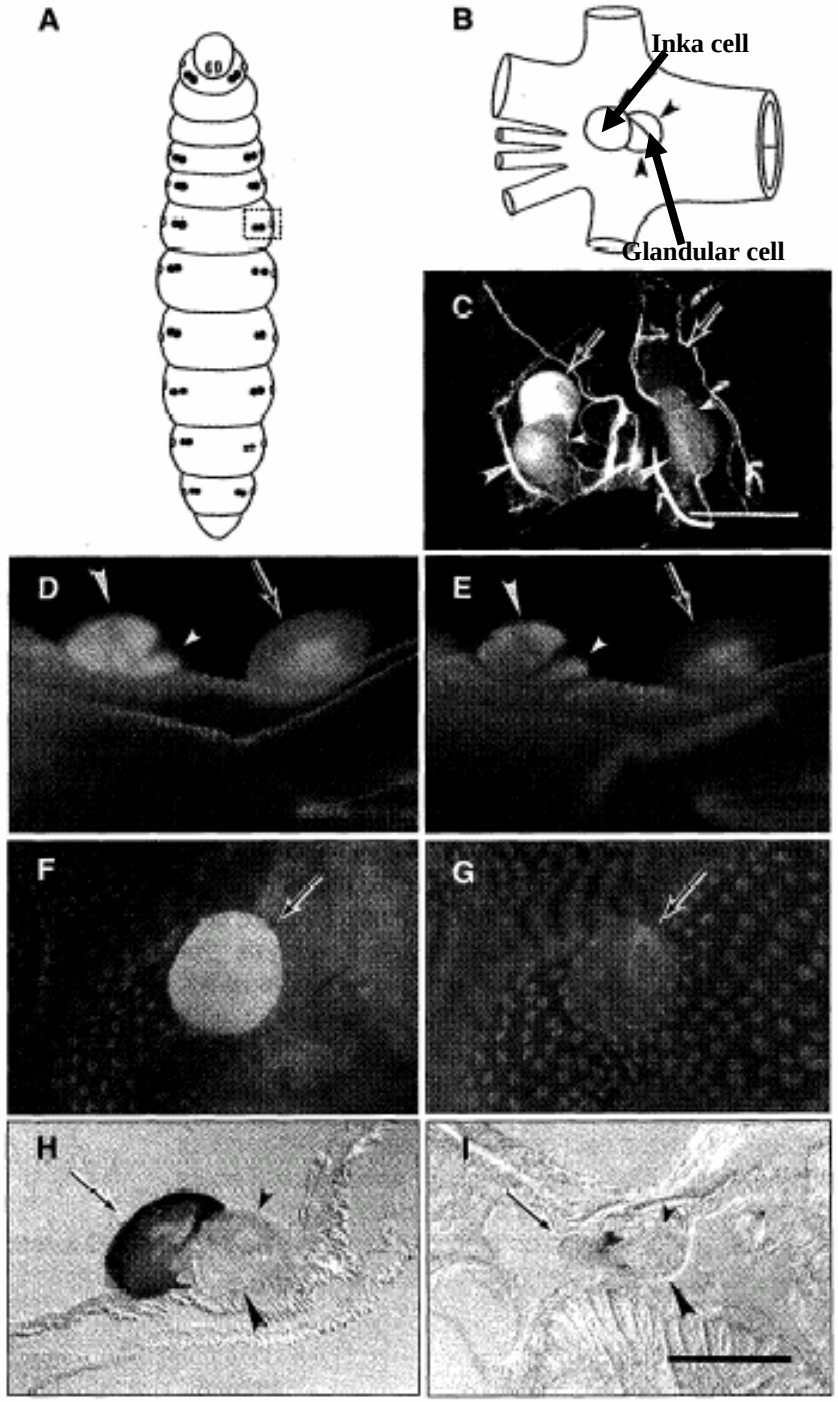
D+E=Both inka and glandular cells are found in pupa

F+G=Glandular cells are absent in pharate adult

H=Dark stained inka cell in pharate pupa 3 hrs before ecdysis

I=Inka cells of pharate pupa just at the initiation of ecdysis behavior. All of its secretion has been released

Inka glands are present in larvae, pupae and adults



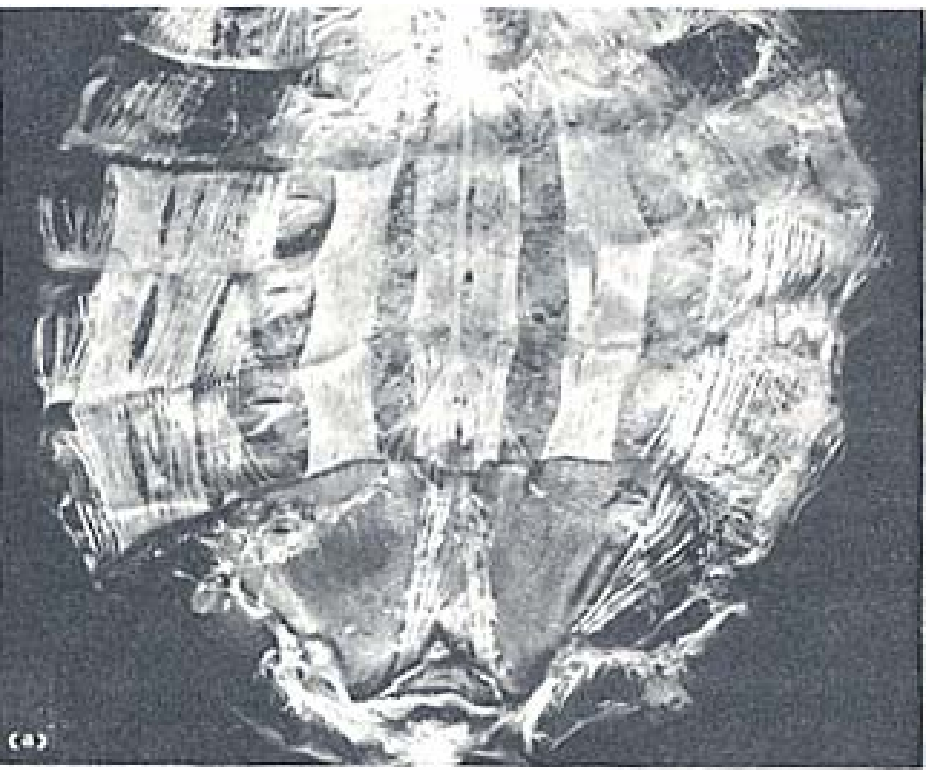
In addition to getting out of the pupal case and cocoon, plus expanding its wings, do you remember something about the muscles used by the moth to exit the pupal case and getting out of the cocoon.

A signal (**eclosion hormone**) serves to turn off the motor neurons that supply the abdominal intersegmental muscles and these muscles undergo pre-programmed cell death (see next set of slides)

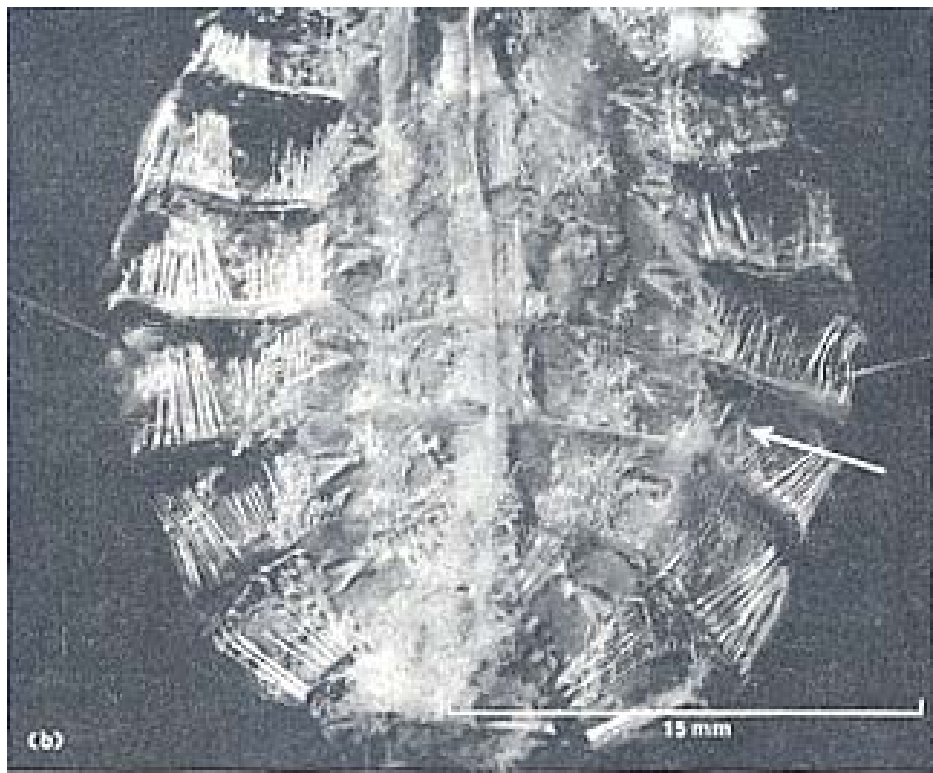
Antheraea polyphemus moth-newly emerged adults still have the 4-6th abdominal longitudinal muscles while in an adult 4 days following emergence these muscles are absent. Where did they go?

From Finlayson, 1956. Quart. J. micros. Sci. 97:215-233
This was a morphological study that reported that something happened to the muscle sets in abdominal segments 4-6.

Newly emerged adult



Adult 4 days after emergence



In 1960, ligation experiments showed that a factor from the brain and thorax influences degeneration of the intersegmental muscles in segments 4-6

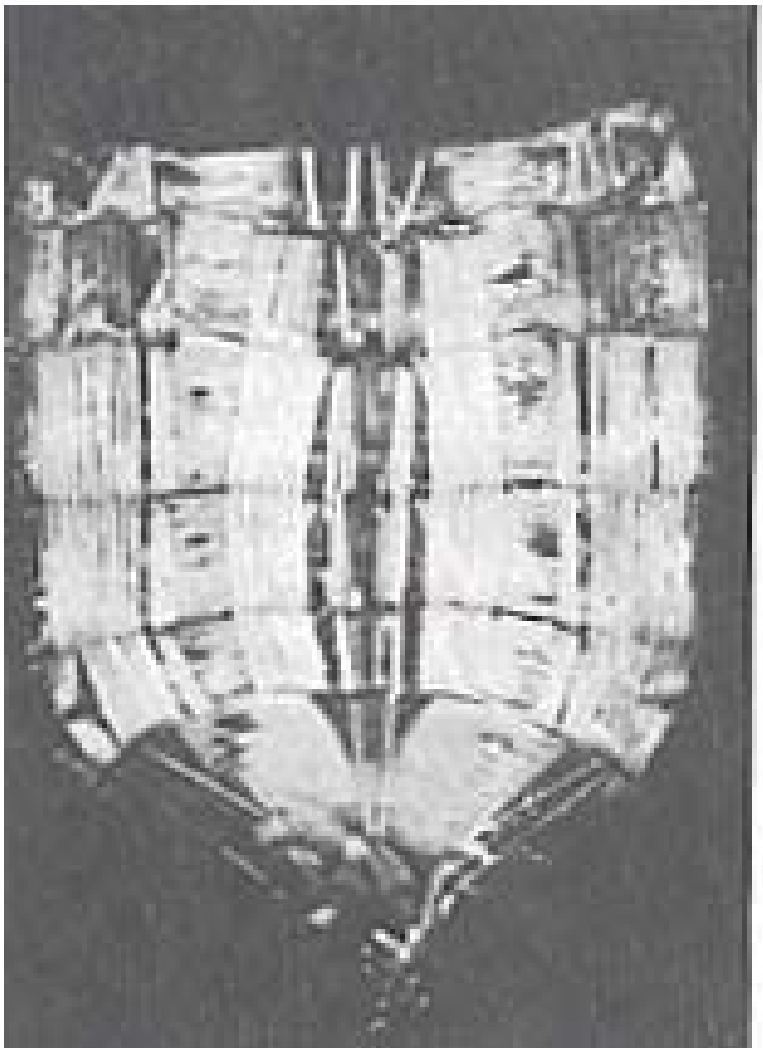
Ligated the head and thorax from the abdomen at different times

Before adult eclosion	After adult eclosion
Muscles did not degenerate	Muscles degenerated within 30 following eclosion

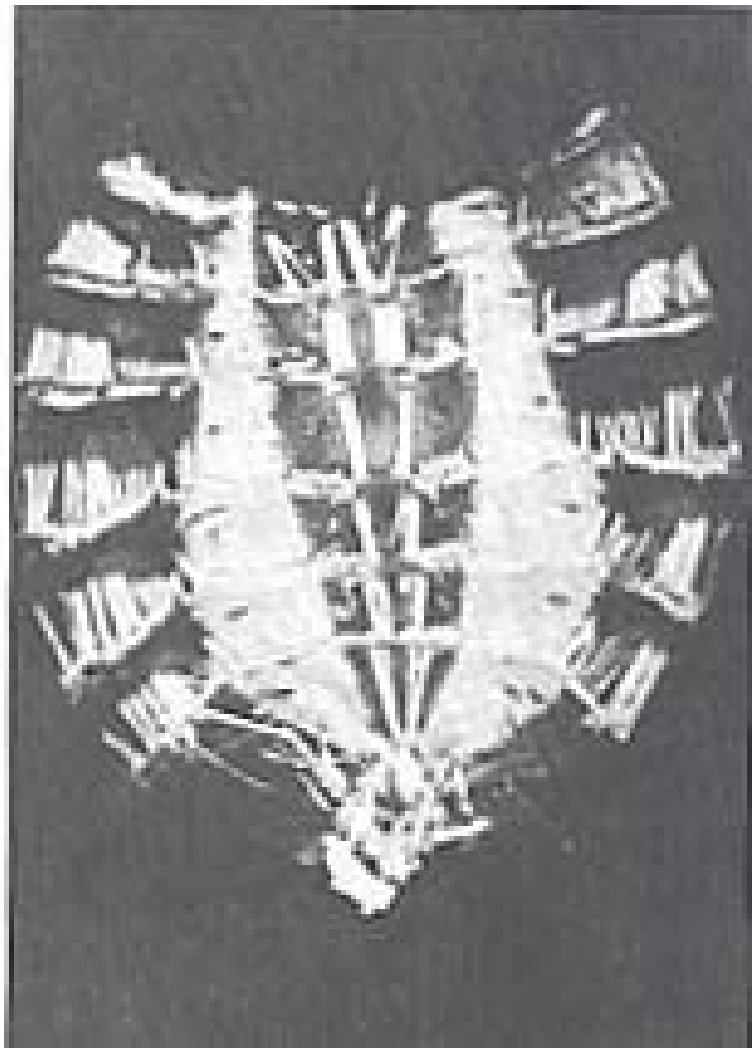
CONCLUSION:A factor from the brain and also one from the thorax are released at eclosion and are involved in muscle degeneration

Schwartz and Truman-*Manduca sexta*-

Before adult eclosion



36 hrs after adult eclosion



Truman's lab. showed that two hormones were involved in muscle degeneration at the time of eclosion and that there were two types of muscle degeneration, each under different control

Slow took about 6 days-Ecdysone hormone from the ecdysial glands activates this process and starts the molt

Fast took about 30 hrs-Involves both eclosion hormone and a new peptide eclosion hormone called the ecdysis-triggering hormone.

Just prior to adult eclosion the ecdysone titer declines
This decline sets the stage for the muscles (nerves going to them) to respond to the ecdysis-triggering hormone

Kafatos, F. C. and C. M. Williams. 1964. Enzymatic mechanism for the escape of certain moths from their cocoons. Science 146: 538-540.

Kafatos noticed that prior to emergence from the cocoon that the one end of the cocoon became wet. Further examination resulted in the discovery of an enzyme (cocoonase) that came from the anterior of the moth and was essential in softening that area of the cocoon that permited the moth to escape.

Notice the wet spot at the anterior End of the cocoon that is probably a cocoonase similar to that found by Kafatos and Williams in cecropia. This aids in digesting and softening that are of the cocoon, thus permitting the escape of the adult moth

Holometabolous insects emerge with the wings in a folded state and must expand them. In order to do this, the following sequence is turned on for wing spreading:

1. **The release of CCAP (crustacean cardioactive peptide) from the subesophaeal ganglion**
2. **Tonic contraction of abdomen forces hemolymph into wings**
3. **Bursicon released from ventral abd. Ganglia that causes plasticization of wing cuticle and starts tanning process**
4. **Release of bursicon is prevented by mechanical contact with cocoon or as in Diptera, the pupal case**
5. **Cardioactive peptide increases heat beat, thus aiding in wing expansion.**
6. **Once expanded tanning is completed and wings become functional**

HORMONAL CONTROL OF EVENTS AT A MOLT. JH IS NOT SHOWN.

CCAP=CRUSTACEAN CARDIOACTIVE PEPTIDE
EH=ECLOSION HORMONE
ETH=ECDYSIS TRIGGERING HORMONE
PTTH=PROTHORACICOTROPHIC HORMONE

CONTROL OF APOLYSIS AND CUTICLE PRODUCTION

1 PTTH stimulates synthesis and release of ecdysone

2 ecdysone in hemolymph

3 ecdysone hydroxylated at tissues

4 20-hydroxyecdysone regulates genes producing cuticle

CONTROL OF ECDYSIS

5 ecdysis triggering hormone causes release of eclosion hormone

5a ecdysis triggering hormone switches on pre-eclosion behavior

6 positive feedback loop between ETH and EH results in massive release of EH

7 central release of EH causes release of CCAP

7a EH acting via hemolymph plasticizes cuticle

8 CCAP switches on eclosion behavior and switches off pre-eclosion behavior

CONTROL OF EXPANSION AND SCLEROTIZATION

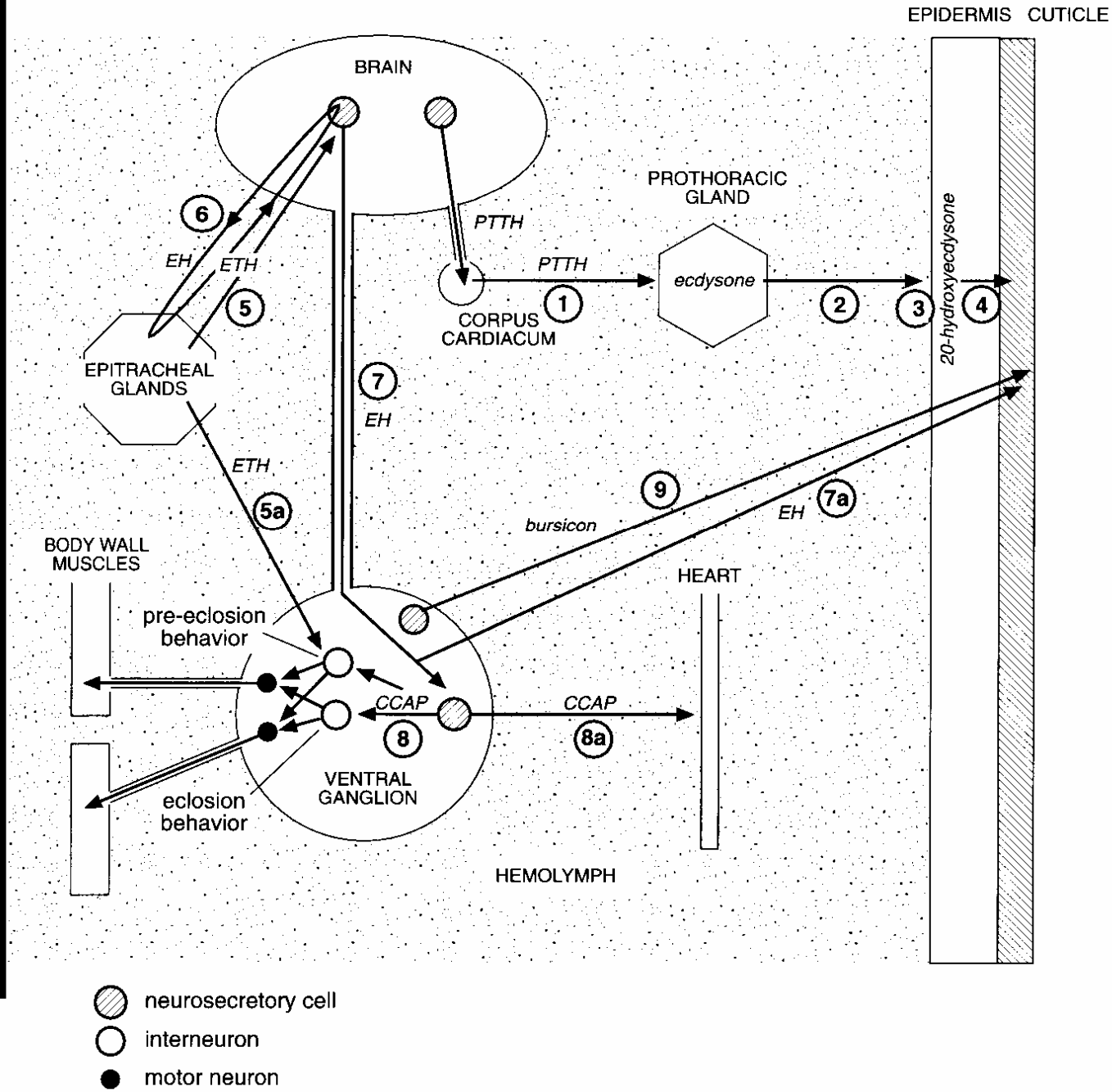
8a CCAP acting via hemolymph increases heartbeat

9 bursicon first plasticizes cuticle, then switches on cuticular sclerotization

Fig. 15.31. The hormones involved in regulation of events at a molt. Juvenile hormone is not shown. Names of hormones are italicized. CCAP, crustacean cardioactive peptide; EH, eclosion hormone; ETH, ecdysis triggering hormone; PTTH, prothoracicotropic hormone.

HORMONAL CONTROL OF EVENTS AT A MOLT. JH IS NOT SHOWN

CCAP=CRUSTACEAN CARDIOACTIVE PEPTIDE
EH=ECLOSION HORMONE
ETH=ECDYSIS TRIGGERING HORMONE
PTTH=PROTHORACICOTROPHIC HORMONE



CONCLUSIONS:

1. Behavior is the manifestation of physiology and how it plays out and is under the control of genes
2. Diet, hormones and biogenic amines can influence the path behavior takes
3. Hormones can act as releasers or modifiers of behavior
4. Biogenic amines can act as neuromodulators of behavior
5. Studies on feeding behavior, mating behavior and eclosion (plus ecdysis) clearly show that these are very complex behaviors that must be very carefully regulated in order for the insect to survive
6. Integrative, physiological studies on these three systems have used very diverse technologies and strategies to unravel the complexity of each system