INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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IBO2017 – Practical 3

Developmental Physiology marking crib and justification

Question 1 – Identifying tissues of a fly larva

Tasks 1a & 1b – Identify the axes of a larva. **(7 Marks)**

Tasks 1c, 1d & 1e – Dissect *Calliphora vicina* larva to isolate identified tissues. **(38 Marks)**

(45 marks in total)

Question 2 – Physiological responses of a larval heart

Task 2a, 2b & 2c – Dissect a *C. vicina* larva to reveal beating dorsal vessel (larval heart) **(10 Marks)**

Task 2d, 2e & 2f – Design and perform an experiment to identify the activity of pharmacological agents acting on the dorsal vessel **(45 Marks)**

(55 marks in total)

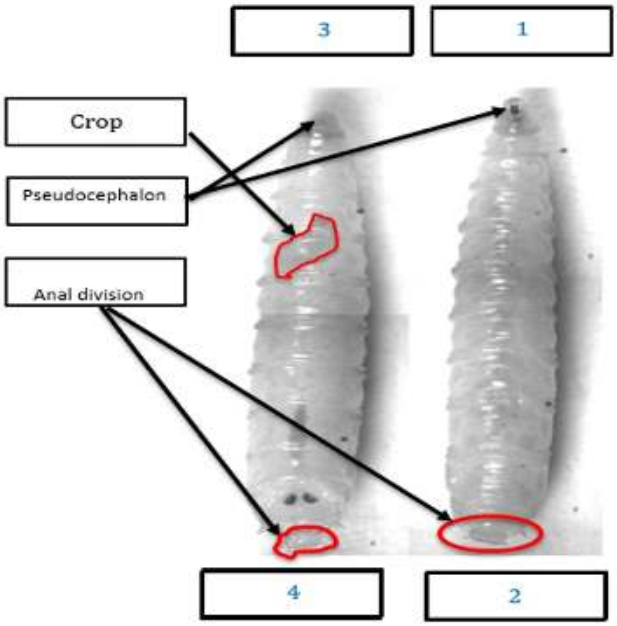
Examination total = 100

The overarching aim of this examination is to test fine dissection skills, attention to detail and understanding of the scientific principles of experiment design and procedure. There is no assessment of prior academic knowledge.

As the exam progresses the complexity and time pressure increase. We have structured this examination in a very specific way to allow some skills development and so candidates should follow the chronological order of the exam tasks.

Question 1: Identifying tissues of a fly larva

Task 1a & -b Identify the body axes of a fly larva



7 Marks

Figure 5. External morphology of *C.vicina* 1

The images given in Fig 2 and -3 are to guide the students; there are subtle differences between species such as size of salivary gland, exact position of crop and number of teeth on the mouth hooks. A possible difference is alluded to in the legend of Fig. 3B. If candidates simply copy from the figures provided, they will get incorrect answers.

Task 1c – Dissection; Task 1d Isolation of organs/tissue; Task 1e complete the table.

Table 1 Recording of identified larval tissues

Candidates will be scored on the correct intact tissues being in the wells, as well as the answers in the table below

Slide Position:	Tissue	Observations to record WRITE YOUR ANSWERS IN THIS COLUMN	MARKS
1	Crop	(1) for crop tissue (evidence of crop tissue, can be partly damaged) Or (2) for whole intact undamaged tissue (1) = Anterior	/3
2	Salivary glands	(1) for one salivary gland (evidence of tissue, can be partly damaged) Or (2) for whole intact undamaged tissue, this is very delicate tissue so holds a bigger value (2) for correct ratio = 1/3 or 3:1 (should be measured in situ, tissue will shrink a little when in gelvitol)	/4
3	Brain	(1) for brain (evidence of tissue, can be partly damaged) Or (2) for whole brain with some nerve roots attached. Or (3) for whole intact undamaged brain with all nerve roots attached.	/6
		This is very delicate tissue and secured by a number of connections, removing intact is difficult so holds a bigger value. (1) for total number of nerves = 20 (has to be exact) (1) for dorsally originating = 12 (1) for ventrally originating = 8 **Justification for three counts**: the correct dorsal/ventral count combination can be difficult if the tissue is not manipulated well. It is still however possible to count the total root projections.	
4	Spiracle and Trachea	(1) for number of slits within a spiracle = 3. (This can be counted on an un-dissected larva). (1) for spiracle (evidence of tissue, can be damaged) with no trachea. It is almost impossible to not have trachea attached, therefore a separate free floating piece from another part of the preparation will not be accepted. Or (2) for spiracle (evidence of tissue, can be damaged) with trachea	/7

		attached. Or (3) for whole spiracle with attached trachea with all three slits damaged. Or (4) for whole spiracle with attached trachea with 2 slits damaged. Or (5) for whole spiracle with attached trachea with 1 slit damaged. Or (6) for whole spiracle with attached trachea no slits damaged.	
5	Mouth hooks	(1) for the correct number of teeth = 1 tooth Localization. (1) for on the nub = 1 (1) for anterior to the nub = 0 (1) for posterior to the nub = 0 (1) for each mouth hook with damage and or not cleared of all muscle. (2 marks) Or (a combination of) (2) for each mouth hook intact and cleared of muscle. (**4 marks**)	**/8**
6	Wing disc	(1) for the isolation of any other imaginal disk (not wing disk) can be damaged. (2) for isolation of any other imaginal disk (not wing disk) intact. (5) for wing disk can be damaged (10) for intact wing disk with no damage **Justification:** imaginal disks are difficult to find and differentiate between, they are also difficult to pick up and transfer effectively they therefore hold a much bigger value.	**/10**

38 Marks **Total Marks for Experiment 1** 45 Marks

Question 2: Physiological responses of the insect heart.

Task 2a,-b & -c Dissect *C. vicina* larva to reveal beating dorsal vessel.

Here the candidates are required to dissect an lethally anesthetised larva, this time in a ventral plane in order to open the larva up gently move aside the internal organs to reveal the two dorsal tubes. The larva needs to be pinned out carefully, not twisted or too stretched and the internal organs moved gently, this will minimise any disruption of the connections from the brain to the dorsal vessels which can cause the heart to stop. Candidates are given 10 larvae, if they have not managed to expose a beating heart after ~5 attempts they will run out of time before they run out of larvae.

Correctly exposed dorsal vessel **(5 marks)**

Dorsal vessel beating **(5 marks)**

Justification: This mark will not be broken down further to assess quality of dissection/dorsal vessel exposure as this could lead to subjectivity between markers.

Any amount of dorsal vessel exposed and beating is suffice for the candidate to continue. This will be evidenced by a demonstrator who will write a standardised code on the exam paper. This will stop students from continuing with the experiment without the correct tissue being exposed. Or simply continuing with no viable tissue and making up their results. If there is no official code and the student has attempted to continue with the exam, any further work beyond 2d and 2e will not be recognised. The marker will strike through these answers.

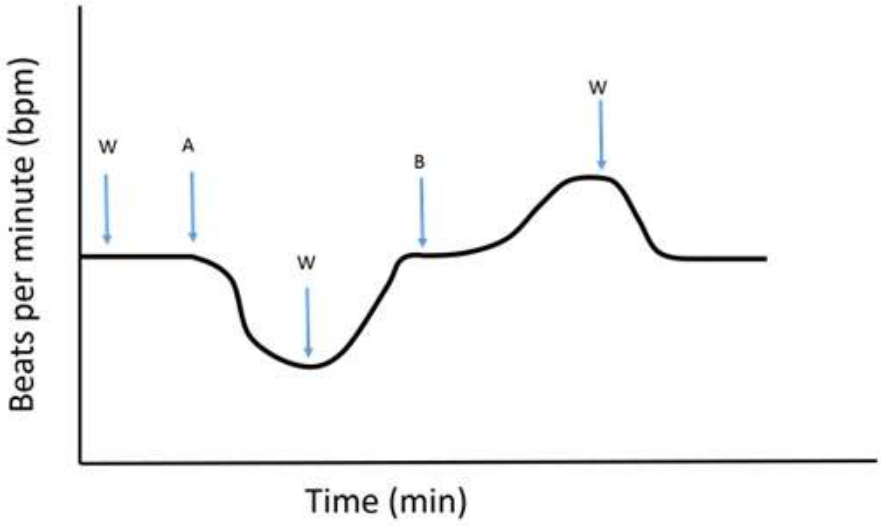
Task 2d, -e & -f Design and perform and experiment to identify three pharmacological agents' activity on the dorsal vessel.

Task 2d

We are looking for candidates to consider appropriate experimental design and representation of experimental data, in addition, to demonstrating their expectations of the action of acetylcholine and adrenaline on one tissue preparation. This will help them to consolidate their expectations of the pharmacology of the experiment and will prepare them for the Task 2e.

They must design their experiment for just one preparation to test multiple agents, using a robust methodological approach. For example, identifying baseline data, initiating a response i.e. adding a reagent, washing to bring bpm back to resting, before continuing with either a repeat of the same agent or the next agent.

The ideal expected sketch is shown below.



If a candidate misinterprets the instructions and draw two sets of data overlaid on one graph this will be recognised and will be scored similarly using the below criteria.

(1) for not starting at 0
(1) for an initial resting phase
(1) for annotations for the addition of each agent A and B
(1) for a decrease with A (Acetylcholine)
(1) for back to resting
(1) for an increase with B Adrenalin
(1) for last wash and back to resting

(7 marks)

Task 2d cont... Experimental design

Candidates will be required to divide an experimental timeline into the relevant number of blocks. Then assign an appropriate letter to each block to represent the addition of solutions (A, B or C) or PBS wash (W) to their single specimen. This will be their experimental design.



Figure 7 – Experimental design. *Relative times of the addition of PBS washes (W) and agents A, B and C*

Candidates will be specifically judged on **application** of all three agents taking into consideration repetitions, **washing** and **interleaving**.

(A) Application of Agents:

(i) (1) for the application of each agent x1

E.G W A W B W C W (or variations of)

(ii) (2) for the application of each agent x2

E.G. W A W B W C W A W B W C W (or variations of)

(iii) (3) for the application of each agent x3

E.G. W A W B W C W A W B W C W A W B W C W (or variations of)

BUT NOT W A A A W B B B W C C C W. This will be classes as option (i) =1 mark

(W) Application of Washes:

(iv) (1) for the application of wash but not after each agent

E.G. W A B C W A B C W

(v) (2) for application of wash after each class of agent

E.G W A A A W B B B W C C C W

(vi) (3) for application of washes after individual agent applications

(vii) (+1) for having end wash

(I) **Interleaving:**

(viii) (1) for interleaving agents (x3)

E.G. W A A A W B B B W C C C W

(ix) (5) for interleaving individual agents (x1)

E.G W A W B W C W

(**Maximum marks = 12**)

Examples

#1

W	A	W	B	W	C	W

A = 1: W = 3+1; I = 5 (Total 10). If the student write X2 or x3 next to it to represent repetitions this will not be recognised as this would indicate more than one larvae preparation (experimental repeats) which in this instance has not been requested.

#2

W	A	W	B	W	C	W	A	W	B	W	C	W

A = 1; W = 3 +1; I = 5 (Total = 11)

#3

W	A	W	B	W	C	W	A	W	B	W	C	W	A	W	B	W	C	W

A = 1; W = 3 +1; I = 5 (Total = 12)

#4

W	A	W	A	W	A	W	B	W	B	W	B	W	C	W	C	W	C	W

A = 3; W = 3 +1; I = 0 no interleaving (Total = 7)

#5

W	A	A	A	W	B	B	B	W	C	C	C	W

A = 1; W = 2 +1; I = 0 (Total = 4) Effectively the same as #1 but with no washes in between each application

Task 2e. Conduction of the experiment and completion of Table 2

The candidates must ensure that they make the correct dilutions; it is a simple 1 in 4 dilution and they will only be awarded marks for the first set of volumes entered in the table below. They need to conduct their experiment following their experimental design or a version of this depending on time. They need to calculate the average BPM for each treatment in order to select the appropriate descriptor for the effect that they identify.

Agent	Resting W	A	B	C
Working concentration volumes	-	Stock: 250 µl PBS: 750 µl **(2)**	Stock: 250 µl PBS: 750 µl **(0)**	Stock: 250 µl PBS: 750 V **(0)**
Record your raw data counts here				
Average bpm	Average = **(1)**	Average = **(1)**	Average = **(1)**	Average = **(1)**
Select the appropriate descriptor	Increase No change within 10% Decrease	Increase No change within 10% Decrease	Increase No change within 10% Decrease	Increase No change within 10% Decrease
	(1)	**(2)**	**(2)**	**(2)**
Identify active receptors	-	1 **(1)**	0 **(1)**	1 **(1)**

(16 marks)

Table 2: BPM of resting tissue, effect of agents A, B and C and identification of agents.

This is where they get credit for the generation of their data.

Task 2f Line graph of their experimental data.

We are looking for a reflection of their experimental design or a suitable adaptation of this depending on the time they have left. We have asked them to record their raw data in the table, we would expect to see evidence of a number of counts taken for each solution. They should plot these on their graph paper using the appropriate annotations. It could look something like the sketch graph in **Task 2d** with the addition of Octopamine data. Alternatively, if they record their data discreetly then they may produce a histogram.

If students conduct their experiment and collect data but run out of time and do not complete the graph they can still be awarded credit for the generation of their data set if they are able to enter this into the table.

Discrete

Histogram using average data – **(4)**
Histogram showing variation **(6)**
Or Box and Whisker – **(6)**

Continuous
Line graph using average data – **(2)**
Line graph using all raw data counts – **(6)**

Annotation – A, B, C and W and labels – **(2)**
(8 Marks)

Task 2f cont...

The two hypotheses (H1 and H2) for this experiment were that the *C.vicina* dorsal vessel will respond to acetylcholine negatively chronotropically (H1) and second, will respond to adrenaline positively chronotropically (H2), as would a mammalian heart.

From their data, they are required to accept or reject these hypotheses.

H1 should be accepted, if their data supports this (we will check their Table 2 answers) and they accept H1 they will get 1 mark, if they go against their data for any reason and reject H1 they will be negatively marked. If their data refutes the hypothesis and they also reject the hypothesis they will get 0 marks because they have conducted the experiment incorrectly.

H2 should be rejected and the marking follows the same rules as above.

Therefore **2 marks** for correctly accepting/rejecting the hypotheses.

H1 (Accept)	Data accepts H1	H1 Accept selected by student	H1 Reject selected by student
		(1)	**(-1)**
	Data rejects H1	H1 Accept selected by student	H1 Reject selected by student
		(-1)	**(0)**

H2 (Reject)	Data accepts H2	H2 Accept selected by student	H2 Reject selected by student
		(0)	**(-1)**
	Data rejects H2	H2 Accept selected by student	H2 Reject selected by student
		(-1)	**(1)**

(2 marks) Total marks for Question 2 = 55 **Examination total marks = 100**