MANCHESTER 1824

The University of Manchester

Faculty of Biology, Medicine & Health

BIOL20332
GENETICS RSM

BIOL20972
DEVELOPMENTAL BIOLOGY RSM

MODULE 1

06-09 February 2023

Andreas Prokop

MODULE 1: Understanding *Drosophila melanogaster* as a model organism for biomedical research

1. GENERAL INFORMATION

1.1. Location & Contacts

This part of the RSM will be delivered by Zoom, organised and taught by Dr. Andreas Prokop (Andreas.Prokop@manchester.ac.uk). It consists of 1 unit lasting 3 days (*see schedule next page*).

Course assistants: Liliana Correia, Nikki Harper, Georgina Pearson, Kate Campbell

1.2. Aims

The aims of this course module are:

1) to introduce to strategies of and develop skills in data acquisition, data management and interpretation (*introduction to immunohistochemistry approaches in Drosophila, phenotype classification, data filing strategies, ImageJ-based image analysis, use of GraphPad*)
2) to introduce to good scientific practice and necessary skills in presenting scientific work (*figure and figure legend exercise*)
3) to gains some experience and develop an understanding of how the use of model organisms (here exemplified for *Drosophila*) can be used to address biomedical problems (*data mining, genetics, cell culture experiments in the context of nervous system development*)

1.3. Intended learning outcomes

By the end of this course you should...

1) .. be able to plan and execute genetic crosses as used on a daily basis in *Drosophila* laboratories across the world.
2) .. have gained a thorough understanding of how to carry out:
   a. basic immunohistochemical and histological experiments in *Drosophila* and beyond.
   b. filing, documentation, analysis and presentation of microscopic specimens.
   c. present images in scientific publications including your final year reports.
3) .. have a principal understanding of how to use genetics and cell biological strategies to study developmental mechanisms.
4) .. be able to appreciate the use of model organisms such as *Drosophila* as an important model for the study of genetic and developmental mechanisms.

1.4. Assessment

A document is handed out which summarises all required assignments; these will comprise:

Completed during the course:

- **A1:** Calculating dilutions (completion of task; 4 pts of module, 1% of course)
- **A2:** Embryo analysis (completion of task; 4 pts of module, 1% of course)
- **A3:** BLAST and data base searches (completion of task; 16 pts of module, 4% of course)
- **A4/5:** Primary neuron analysis (completion of task; 20 pts of module, 5% of course)

Completed outside the course:

- **Assignment 1:** Figure and legend (assessed task; 28 pts of module, 7% of course)
- **Assignment 2:** Mating scheme solution (assessed task; 28 pts of module, 7% of course)

1.5. Preparation for the course

The fruit fly *Drosophila* is a highly efficient and cost-effective model organism to explore fundamental mechanisms of biology, which then often apply to higher organisms - to a degree that genes of mice or humans can often rescue the loss of the equivalent gene in flies. Many advances in modern biology would not have been possible without *Drosophila*, and 10 Nobel laureates awarded in "Physiology or Medicine" for work in fruit flies clearly illustrate this statement. For further details, please see:

- droso4schools.wordpress.com/why-fly
- Prokop, A. (2018). Why funding fruit fly research is important for the biomedical sciences. *Open Access Govern* 20, 198-201 -- tinyurl.com/y7b25jpm
- Prokop, A. (2016). Fruit flies in biological research. *Biological Sciences Review* 28, 10-14 -- tinyurl.com/ybvpoqmw

During this module you will be introduced to key strategies of state-of-the-art biomedical research using fruit flies. You will be trained by executing a number of activities which will include applied genetics, image analysis, statistical data evaluation and the use of data bases. Please, note that this module is entirely online which means that you will not get wet lab experience, but be introduced to aspects of experimental design and data analysis/management which are pivotal for successful research.

In order to fully capitalise on the training within this module, please prepare the following well in advance of the course:

- **GENETICS MANUAL:** An essential strategy in most labs using fruit flies for their research is the use of applied *Drosophila* genetics: employing classical genetics strategies to generate fly stocks that carry combinations of different genes or genetic tools, which can then be used for experimental studies. The training applied on this course is being used in *Drosophila* labs across the globe and its resources have been downloaded over 40,000 times (more info here: https://sites.manchester.ac.uk/fly-facility/training). Please, download the genetics manual from our repository (https://ndownloader.figshare.com/files/3697503) and study it before the course starts: having studied the contents will enormously improve your experience and facilitate your performance on the module, will help with one of the assessments, and will improve your understanding of classical genetics also in the context of other model organisms.
- **GRAPHPAD PRISM:** This state-of-the-art statistic program is freely available to full-time students. If you do not yet have it, please go to https://www.itservices.manchester.ac.uk/software and click the link ">Applications website" which prompts you to log in. [Note: if you are off-campus, you need to activate your VPN; follow the ">Virtual Private Network (VPN)" link on the same page if you do not yet have it.] Once logged in under ">Applications website", choose "Browse Applications" in the left-hand menu and click "Statistics & Qualitative Methods", then select "Graphpad Prism". Select the "Installation" tab and click the "Knowledgebase..." link. It will prompt you to log in again [sometimes it only opens a default page: go back to the previous page and click the "Knowledgebase... " link again]. It should now open the "Graphpad Prism: Installing and licensing" page with licensing and installation information and functionality. Note that the process involves that you are sent an email with an activation code: if you had GraphPad installed already but have no longer access, check for a renewal email you may have received a while ago.
- **IMAGE J/FIJI:** This image analysis program is used by biologists worldwide and provides countless plugins for all kinds of applications. If you do not yet have it, please go to https://imagej.net/software/fiji/downloads and download the version appropriate for your

system (note: >300 MB will take some time). Drag the "Fiji.app" folder out of the zip folder and copy it onto the desktop or into your "Documents" folder. Open the folder and click the "ImageJ.exe" file; the program will open, usually asking you to update when opening the first time. Of note: MAC users might find this link to work better to download it bundled with java8 instead: https://imagej.nih.gov/ij/download.html; if it does not let you open the application because it cannot "*verify the app developer and that the app is free from malware*", do the following:

1. Go to 'System Preferences / Security & Privacy'.
2. Stay in 'General' tab and click on the lock (bottom left) to 'unlock' (*it will ask you for your password*).
3. This should give you a notification asking if you want to bypass the security to allow ImageJ to be installed.

If you do not have a suitable computer or experience other difficulties, please contact Andreas.Prokop@manchester.ac.uk well ahead of the course. Finding solutions might take a few days and cannot be arranged on the day when the module starts.

1.6. Expected timetable of the module (***P*** *= PPT presentation for all;* ***L*** *= live presentation for all;* ***A*** *= activity in breakout session;* ***O*** *= off line*)

Monday 06 Feb (*https://zoom.us/j/99952505902 Passcode: Mod1*)

11.00-11.30 Introduction: ***P1*** *- Welcome & intro to Drosophila as a model in general and for the study of nervous system development in particular*
11.30-12.30 Demonstration: ***P2*** *- Preparing embryos for immunohistochemistry*
Activity: ***A1*** *- Calculating dilutions* (4 pts)
12.30-13.00 Lunch
13.00-13.20 Demonstration: ***P3*** *– Background on antibodies, embryo staining, data filing*
13.30-14:20 Activity: ***A2*** *- Analysing staining results* (4 pts)
14:30-15:10 Results/Discussion: ***P4*** *– Discussing results and their meaning*
15.20-16.00 Instruction: ***P5*** *- How to design a figure & legend* (assessed 28 pts)

- Download documents for 1st day: *available on the day*

Tue 07 Feb (*https://zoom.us/j/99952505902 Passcode: Mod1*)

11.00-11.20 Background: ***P6*** *- Genetics introduction* (later assessed 28 pts)
11.30-13.00 Activity: ***A*** *(download) - Genetics training - PowerPoint [LINK]*
13.00-13.30 Lunch
13.30-14.00 Demonstration: ***L*** *- data bases*
14.00-15.00 Activity: ***A3*** *- own BLAST & database work* (16 pts)
15.00-15.45 Discussion: ***L*** *– outcomes of data base work*
Demonstration: ***L*** *– solving genetic tasks*
15.45-17.00 Activity: ***A*** *(download) - solving genetic tasks (training) [LINK]*

- Download documents for 2nd day: *available on the day*

Wed 08 Feb (off-line)

13:00-17:00 *Re-read the Genetics manual*
Compose figure and legend
(all assignments will be jointly submitted by Fri 17 Feb)

Thu 09 Feb (*https://zoom.us/j/99952505902 Passcode: Mod1*)

11:00-12.00 Background: ***P7*** *- primary neuron culture*
Demonstration: ***L*** *- culture analysis*
12.00-13.00 Lunch
13.00-14.20 Activity: ***A4***, ***A5*** *- own primary neuron analyses* (assessed 6 pts)
14.30-15.50 Activity: ***A*** *(download) - solving genetic tasks (training) [LINK]*
16:00- Solve the **assessed genetic task** *(will be provided at the end of your breakout session and briefly discussed with your tutor)*

- Download documents for 3rd day:
  - Download activity documents: *available soon*
  - Download the Genetics task assessment: *available on last course day*
  - Genetics solutions: *available on last course day*

2. BACKGROUND INFORMATION

2.1. Addressing the genetic basis of two distinct biological processes

All biological processes and phenomena - from development to tissue maintenance, from metabolism to neuronal activity - are orchestrated through genes and the factors they encode. Which are the genes required in each particular context? How are these genes regulated? Which functions do their products execute? How do they interact with other gene products? Genetic model organisms can be used effectively to give profound answers to these questions. This RSM module will focus on one such model organism, the fruit fly *Drosophila*, and demonstrate how *Drosophila* can be used to identify and study genes in certain biological contexts. Here we will focus on the **guided growth of axons** as a key developmental process that ensures functionality of a nervous system (*Prokop et al., 2013, J Cell Sci 126, 2331ff.*). Axons are the cable-like, often meter-long cellular processes of neurons that wire the nervous system, hence form the core of nerves and nerve tracts. They are the cables that conduct electrical messages in form of action potential/nerve impulses and pass them on at synaptic contacts with appropriate target cells (*other neurons, muscles or gland cells*) which can be up to a meter away in our body (*Fig.1A,B*). This organisation is the cellular basis of coordinated behaviour and requires that axons wire up correctly. The precision of this wiring process is achieved during development through the guided growth of axons along paths which are specific for every neuron. For this, the growing tips of axons, called **growth cones** (*arrow heads in Fig.1A*), make stereotypic step-by-step directional decisions along their paths. They interpret chemical and physical properties of the surrounding tissues, in form of diffusible or contact-mediated molecules which have been precisely arranged in space and time through the processes of pattern formation. To sense these cues, each growth cone must be equipped with its appropriate individual set of signalling receptors, and this is a function of their birth history. Therefore, it is pivotal that patterning processes, during neurogenesis in the nervous system as well as in all tissues that axons grow through, are precisely coordinated. In this RSM module we will look at genetic mechanisms

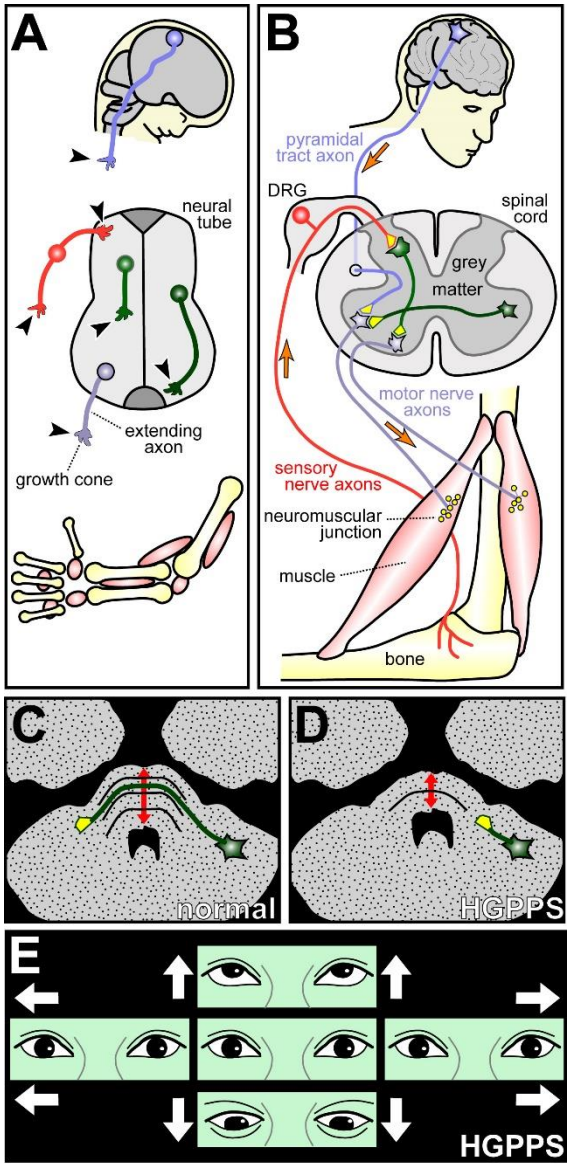

Figure 1. *Examples of inherited human diseases relevant to this Genetics RSM module:*
A*) In the developing nervous system, neuronal axons grow out along reproducible paths via growth cones at their tips (arrow heads).* ***B****) Stereotypic guidance of axonal growth ensures that sensory neurons (red), interneurons (green) and motorneurons (blue) arrange into correctly hard-wired circuits that conduct action potentials along cable-like axons over long distances (orange arrows) and pass them on at synapses to their appropriate target cells (yellow triangles and circles).* ***C-D****) Mutations of the* robo3 *gene cause Human gaze palsy with progressive scoliosis (HGPPS; Jen et al., 2004, Science 304, 1509ff.), and HGPPS patients have a severe reduction of commissures (red double-arrows), suggesting that commissural neurons (green) fail to cross the CNS midline.* ***E****) HGPPS patients show a coordination deficit across the body axis, for example the inability to move eyes horizontally. Work in* Drosophila *was instrumental in paving the way to a molecular understanding of this disease (Fig.3).*

governing one particular choice event that occurs at the midline of the CNS, where growing axons either stay on the same body side (*red, blue, light green in Fig.1*) or cross over to the other half (*dark green in Fig.1*). As will become clear during this RSM module, the genetic mechanisms underlying pattern formation or guided neuronal growth are often of relevance to human disease, and aberration of such mechanisms will cause malformation or malfunction (*Figs. 1C-E*).

Apart from their correct development, axons are usually maintained for a lifetime of an organism. This means that these delicate cellular structures of up to 1 m length with a diameter of <15 µm (*as found in our peripheral nerves or the white matter of the spinal cord*) survive for up to a century. Therefore, axons are the prime lesion sites in ageing, injury, coma and neurodegenerative processes, such as dementias (*including Alzheimer's disease*), spastic paraplegias and other hereditary diseases, multiple sclerosis, diabetes, chemotherapy or other toxin-induced neuropathies. Accordingly, we lose 40% of axons towards high age and even more in neurodegenerative disorders. Key roles are played by bundles of microtubules running all along the axon shaft, and we will analyse genetically induced aberrations of microtubule bundles during this RSM. See below a model developed to explain axon degeneration:

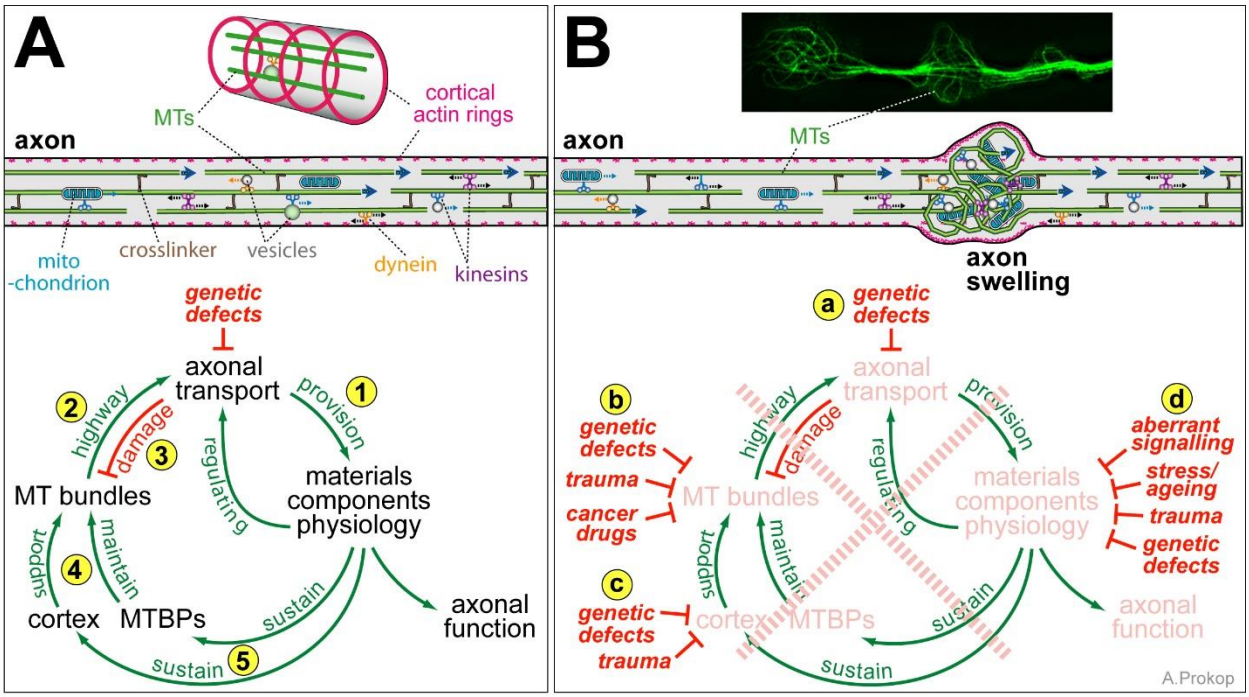

Fig. 2 *The dependency cycle of axon homeostasis. (**A**) An axonal segment is shown at the top with bundles of polar MTs (directional polymerisation shown as blue arrows), motor proteins and transport cargoes; the three-dimensional image at the very top shows the spatial relation of cortical actin rings and MTs. The bottom image shows the "dependency cycle of axon homeostasis": (1) axonal transport provides cargoes essential for axonal function and physiology; (2) this transport requires MT bundles as essential highways; (3) axonal transport causes mechanical damage to MT bundles; (4) MT-binding proteins (MTBPs) and the cortical actin sleeve maintain and support MT bundles; (5) MT bundle maintenance requires axonal transport-dependent materials and physiology. (**B**) The sketch at top shows an axonal swelling containing disorganised MTs (see also super-resolution image above) and accumulated organelles. The image below illustrates the fact that the cycle of axon homeostasis shown in A can be lesioned in many positions, causing a chain reaction of auto-destruction; examples for lesions in positions (a) to (d) are provided in the main text* (*Prokop, 2021, Cytoskeleton ff.*).

2.2. The fruitfly *Drosophila melanogaster* as a genetic model organism

The use of model organisms is common practice in biomedical research and, at least since full genomic sequences were available, it has become clear that many genes are evolutionary conserved and insights are translatable between organisms (*Fig.3*. In particular, the use of genetically tractable invertebrate model organisms, such as the fruit fly *Drosophila melanogaster*

or the worm *Caenorhabditis elegans*, enormously advanced our knowledge about genetic mechanisms underlying biological processes, with important implications for medical research (*Fig.3*)[1]. Therefore, model organisms like *Drosophila* are often seen as tools or "test tubes" for human genetics. Further advantages of using fly are explained in detail in the accompanying document "FlyGenetics-IntroStudents.doc" (available on shar.es/Yis4C) or read the recent advocacy articles[2,3].

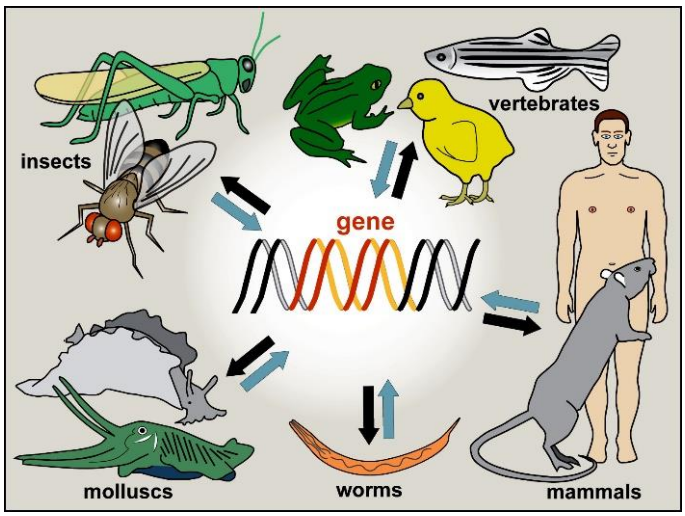

***Figure 3**. Using model organisms:*
Different model organisms are being used in biology. Researchers must choose the most suitable organisms for their scientific questions, considering their particular pros and cons. Since genes are well conserved across the animal kingdom, insights obtained in simple model organisms tend to be translatable into the biology of higher organisms and humans, speeding up investigation and improving understanding of fundamental biological mechanisms and processes - often relevant for studying disease.

During this RSM module, you will experience three important aspects of work with flies:

- You will learn **how *Drosophila* genetic tools can be employed to decipher principal mechanisms** underpinning biological processes. To illustrate this, axonal growth guidance at the CNS midline will be used as examples (*see Section 2.3*). You will interpret genetic mutant phenotypes and get introduced to the use of available data bases to extract information about these genes and their orthologues in humans.
- You will be introduced to and apply strategies to analyse **cultured primary *Drosophila* neurons**, analysing their axon lengths and microtubule bundle organisation, a procedure that is used daily in my own laboratory. You will use **statistical programs** to assess the quality of your data.
- You will learn how **applied genetics in *Drosophila*** is used to achieve the above outcome. To understand and appreciate this strategy, you will be trained in **mating scheme design**, which will introduce you to the ways in which the rules of classical Mendelian genetics are applied to capitalise on mutations and transgenic tools. This is relevant for **state-of-the-art research** in fly laboratories and introduces you to **principles of work with all genetic model organisms including mice**. This training is supported by a fly genetics manual and a PowerPoint presentation available as eLearning resources (see page ii).

2.3. The midline of the *Drosophila* CNS

The spinal cord of vertebrates and the ventral nerve cord of *Drosophila* show certain commonalities in their organisation (*Sánchez-Soriano, N. et al., 2007, Neural Develop 2, 9*). One common principle is that axons of certain classes of neurons cross the midline of the nervous system, whereas axons of other sub-groups of neurons never cross but stay ipsilateral (*on the same side of the body where their soma lies; Fig.4*). Mechanisms underlying this simple choice

[1] see droso4schools.wordpress.com/why-fly

[2] Prokop, A. (2016). Fruit flies in biological research. *Biol Sci Rev* **28,** 10-14 -- tinyurl.com/ybvpoqmw

[3] Prokop, A. (2018). Why funding fruit fly research is important for the biomedical sciences. *Open Access Govern* **20,** 198-201 -- tinyurl.com/y7b25jpm

are shared between vertebrates and *Drosophila*. They have been discovered first in *Drosophila* through the use of genetic screens (*Fig.2 of "FlyGenetics-IntroStudents.doc")*. Here, you will get to know some of these genes and experience how their functions can be studied.

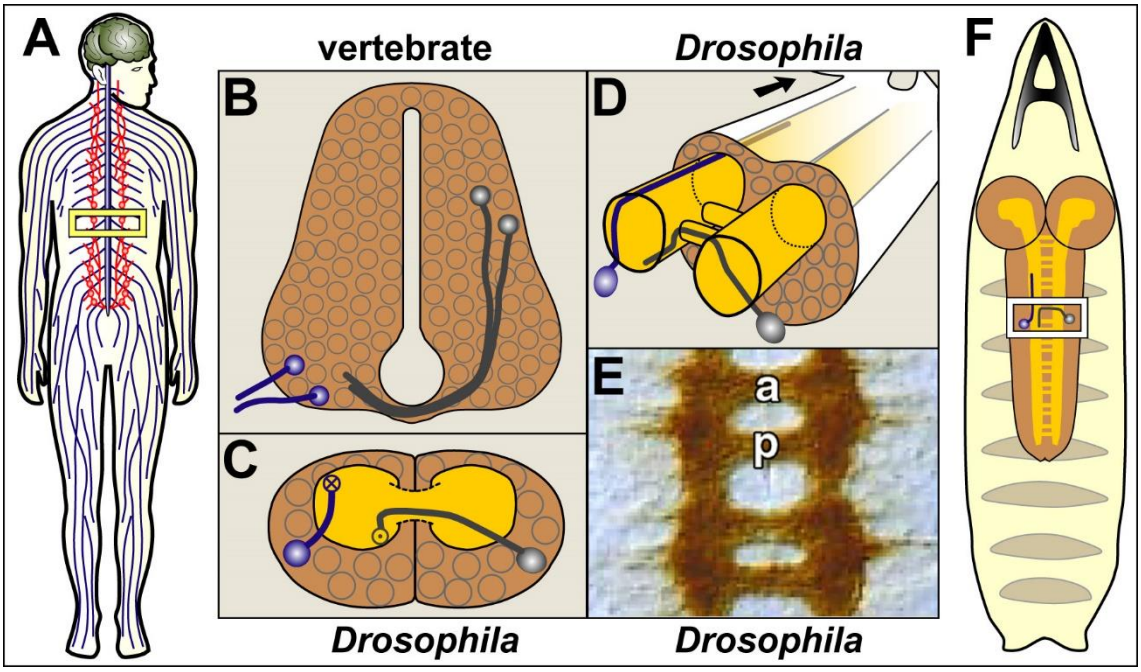

***Figure 4**. Axon crossing at the CNS midline. **A)** Schematic view of the human nervous system with brain (green), spinal cord and nerves (blue), and the autonomous nervous system (red). **B**) Cross section through the developing vertebrate spinal cord (corresponding to boxed area in A) with grey commissural axons crossing the midline and blue motoraxons projecting ipsilaterally. **C-E)** Different views of the developing ventral nerve cord of* Drosophila *(corresponding to boxed area in F); axons crossing the midline are shown in grey, ipsilateral axons in blue; the specimen in E is stained with BP102 antibody (brown) which highlights the axonal area called neuropile (orange in C, D, F; a, anterior; p, posterior commissure). **F**) Top view of a* Drosophila *larva.*

2.4. Overview of 'experiments'

You will study the functions of genes involved in axonal growth guidance at the midline of the embryonic *Drosophila* CNS. For this, you will identify and describe mutant embryos in which the 'pathfinding' or 'guidance' genes A, B and C are dysfunctional. As readout, you will use antibodies which mark preferentially the neuropile (*BP102, α-Fas2; Figs. 4 and 5*). Complementary to this, you will see antibody staining of wildtype embryos to analyse the natural expression of proteins A and C. Using data base research, we will unravel the nature and function of these genes.

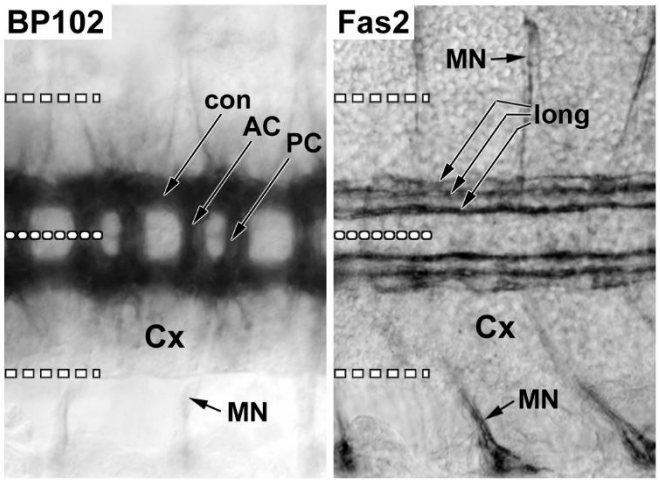

***Figure 5**. Staining patterns of BP102 and Fas2 in the embryonic CNS (compare Fig.3, D-F). Horizontal views of CNSs (dashed lines, lateral borders; dotted line, midline) stained for BP102 labelling the synaptic neuropile, or Fas2 labelling motornerves (MN) a subset of 3 longitudinal axon bundles within the connectives of the neuropile (long). Further abbreviations: AC, anterior commissure; con, connective; Cx, cortex; PC, posterior commissure.*

APPENDIX

Since you will have little laboratory experience this year, it is nevertheless important that you make yourself aware of some good practice to build on if you enter laboratories at later stages, for example for your final year project. Here are some tips for you to consider for practical work.

A1. DOCUMENTATION OF EXPERIMENTS AND FILING OF DATA

A1.1. Why bother? A short introduction

It is a frequent requirement in scientific life to revisit data or specimens that were generated years ago. In such a situation you must be able to find and access relevant materials, understand your notes, and reproduce experimental procedures or conclusions. It is pivotal that you take good notes about your experiments and file your specimens and data efficiently and reliably. This requires discipline, care and feasible strategies, all of which can be trained on this course. Notably, your documentation and protocols will be essential proof of your work and must be good enough to withstand critical or even legal investigation by others.

A1.2. Guidelines for the laboratory protocol

You must keep a laboratory note book which is up-to-date at any stage of the experiment. An experiment that has not been properly recorded has not been done! However, consider that in a real laboratory situation you will not have the time to write long texts and explanations. Instead, try to keep your notes concise but nevertheless precise and understandable. Try whether others can understand them, since this is a good test demonstrating how likely it is that you will understand your own notes in years to come. Consider the following, which will also be discussed repeatedly during the course:

a) NUMBER and DATE each page used. Use permanent ink and no correction fluid. Any errors must be crossed out with a single line so that the original text is still visible. The correction must be justified in words.

b) In case several experiments run in parallel, develop a system to keep them separate in your notes in a clear and understandable way.

c) Before starting, be clear about your experiment from its design through to its documentation. Write down the AIM OF EACH EXPERIMENT before you start. This is a good way to make sure you really understand the rationale. If you are unclear or disagree, re-discuss with your supervisor. Consider that you want to invest YOUR time ONLY in sensible experiments!

d) For each day, give a BRIEF OVERVIEW of the day's task, so that the reader can make sense of detail that follows; for example "*Preparing stage 16 embryos of different genotypes for antibody stainings*"

e) Note down SINGLE STEPS OF YOUR EXPERIMENTS. If you use standard protocols you may not have to explain each step but rather refer to the protocol in a precise manner; for example "*embryos were released from their chorion and vitelline membrane and then fixed as described in protocol 1 of the BL20332 RSM manual 2018, module 1"*.

f) However, note down INDIVIDUAL PARAMETERS & DEVIATIONS FROM THE PROTOCOL. For example, in this module you must note down the GENOTYPE (*wild type, mutant, transgene?*), the DEVELOPMENTAL STAGE of specimens (embryonic, larval), the MATERIALS/CHEMICALS used (*e.g. fixatives, antibodies with relevant concentrations*) and potentially also equipment used. Also keep written track of where you are at any stage of the protocol by noting down the TIMES WHEN YOU CARRIED OUT THE INDIVIDUAL STEPS.

g) Note down the intermediate and final OUTCOMES of your experiments: Did the staining work? Note down how many slides with specimens you generated, what their quality was and where exactly they were stored (*see Section 4.3*). Which STATEMENTS can you deduce? Has the aim of the experiment been reached (*see c*)? Is further work required (*e.g. repeat of experiment using different parameters, control or validation experiments*)?

f) SPECIAL OBSERVATIONS, PROBLEMS, TIPS, TRICKS, EXPLANATIONS or THOUGHTS that cross your mind might help you in future, and you might want to write them down. However, make sure you separate them out from the actual experimental details (*e.g. box them in as "side note"*).

A1.3. How to file microscopic specimens

Immediately after you have embedded your specimens, you MUST LABEL the respective slides with PENCIL on the FROSTED SLIDE EDGE; please, write down the following items (*letters refer to accompanying Figure*):

a) The DATE at which you carried out the experiment. This information will link directly to the respective notes in your protocol book and will help you to recall the conditions and details of the respective experiment.

b) The DEVELOPMENTAL STAGE of the specimens (*e.g. "stage 16" embryo or "L3" = 3rd instar larval stage*) and their GENOTYPE (*e.g. "wt" = wild type, "mutant A", or "C-lacZ"*).

c) The EXPERIMENTAL TREATMENT (*e.g. "BP102" = stained with anti-BP102*); information under b) and c) provides a quick overview and reassure the identity of specimens.

d) A QUALITY JUDGEMENT (*given by course assistants before you file the slide*):

"-" = no staining or too much background (*usually discard right away*)

"+/-" = weak but identifiable specific staining

"+" = in principle good staining, but tissue preservation or orientation not optimal

"++" = all aspects of high quality

This rating will make sure that, during the documentation sessions, preparations of highest quality are used most and no time is wasted on bad preparations.

e) YOUR GROUP NUMBER; this allows other groups to consult with you about specific aspects of your experiment or reassure the specimens' identity.

f) Once the slide has been inspected and all information noted on the frosted slide edge, bring it to the common slide box, choose the next available and appropriate slot and write the SLOT NUMBER onto the slide ("f" in Fig. 10). This ensures that slides will be returned to their place of origin and can be reused by others.

g) Insert key information (*genotype/stage, experimental treatment, quality of specimen*) into the appropriate space of the LIST IN SLIDE BOX LID (Fig. 10, left). This facilitates quick browsing for suitable specimens.

h) Remember to write down the SLIDE BOX SLOT POSITION IN YOUR LABORATORY NOTE BOOK, to be able to find and revisit your own specimens.

i) When documenting, make sure also others can work. Therefore, NEVER HAVE MORE THAN TWO SLIDES AT YOUR PLACE and always RETURN SLIDES INTO THE CORRECT BOX AND SLOT.

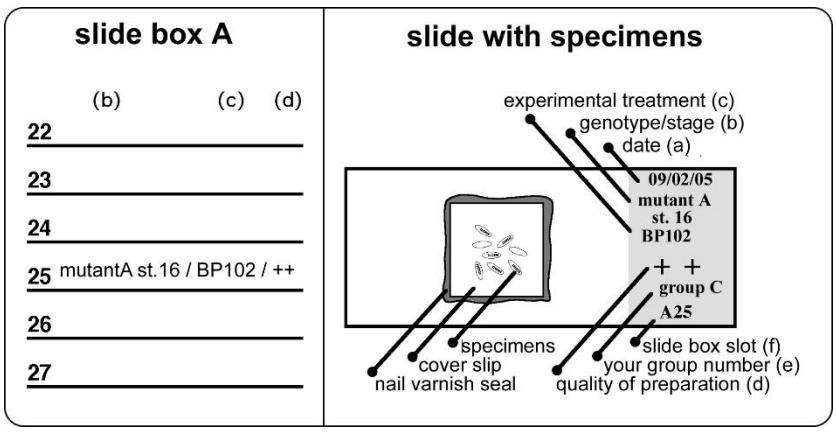

Figure 10. *How to file slides. A slide with embedded specimens (sealed with nail varnish) is shown on the right; the frosted edge (grey) contains important information (see text). In this example, BP102 staining of stage 16 embryos of the mutant strain A has been carried out by group C, they are of high quality and the slide belongs into slot 25 of slide box A. The lid of the slide*

box is shown on the left; it lists information (b), (c) and (d) from the slide, allowing all students to browse efficiently for appropriate preparations.

A2. COMPOSING A FIGURE AND FIGURE LEGEND FOR SCIENTIFIC PUBLICATIONS

Examples of figures and figure legends are given throughout this manual. However, also have a look at scientific publications and look at their figure designs and legends. You will find good and bad examples, but think about the criteria that have determined your judgement. Please, find below a number of rules or suggestions you can follow when composing a figure and its legend. This information can also be downloaded as a PowerPoint presentation in which you will find images illustrating each statement (*"05 - Figure legend.ppt" - available on shar.es/Yis4C*).

1) Think of a statement you want to make with a figure and choose the images to support it.
2) Formulate a legend title (*e.g. 'Mutations affecting the embryonic neuropile in Drosophila'*).
3) Arrange images in a logic sequence and in right orientation (*anterior left/up, dorsal up/right*).
4) Label single images with capital letters, and refer to these letters in text and legend.
5) Explain what is to be seen (*e.g. which species, tissue, stage, staining*); to this end, ..
   a) .. group statements common to all/some images, instead of repeating them for each image separately (*e.g. 'All specimens are stained with anti-X', or 'A-C show embryos at stage 16, D-G late larval CNSs'*); this strategy saves space and facilitates reading.
   b) .. Make extensive use of symbols or abbreviations in the figure to guide the reader unequivocally and efficiently through your images [*e.g. 'The neuropile of wild type embryos is composed of commissures (white arrow heads in A) and connectives (white arrows in A), whereas commissures are missing in mutant X (black arrow heads in B) and connectives are absent in mutant Y (black arrows in C)'*]; use symbols consistently throughout your figure.
6) You may indicate further information directly within each image, especially if it concerns features differing from image to image (*e.g. indicate abbreviated genotype in the top right corner of each image and the respective antibody staining in the bottom right corner*). This allows a specialist reader to grasp the content of a figure without having to read through the complex figure legend.
7) All used abbreviations must be explained in the legend.
8) A figure must show a scale bar if biological material is displayed.