INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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Student name:	Student code:	Country:

28th International Biology Olympiad

July 23-30, 2017
University of Warwick
United Kingdom



Practical Exam 3

DEVELOPMENTAL PHYSIOLOGY

The exam will start and end with a whistle.

Total points: 100
Duration: 120 minutes

GENERAL INSTRUCTIONS

In this practical test you have **TWO hours** to do **TWO Questions**.

You must perform the tasks in the order given here, Larvae for Q2 will be available from 30 minutes after the start of the examination:

Question 1 – Identifying tissues of a fly larva (45 Marks available)

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

Tasks 1c, d and e – Dissect *Calliphora vicina* larva isolate and identify tissues. (38 Marks)

Question 2 – Physiology of a larval heart (55 Marks available)

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

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

This is a test of fine dissection skills, observation and experimental design.
Good luck!

Important Information:

- Please remember to write your name, your student code and your country in the given boxes.
- Write your answers in this question booklet and place your dissection specimens into the 6 welled microscope slide. Both items will be collected.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your Red card immediately.
- During experiments, ensure to handle equipment properly. Any spilled solutions or equipment damaged by you will not be replenished.
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
- Attach your graph paper and your plain paper on to this question booklet with a paper-clip and put in the envelope provided. Ensure to stick your small identification sticker onto the white section of the 6 welled microscope slide.
- No paper, materials or equipment should be taken out of the laboratory.
- **An English translation of this paper is available upon request.**

MATERIALS AND EQUIPMENT

- 1 x Dissection Stereo Microscope
- 1 x Desk Lamp
- 2 x Pairs Dissecting forceps
- 1 x Pair fine forceps - for moving larvae
- 10 x Dead larvae in a Universal tube. Those have been treated overnight with ethyl acetate (Task 1). More can be made available if required.
- 10 x Larvae treated with a lethal dose of anaesthetic in a glass petri-dish (Task 2). These have been treated for I hour with ethyl acetate – these will be provided when requested
- 2 x Plastic dissecting dish filled with a black silicone base
- Approximately 20 minutian pins have been stuck around the edge of each dissecting dish. To move them grip the pins with dissecting forceps close to the the bottom of the pin where it penetrates into the black silicone
- 1 x P1000 pipette plus tips
- 1 x P200 pipette plus tips
- Three 1.5 ml Eppendorfs - labelled A, B, and C, containing Acetylcholine, Adrenaline and Octopamine respectively
- 1.5 ml Eppendorfs for your dilutions
- PBS – Phosphate Buffered Saline – 100 mls
- Gelvitol - mounting media
- Counting clicker - available for beat counts
- 1 x timer
- Microscope slide with six hydrophobic wells marked 1-6 (Do not touch the hydrophobic wells)
- Sheets of paper towel
- 1 x Marker pen
- 1 x Graph paper
- 1 x Plain paper sheet
- 1 x Paper clip
- 1 x Red Flag (dark if colourblind)
- 1 x Green flag (light if colourblind)
- 1 x Waste beaker

QUESTION 1: IDENTIFYING TISSUES OF A FLY LARVA

Introduction:
The Calliphoridae are typical members of the Order Diptera. Larval anatomy, while often different to the adult, retains many of the adult features. In the first set of experiments you will be required to identify a number of tissues in the larval stages of a *C. vicina.*

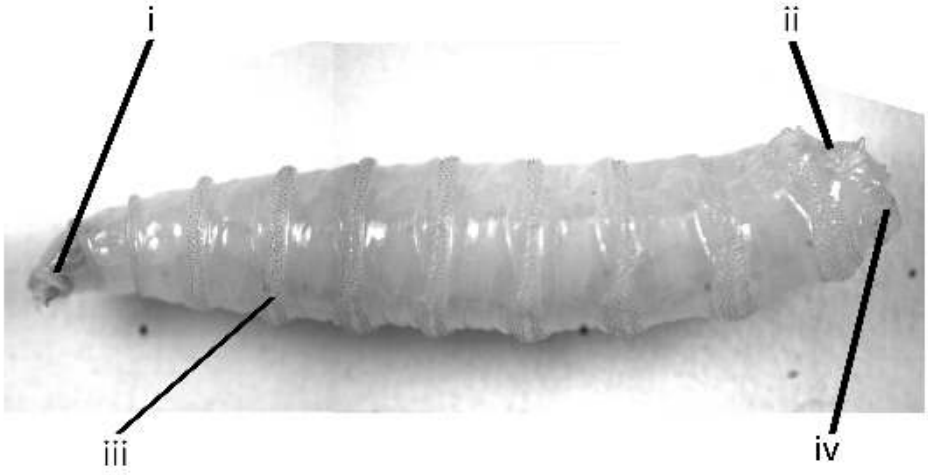


Figure 1. Lateral view of a third-instar larva (a developmental stage) of Calliphora vicina, anterior is to the left. *i) Pseudocephalon ii) Spiracle field iii) Spinose band iv) Anal division*

Anterior to the anal division is the spiracle field where access of air into the tracheal system is gained through a pair of small brown ringed structures called spiracles containing a number of brown slits.

Following dissection using a stereo dissecting microscope, the anatomy of the internal tissues of a larva can be viewed. The structures are represented in Fig.2. Of importance to Experiment 1 is the nervous system, the fat bodies, the alimentary system, the tracheal system and the imaginal discs. The imaginal discs are discrete sheets of epithelia that will form the adult integument following metamorphosis.

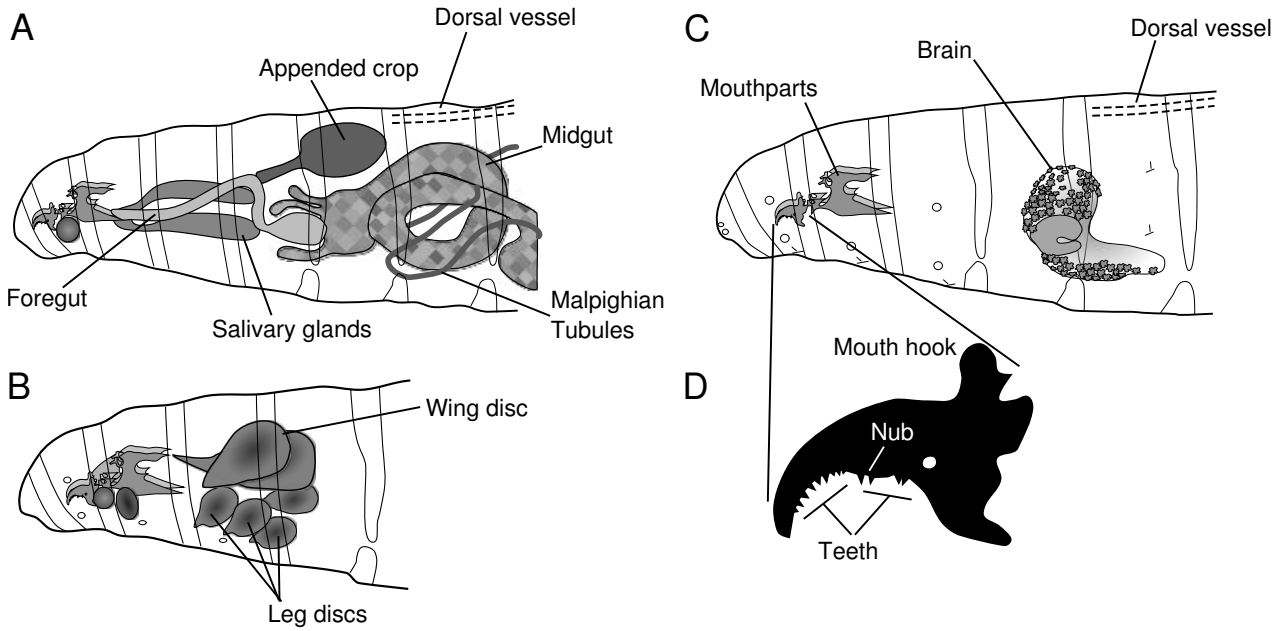


Figure 2. Lateral diagrams of anterior internal structures of a highly derived dipteran larva. A. *Note position of crop and salivary glands.* ***B****. Note the position of the wing disc in relation to the mouthparts and brain.* ***C.*** *The anterior part of the mouthparts, the "mouth hook", is decorated by a number of teeth both on the hook and on the nub. These number from 0 – 12 depending on the species and developmental stage e.g. in* ***D.***

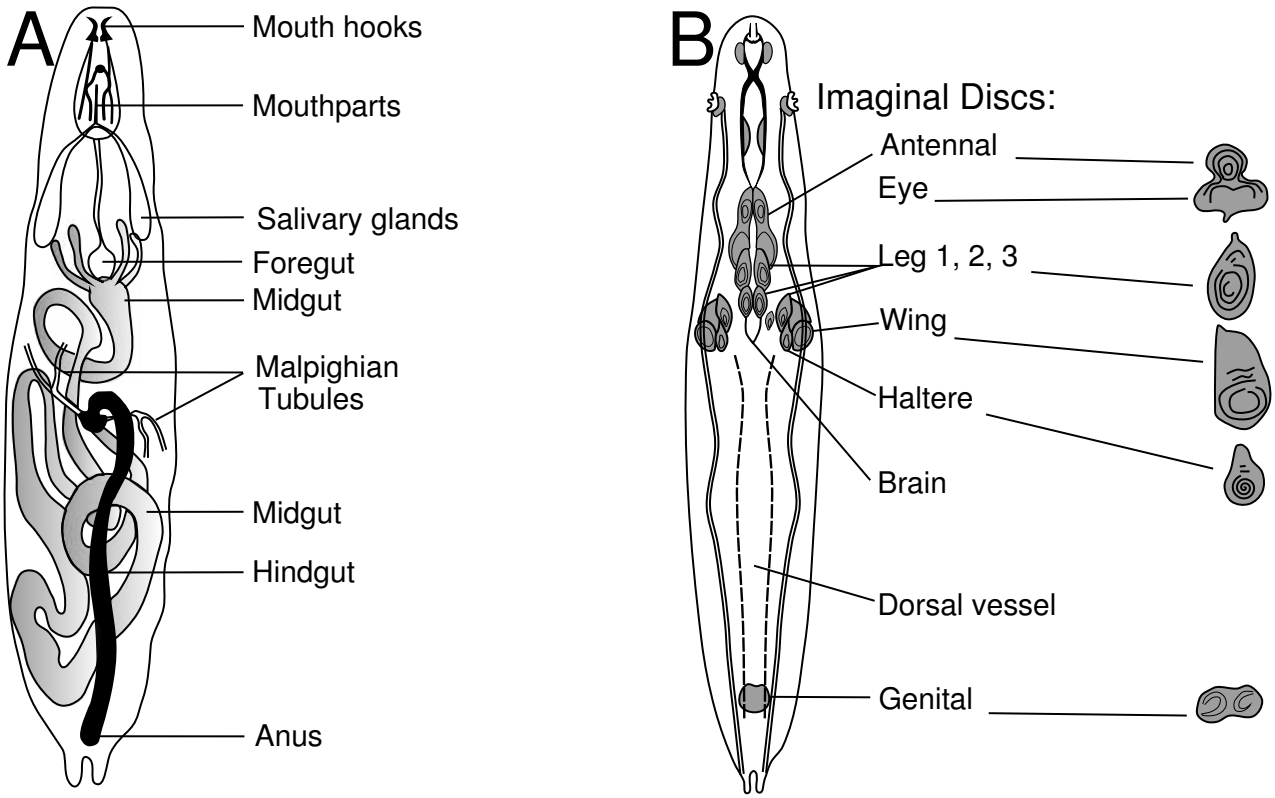


Figure 3. Dorsal representations of a dipteran larvae *(not C.vicina)* ***A****. Alimentary system (without crop)* ***B****. A dorsal representation of the relationship between the brain, major tracheal tubes, dorsal vessel and imaginal discs. Note the relative size and position of the wing disc in the diagrams – in C.vicina it is found attached to lateral trachea slightly more anterior to the brain than the diagram here (compare to Fig. 2B).*

In the diagrams above, **the fat tissue is not shown**. Fig. 4 shows two partially prepared larvae showing this material – and the natural lack of colour and shading in such preparations.

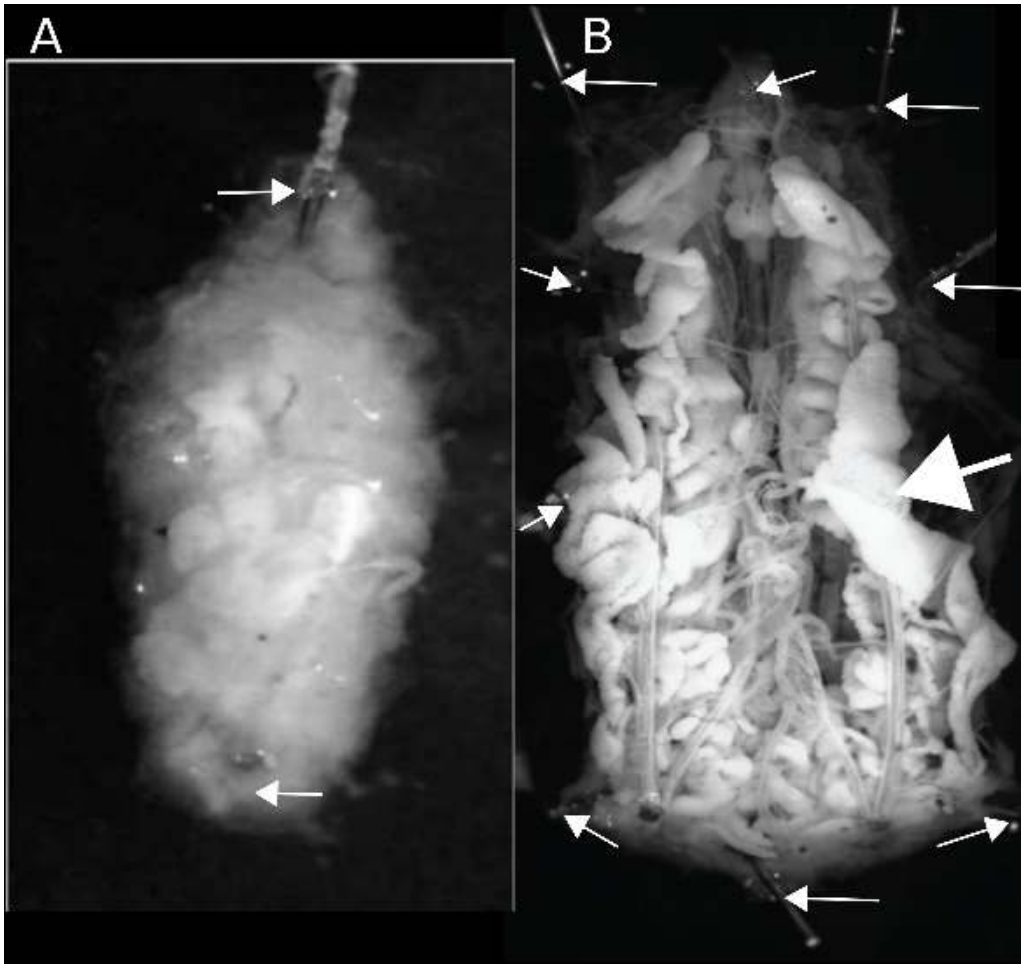


Figure 4**. **Initial Dissection A**. Freshly pinned larva, following careful ripping of cuticle.* ***B. *Filleted and pinned preparation – larger white arrow shows the bright white fat body material making up much of the internal contents. Small white arrows point to minutian pins. Note the thin white/silver trachea throughout the body*

Task 1a

Figs 1 and 5 shows the external morphology of a *C.vicina*.

Place a dead larva on the black silicon dissecting petri-dish – observe the structures seen externally. Using both your observations and the above figures, identify the anterior and posterior ends of the larvae as well as the top (dorsal) and bottom (ventral) surfaces.

Task 1b

i) In Fig. 5 place one of the following identification numbers (1, 2, 3 or 4) into the relevant boxes below to denote the orientation of the larvae in the images. You may use numbers more than once. 1 = Ventral anterior; 2 = Ventral posterior; 3 = Dorsal anterior; 4 = Dorsal posterior.

ii) Draw a line from each of the 3 labels (i, ii and iii), in between the images, to the relevant part of either image.

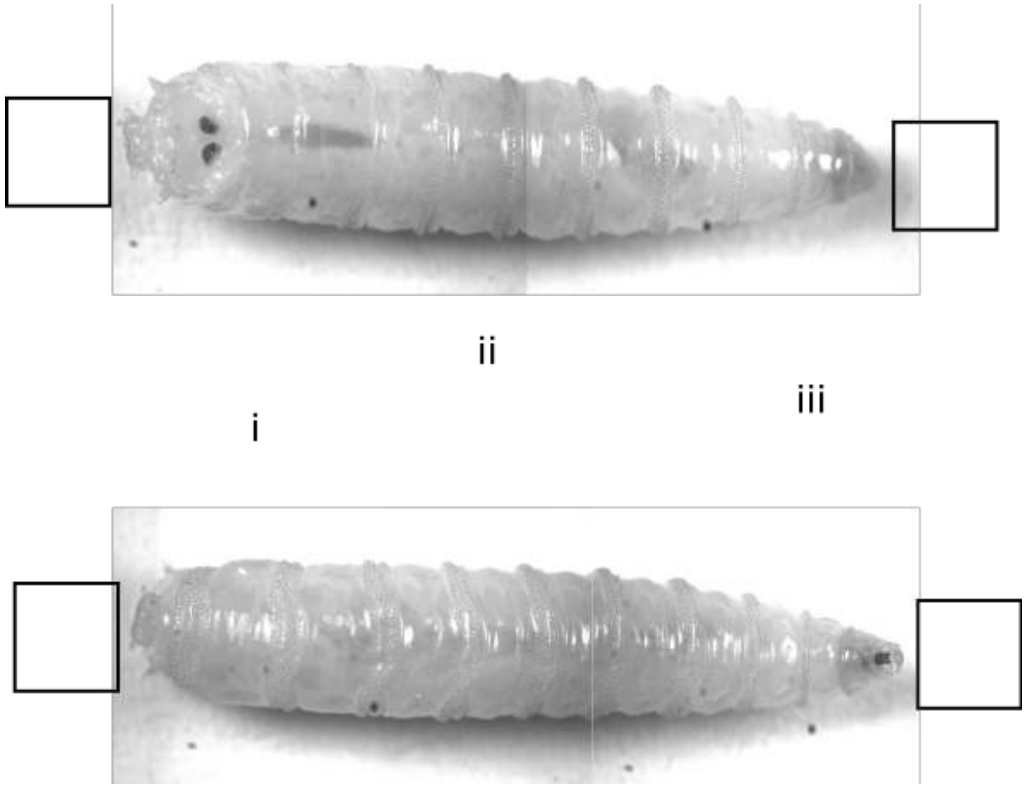


Figure 5. External morphology of C. vicina*. i) Anal division ii) Pseudocephalon iii) Crop*

(7 Marks)

Task 1c + Task 1d

You are required to identify, perform simple measurements and to transfer specific structures to the six-welled microscope slide. You are advised to do all of this whilst looking down the microscope and using your dissecting forceps.

IMPORTANT: The minutian pins should only be moved using forceps whilst looking down the microscope. When pushing them into tissues ensure to hold them closer to the sharp bottom end than the top – they will bend if you do not do this and they will be harder to use. **HINT – ALWAYS** use two pairs of dissecting forceps for the manipulation of the larvae and pins.

BE PROTECTIVE OF YOUR FORCEPS and the preparation. **DO NOT** penetrate the larva too deeply and always tear sideways or up and away from the preparation. It should look similar to Fig. 4A

General Dissection Instructions

1. Using the same larva as in Task 1a, or a new one, put the larva with its dorsal aspect uppermost on your black silicon dish, under the microscope. Using dissecting forceps, pick up a minutian pin and place in the posterior end of the larva through the anal division, just behind the spiracles. With your forceps, gently stretch the larva at the anterior end and insert a pin immediately behind the mouthparts.
2. Pick-up the cuticle between the last two posterior spinose bands and tear apart – the first tear is difficult. This achieved by using the two pairs of dissecting forceps with the larva's posterior end towards you.
3. Using a pipette gently cover the preparation in PBS.
4. Using dissecting forceps rip the cuticle along the mid-line, from the posterior tear to the anterior pin. CAREFULLY place one tip of each forcep just under the cuticle and the other tip on top and gently rip the cuticle by gripping and pulling slightly. Do this in short stages repositioning the forceps after each segment is ripped. Finally turn the dish around and complete any ripping of the cuticle to the posteriorly placed pin.
5. Open out and pin the cuticle flat against the black silicone, producing a "fillet" and exposing the internal structures. Use one pair of forceps to pull back both the cuticle and muscles and the other to manipulate a pin into position. It should look similar to Fig. 4B.
6. Remove excess fat, being careful not to disrupt the brain and gut. Pick up the fat with one forcep, remove and wipe onto tissue or place to one side of the dish.

NOTE: In preparation for this next section place a small drop of gelvitol within each of the hydrophobic wells on your slide. When transferring any tissue from the dissection plate to the microscope slide, aim to pick up the tissue in some fluid so the fluid is held between the tips of the forceps by surface tension. Upon addition to the gelvitol, open the forceps and the tissue will transfer in to the gelvitol.

Task 1d

1. Remove the Appended crop and put at position 1 on your microscope slide. In Table 1, report whether this connects to the alimentary system anteriorly or posteriorly to the brain.
2. Identify the salivary glands, remove one and place at position 2 of your slide. Estimate the ratio of one salivary gland's length to body size length and write this in Table 1.
3. Gently dissect out the brain by removing trachea and breaking the attached nerves and place at position 3 on your microscope. Count the total number of nerves originating dorsally and ventrally from the brain, insert this information in to Table 1.
4. Dissect a posterior spiracle with a small attached section of trachea, place at position 4 on your microscope slide. In Table 1 state the number of slits in one spiracle.
5. Remove the mouthparts. Carefully tease away excess muscle material, free the mouth hooks, separating them from the mouthparts and from each other. Place both mouth hooks into position 5 on the microscope slide. Carefully observe, using transmitted light, to confirm the number and position of teeth on a mouth hook. Record this in Table 1.
6. Find and dissect a wing disc, carefully place into position 6.
7. **IMPORTANT** - You must firmly attach your identification sticker to the white part of the microscope slide. Your slide will be collected and marked at the end of the examination.

Task 1e

You will be scored on the correct tissue in the gelvitol drops and whether it is intact, as well as the answers you give in the table below.

Table 1 Recording of identified larval tissues

Slide Position:	**Structure**	**RECORD YOUR OBSERVATIONS AND ANSWERS IN THIS COLUMN**
1	Crop (3 marks)	Is the connection of the crop to the alimentary canal posterior or anterior to the brain? Circle the correct answer. ANTERIOR POSTERIOR
2	Salivary glands (4 marks)	What is the approximate ratio of salivary gland length to length of the larval body?
3	Brain (6 marks)	Total number of nerves: Dorsally originating: Ventrally originating:
4	Spiracle and Trachea (7 marks)	Number of slits within a spiracle:
5	Mouth hooks (8 marks)	Total number of teeth: Directly on the nub: Anterior to the nub: Posterior to the nub:
6	Wing disc (10 marks)	Nothing applicable

(Table Total - 38 Marks)

QUESTION 2: PHYSIOLOGICAL RESPONSES OF THE LARVAL HEART.

Introduction

Larvae in the glass petri-dish have been exposed to a lethal dose of anaesthetic. However, the tissues of these animals can, for a short time, still respond to pharmacological agents in a similar way to mammalian systems, with some possible differences. You are required to demonstrate the effects of three pharmacological agents on the beating dorsal vessel: Acetylcholine (A), Adrenaline (B) and Octopamine (C). This organ has evolved in insects to circulate hemolymph through their open circulatory systems. It is equivalent to a mammalian heart. Similar genes are required for development in both insects and mammals. Some mammalian hormones and neurotransmitters can act directly on the insect tissue through homologous receptor proteins.

Task 2a

When you are ready, hold up your green flag and a demonstrator will give you 10 freshly anaesthetised larvae in a glass petridish. Under your dissecting microscope pin a larva with its **ventral** surface uppermost. First place a pin through the anal division. Holding the head/mouthparts, stretch the larva a little without twisting or rotating it and pin just behind the pseudocephalon.

Task 2b

Use the same dissection technique as in Question 1, this time with the ventral surface uppermost. Once you have the fillet, this time CAREFULLY pick-up the gut and Malpighian tubules and pin to the side. Fat can similarly be moved to one side or removed. When picking up these tissues, you will need to carefully break some tiny trachea. The dorsal vessel is a tube running along the dorsal midline (Fig. 1 and Fig. 6). Do not remove the brain or too many trachea, do not touch or damage the dorsal vessel with your forceps directly or indirectly when moving other tissues. All of these may prevent the “heart” from beating. If the PBS wash is cloudy from lipid droplets, then carefully remove and replace the PBS using a P1000 pipette and tips.

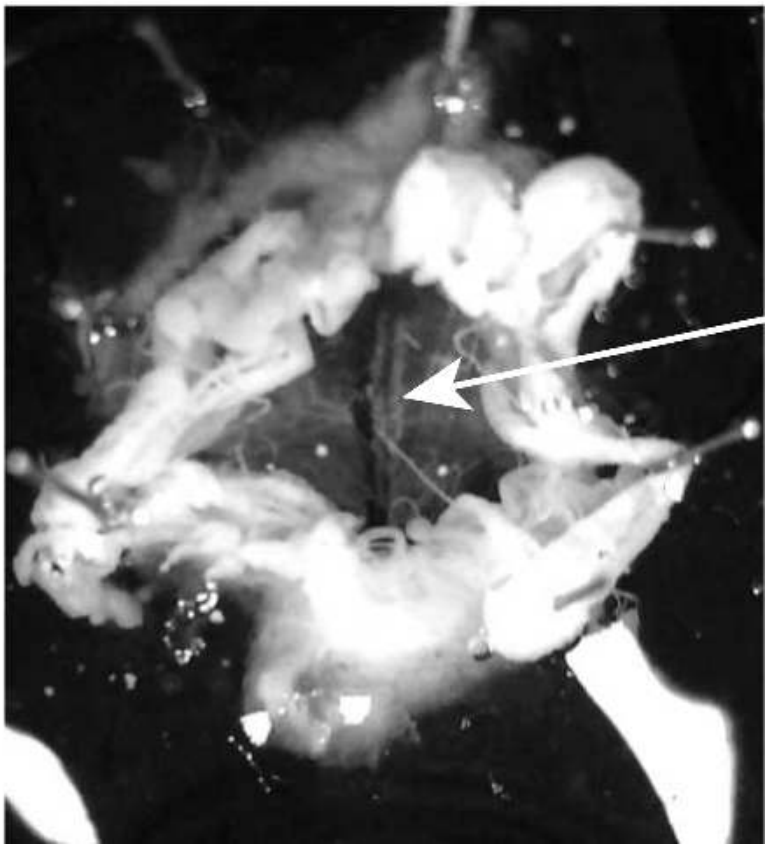


Figure 6. A VENTRAL dissection of a C.vicina larva – following careful moving of the nervous system, gut, trachea and fat, reveals the dorsal vessel (denoted by the arrow)

Task 2c
Observe the dorsal vessel, you should see two parallel lines, if the dorsal vessel is beating it will pulse/move in opposite lateral directions and back together again in synchronisation, this is one heart beat. Once you are satisfied with your preparation, raise your Green flag and a demonstrator will sign on this sheet to confirm that 1) the dorsal vessel has been correctly exposed and 2) it is intact and beating.

Your demonstrator will show you a red flag if the dorsal vessel is not exposed
or
Your demonstrator will show you both a red and a green flag if you have exposed the dorsal vessel, but it is not beating
or
Your demonstrator will show you a green flag if you have successfully exposed the dorsal vessel and it is beating.

You have 10 trials/attempts at this dissection, once you have successfully exposed a beating dorsal vessel and have had this confirmed by a demonstrator (they will write a specific code on your exam paper) you can progress onto Task 2d.

If you are running out of time or larvae or you do not wish to attempt any further dissections then <u>you MUST sign below the table before moving on to Task 2d</u> to finish the examination. Otherwise no credit will be given for Task 2d.

	Demonstrator code
Correctly exposed dorsal vessel NOT Beating	(5 Marks)
Alternatively Correctly exposed dorsal vessel beating	Alternatively (10 Marks)

Once the demonstrator has signed and you wish to progress to the next task, please sign below.

Task 2d
Observation - In mammals heart rate is affected by numerous agents. Neurotransmitters such as acetylcholine decrease the strength (negatively inotropic) and the rate (negatively chronotropic). Alternatively, hormones such as adrenaline increase strength (positively inotropic) and rate (positively chronotropic).

Hypothesis - Such observations lead to the hypotheses that: (H1) Acetylcholine will decrease the beats per minute (BPM) of the dorsal vessel. (H2) Adrenaline will increase the BPM of the dorsal vessel in this species. On to Fig. 7 draw a single-line graph of your **expected** results showing the effects of A, B and W (PBS Wash)**,** applied to a **single** preparation**.** Ensure to indicate the changes to bpm of the dorsal vessel during the time course of the experiment.

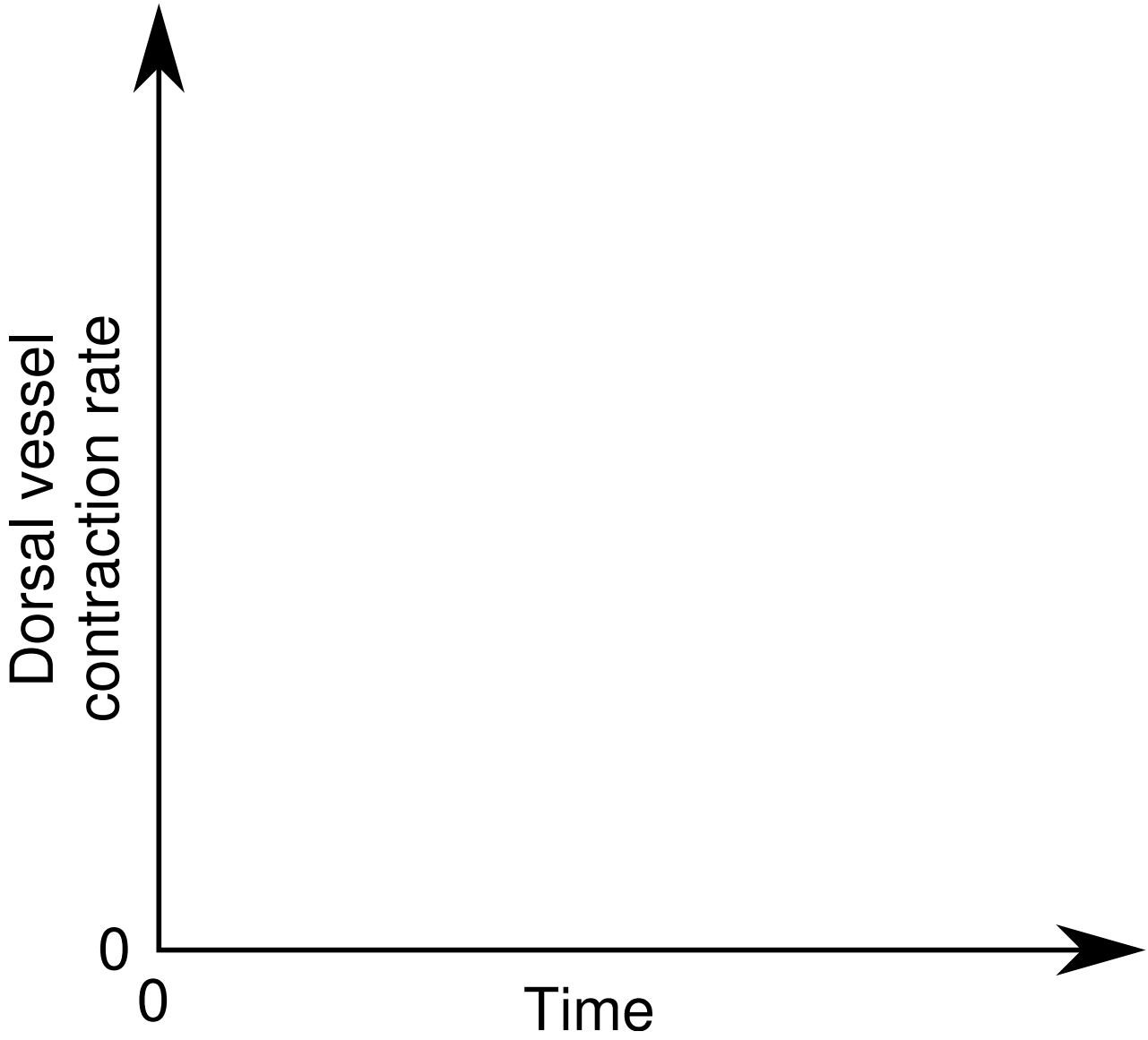


Figure 7. Line graph of expected results for addition of Acetylcholine (A) and Adrenaline (B) and PBS wash (W) to the C.vicina dorsal vessel

(7 Marks)

1. You are required to design and perform an experiment to test the hypotheses H1 and H2 and to discover how the insect hormone, Octopamine (C) acts on the dorsal vessel isolated in Task 2b and confirmed in Task 2c.
2. The block below represents the duration of the ideal experiment you would conduct on your single larva preparation. Divide up this block into sections to represent the order you would apply the respective solutions (A, B and C) and PBS wash (W) on **one** larvae preparation from Task 2c. Write the appropriate letter into each division, the first two have been done for you.
3. When designing your experiment you need to consider and effectively represent, washing of the tissue and repetitions.

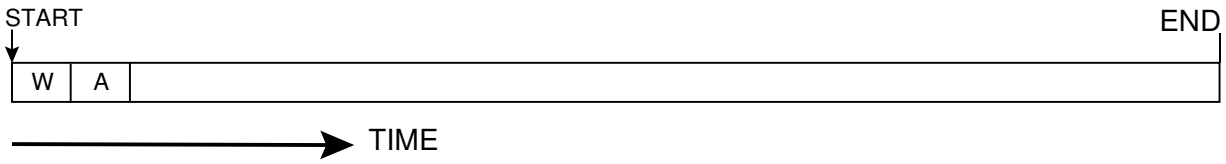


Figure 8. Experimental design*. Planned addition of PBS washes (W) and agents*

(12 Marks)

Task 2e

1. Cover your preparation with fresh PBS (W) and record the baseline beats per minute (bpm) in Table 2.
2. Three eppendorfs marked A, B, and C contain stocks of:
   A = 2 x 10^{-2} M Acetylcholine
   B = 2 x 10^{-2} M Adrenaline
   C = 2 x 10^{-2} M Octopamine

   100 ml PBS is also provided in a medical flat bottle

   Just before use, dilute A, B and C in PBS to their working concentrations of 5 x 10^{-3} M in 1 mL in Eppendorf tubes. For each agent record volumes for dilutions in Table 2 in µL.
3. Considering your ideal experimental design (Fig. 8), evaluate the effects of A, B, C and W on a single dissected preparation taking into account the time you have left. Record your raw data and average bpm in Table 2 for the dorsal vessel at rest and for the effect of each of the agents A, B and C.
4. Based on your data select the relevant response of bpm in the table e.g Increase, No change within 10% or Decrease in the table
5. From your data indicate whether the receptor for each agent is demonstrating activity or not by writing "1" or "0" respectively in the relevant column of row 4.

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

Solutions	**W**	**A**	**B**	**C**
Dilution volumes (µL)	Not required	Stock: PBS:	Stock: PBS:	Stock: PBS:
Record your raw data counts here **Average bpm**	Average =	Average =	Average =	Average =
Select one of the following responses.	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease	Increase No Change within 10% Decrease
Identify agent receptor activity **1 = there is activity** **0 = there is no activity**				

(12 Marks)

Task 2f

1. On the Graph paper provided, plot a suitable graph of your data over the time course of your experiment. Remember this should represent data applied to a single preparation.

(12 Marks)

2. For this experiment H1 was that the *C.vicina* dorsal vessel will respond to acetylcholine negatively chronotropically and H2 was that adrenaline acts positively chronotropically, as would a mammalian heart.
3. You are now required to accept or reject these hypotheses, you **must** base your decision on the data that you have generated.
   Circle your decision below:

H1 ACCEPT REJECT

H2 ACCEPT REJECT

(2 Marks)

END OF EXAM