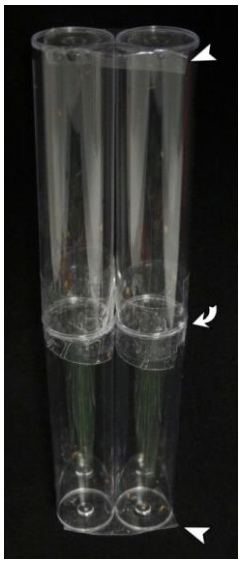
1) FLY CLIMBING WALL: TESTING MOTOR SKILLS

Preparation ahead of event:



- Age wild type *Drosophila melanogaster* for 6 weeks at 25°C in standard fly food vials [1]. To prevent flies getting stuck in the food, have a strip of filter paper in the vial for flies to climb on, and transfer flies to fresh vials every 2-3 days.
- Have vials with new flies hatching in the week before the event.
- To determine required number of flies:
  - consider that you will need 10-15 old and the same amount of young flies per stand
  - consider how many stands you will need for how long (one stand can be used for 1.5 hours): in schools, the number of stands per groups of 2- 4 pupils is the determining factor, on science fairs you will use only 1-3 stands but for considerably longer time.
  - For example, to have 200 old and 200 young flies, you should start with the ageing of 15 well populated vials 6 weeks ahead, and have about 10 vials with hatching flies in the week before the event.
- Prepare the climbing walls: print copies of the '*StandClimbing-Wall.pdf*' (Exhibition folder) and glue them onto appropriately sized cardboard trays or lids. Place a weight behind the trays to maintain them upright (we use tape dispensers).

- Preparation before the event:
  - Ideally the evening before the event: use ether, the freezer method or a CO_2 pad to anaesthetise flies and transfer 10-15 flies to a fresh food vial. The following morning transfer the flies to an empty plastic vial [2] using a small paint brush or pooter [3]. Attach the open end of the vial to a second vial to create one long enclosed tube and secure the tubes with sticky tape (*curved arrow in image*).
  - Do this in parallel for aged and young flies; mark the top of the tube with aged flies to distinguish them from the young ones.
  - Join the two tubes in parallel and hold them together with sticky tape at the top and bottom (*arrow heads*).
- Carrying out the experiment (see: '*04-ClimbingAssay.wmv*' in 'Schools' folder)
  - Tap flies down and place vials upright in front of the climbing wall.
  - After 15 seconds, take a picture from frontal, so that vials align with the scale of the climbing wall.
  - Show images online or print them out.
  - Visitors/pupils count numbers in each sector of the height scale, note them down on the print or a separate paper, and plot them as a graph ('*Climbing-Plot.pdf*').
  - Data are cumulatively entered into a spread sheet ('*Climbing-StatsSheet.xls*') to increase sample numbers and improve the statistical outcome.
  - In schools, the **presenter** will require **one helper** to take pictures, print sheets, enter data into the spread sheet. On science fairs, **2 people** can run a stand with one set-up.
- Alternative way to carry out the experiment (race version)
  - Visitors/pupils find a common rhythm to tap the fly vials down three times synchronously

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[1] How to get fly food: www.flyfacility.ls.manchester.ac.uk/documents/Manchester%20Fly%20Food.pdf

[2] To purchase empty vials: flystuff.com/drosophila-products/vials/

[3] How to make a pooter: ndownloader.figshare.com/files/3306824

- A stop watch is shown on screen [1].
- Visitors/students work in teams of at least two.
- Flies are tapped down synchronously across all teams and the stopwatch is started.
- One person in each team observes the flies and gives a signal when the first young and the first old fly reach the top; the other person looks at the stop watch and notes down the time for young and old.
- A plot is drawn on the board indicating "young" and "old" on the X-axis and 0 to 40 s on the Y-axis
- Groups are asked to provide their two times and data are plotted as they come in..
- A brief discussion can take place talking about data clouds typical of biology and simple ways to describe them (mean or median as the most obvious first ones).
- Make pupils especially aware of the statistics web page [2].

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2) LOOKING AT GENETIC MARKER MUTATIONS

- Preparation ahead of event:
  - Obtain [3] and maintain stocks of flies carrying different marker mutations. For example, we use wildtype, *Curly* (curved wings), *Krüppel*$^{lf\text{-}1}$ (slit/shaped eyes), *white*, *mini white* (orange eye colour), Serrate (notched wing tips), *vestigial* (rudimentary wings) *and eyeless* (no/rudimentary eyes).
  - Organise or buy simple teaching stereomicroscopes; we bought cheap battery-powered stereomicroscopes [4].
- Preparation the evening before or morning of the event:
  - Anaesthetise flies from each stock, using ether, the freezer method or a CO_2 pad [5].
  - Use a small paint brush or pooter [6] to select flies clearly displaying the respective markers into a centrifuge tube.
  - Freeze for 30 min at -20ºC to kill flies, and store them in the refrigerator until ready to use.
  - Stick a strip of double-sided sticky tape across the centre of a 5 cm plastic petri dish (*see image*).
  - Using a pair of fine forceps carefully position the flies laterally and in the correct sequence (*see image*). Press the flies gently down with the forceps to secure them in their position.
  - Place wet tissue paper in the petri dish to prevent fly specimens from drying out (*image*).
  - Put the lid back on the petri dish and secure it with two strips of sticky tape on opposite sides. Do not seal all around to prevent condensation.

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[1] Stopwatch can be downloaded here: www.online-stopwatch.com/full-screen-stopwatch

[2] Statistics webpage: droso4schools.wordpress.com/l2-climbing-assay/statistics

[3] Fly strains are available on request: Sanjai.Patel@manchester.ac.ukailto

[4] Information about affordable microscopes: www2.mrc-lmb.cam.ac.uk/microscopes4schools/

[5] Where to get CO_2 pads: flystuff.com/drosophila-products/anesthesia

[6] How to make a pooter: ndownloader.figshare.com/files/3306824

- These dishes can be stored at 4ºC for 1-2 days and will keep for 8 hours during the event. Therefore, it is sufficient to generate one dish per microscope, but have 2-3 replacement copies just in case.
- Print sufficient copies of '*Genetics-ActivitySheet.pdf*', or generate your own using the Genotype Builder [1].

- Carrying out the experiment
  - Visitors/pupils look at the flies under the stereomicroscope.
  - They assign observed phenotypes to the numbered fly sketches shown on the activity sheet and indicate the order in which they are arranged.
  - The written-down answers indicate whether visitors/pupils were able to see the correct features when looking at the flies, which is a simple but powerful quality control.
  - In schools, the presenter alone can handle this task. On science fairs, this stand can be very busy and **2 helpers** can handle 5-6 microscope stations.
  - Good to use the activity sheet (LINK) which spells out a gene name on busy science fairs as you don't need to individually check everyone's answers

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3) SEIZURE AND PARALYSIS

- Preparation ahead of the event:
  - Obtain and maintain *shibire*^{ts1} / *shi*^{t1s} and *paralytic*^{bss1} / *para*^{bss1} (*bang senseless*) mutant flies [2]. Make sure that *shi*^{ts} flies are kept between 18°C and 25°C and *bss*^{1} flies are always kept at 25ºC.
  - Boost stocks ahead of the event to have plenty of flies: For school visits, you will need 2 vials of each fly stock per student pair; for science events you will need 100 vials of each stock which is sufficient buffer to allow used flies to recover and be re-used for another experiment (see procedures below). For science fairs, we suggest to use wild type flies as controls, rather than use *shi*^{ts} and *bss*^{1} as controls for each other (which is a very powerful approach in schools, demonstrating the specificity of the effect).
- Preparation shortly before the event:
  - Prepare an appropriate number of fresh fly food vials [3] containing ~10 flies of either the *shi*^{ts} or the *bss*^{1} stock, respectively. Label them distinctly with coloured sticky tape to distinguish the vials.
  - Use different coloured tape across the top of the vials (leaving an air gap!) to distinguish the fly stocks and make sure cotton plugs do not fall out.
  - Vials are no longer suitable for use if maggots accumulate and the food gets soggy. In that case, transfer flies to fresh vials. Combine flies from different vials if numbers per vial have gone down.
- Carrying out the Epilepsy/Seizure experiment
  - Use one *bss*^{1} vial together with one *shi*^{ts} vial per visitor/pupil; vials contain ~10 flies each;
  - Visitors/pupils hold fly vials from the top and shake vigorously from the wrist (see '*06-ShakingFlies.wmv*'). The *bss* flies fall to the ground due to seizure, whereas the *shi*^{ts} flies are unaffected

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[1] Genotype Builder: '*Roote+Prokop-SupplMat-5v2.psd*' at dx.doi.org/10.6084/m9.figshare.106631

[2] Fly strains are available on request: Sanjai.Patel@manchester.ac.uk

[3] Fly food vials: www.flyfacility.ls.manchester.ac.uk/documents/Manchester%20Fly%20Food.pdf

- *bss*[1] will need at least 1 hour of recovery time before they can be re-used. Therefore, bring an empty tray to store flies in sequence for recovery; make sure that the flies with longest recovery time are used first
- In schools, the presenter alone can handle this task, but will require time before each session to distribute the fly vials to individual desks. On science fairs, this stand can be very busy and **at least 2 helpers** are required at any given time.

- Carrying out the Paralysis experiment
  - Use one *shi*^{ts1} vial together with one control vial (*para*^{bss1} or wild type) per visitor/pupil; vials contain ~10 flies each.
  - Visitors/pupils place one *shi*^{ts} vial and one control vial under their arm pits for ~5 min with the aim of warming them up to 37ºC. The *shi*^{ts} flies fall to the ground due to paralysis, whereas *bss* flies are unaffected.
  - The *shi*^{t1s} flies will need at least 1 hour of recovery time before they can be re-used. Therefore, bring an empty tray to store flies in sequence for recovery; make sure that the flies with longest recovery time are used first.
  - *shi*^{ts} flies can be very inactive in very warm environments and it is a good idea to store them at lower temperatures until ready to use
  - In schools, the presenter alone can handle this task, but will require time before each session to distribute the fly vials to individual desks. On science fairs, this stand can be very busy and **at least 2 helpers** are required at any given time.

4) OPTOGENETICS

- Equipment & fly stocks:
  - Fly Stocks[1]: for larval contraction cross flies of the driver line *elav-Gal4* or *nSyb-Gal4* with a transgenic stock for the targeted expression of light-inducible optogenetic Channelrhodopsin (ChR), either *UAS-ChR2*^{H134} (responding to blue) or *UAS-ReaChR* (responding to red light). For fly seizure the combination *nSyb::ReaChR* works well. For fly jump response we use the stable stock *w,20XUAS-CsChrimson-mVenus (attP18);17A04-p65ADZP(attp40);68A06-ZpGdbd(attp2)*[2].
  - Light sources (consider health & safety regulations for UV light):
    - As blue light source we use a 470 nm LED[3] with suitable power supply[4], or a fluorescent microscope with appropriate excitation filter. If using an LED, use a stereomicroscope, ideally with camera and either a large monitor or projector to demonstrate the effect to visitors/pupils. On science fairs it might be sufficient that individual visitors observe the response, but having a camera and monitor allows small groups to jointly experience the effect.
    - As red light source we use a red 660 nm LED[5] with suitable power supply[16], or a fluorescent microscope with appropriate excitation filter
  - In addition for larval contraction you will need to prepare containers to hold the larvae:
    - Cut off the lids of two 1.5 ml centrifuge tubes.
    - Paint the lids on the inside with a black, water resistant marker pen.
    - Stick them next to each other on a suitable base (e.g. petri dish lid) with adequate glue or double-sided sticky tape.

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1 Fly strains are available on request: Sanjai.Patel@manchester.ac.uk

2 courtesy of Karen L Hibbard; useful reference: www.nature.com/articles/nmeth.2836

3 Suitable blue LED: www.thorlabs.de/thorproduct.cfm?partnumber=M470L2

4 Suitable power supply: www.thorlabs.de/thorproduct.cfm?partnumber=LEDD1B

5 Suitable red LED: www.thorlabs.de/newgrouppage9.cfm?objectgroup_id=2692&pn=M660L4#4426

- Prepare flies/maggots for experiments:
  - Cross Gal4 driver lines to corresponding UAS-ChR lines
  - Dissolve all trans-retinal powder[1] in 0.88 ml of 100% ethanol to make up to a 100 mM solution. Store at -20ºC in a centrifuge tube protected from light with aluminium foil.
  - Use a spatula to transfer ~6 ml of fly food from a fresh fly vial [2] into a plastic syringe. Inject the food into a second syringe so that it forms a dense clot without air bubbles at the back end. Insert the pistil, so that there is no air between pistil and the food clot.
  - Using the scale on the syringe, eject surplus food to set the volume to 5 ml. Pull out the pistil and pipette 100µl of the trans-retinal solution into the food. Inject the entire volume of the trans-retinal-containing food from the syringe into an empty fly vial.
  - For larval contraction assay:
    - transfer flies of the actual cross into the trans-retinal vial[3]. Add a strip of filter paper to prevent flies from getting stuck in the food. I add a soft mesh on top of the food to stop the flies getting stuck which works well

    - Incubate the flies at RT or 25ºC in the dark until you see maggots in the food (~2 days). Transfer adults to a fresh 2nd vial with trans-retinal food to start another cycle.
    - Meanwhile, transfer the 1st vial to 29ºC and incubate for a further 2-3 days in the dark to raise maggots containing both driver and ChR constructs which have taken up trans-retinal. Use fat white maggots which climb the vial walls (wandering larval stage).
    - For control larvae, maintain wildtype flies or any other stock available to you in standard fly food vials at RT or 25ºC for 4-5 days until wandering larvae appear.
  - For adult seizure or jump assay: transfer flies of the daughter generation (i.e. carrying both the driver and ChR constructs) into the trans-retinal vial .Incubate the flies at 25ºC or 29ºC overnight in the dark

- Carrying out the larval contraction experiment:
  - Add a drop of water to each container
  - Use a fine forceps to transfer ~3 control larvae into a Petri dish with water to wash off any debris and from there into one of the containers
  - Repeat procedure with *elav::ChR* or *nSyb::ReaChR* larvae that have been fed with trans-retinal and transfer them into the other container.
  - Using the LED or fluorescent filter, repeatedly shine blue/red light at maggots for 2-3 seconds followed by a equally long break: the *ChR* maggots will contract upon light, control maggots will not, indicating that the light *per se* has no effect in the absence of Channelrhodopsin.
  - At any given time, **one person** is enough to demonstrate the activity.

- Carrying out the induced adult seizure and fly jump experiment:
  - Take a vial *of the Gal4/UAS-ChR* flies that were fed trans-retinal and control flies in separate empty tubes

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[1] Trans retinal powder: www.sigmaaldrich.com/catalog/product/sigma/r2500?lang=en&region=GB

[2] Performing genetic crosses and fly handling: http://shar.es/YcX2f

[3] Fly food vials: www.flyfacility.ls.manchester.ac.uk/documents/Manchester%20Fly%20Food.pdf

- Using the LED or fluorescent filter, shine red light at the flies to induce seizure or make the flies jump. Flies will need 5-10 mins in the dark to recover from seizure. For the jump response it takes an intense light to activate the giant fiber. A ~3mW/mm2 intensity for 50ms works well

5) THE FLY BRAIN ACTIVITY: TRUMP CARDS & NERF GUNS

- Preparation ahead of the event:
  - Print poster ('*StandBrain-Poster.pdf*' in 'Exhibitions' folder) of sufficient size to use it as target for Nerf guns
  - Print 2 sets of laminated top trump cards ('*StandBrain-TrumpCards.pdf*' in 'Exhibitions' folder)
  - 3 Nerf guns with 20 times Nerf gun ammunition [i1]
  - Lollipops are prize
- Carrying out the experiment:
  - Visitors are given a trump card showing a certain brain area highlighted; these cards also have four categories with scores so that they can be used as top trump cards, when the stand is not busy.
  - Participants identify the region depicted on the card on the large poster.
  - They use single bullet Nerf guns (don't look militaristic and are controllable)
  - Whilst they repeatedly try to hit the target, they are told what the region of the brain they were aiming at is and what its function is.
  - Participants are given three cards in total and the time to hit all three targets is measured.
  - They are then told to record their time in a lab book and win a lollipop.
  - The lab book is a good method of data collection for the task and an incentive for children to challenge their friends and parents such that everyone in the group is drawn into the “game”.
  - The activity is very inclusive as people of all abilities are able to do it, and especially children may come back later to challenge others to the game.
  - This activity works well with **two helpers** at any given time: one person doing the timing and handing out bullets, while the other shows the cards and discusses the brain regions they are aiming at.

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6) DEMONSTRATING HISTOLOGICAL STAINING WITH CELERY

- Materials needed:
  - A simple compound microscope with light source and 10-20X objective
  - Microscope slides
  - A handful of celery trunks
  - Razor blades with one blunt end
  - Protection kit (lab coats, gloves, goggles)
  - forceps
  - Staining solution (0.05% solution of toluidine blue in water)
  - Large Petri dish lids (or equivalent) for staining reaction

[1] Nerf guns: https://www.amazon.co.uk/NERF-N-Strike-Elite-Jolt-Blaster/dp/B009NFH54W/ref=pd_bxgy_21_img_2?_encoding=UTF8&psc=1&refRID=NPAVYPAPV2V4PKAF64DJ

- Carrying out the experiment:
  - With the razor blade cut 1-2 mm thin slices off the celery trunks
  - Transfer them on a slide and view under the microscope
  - Transfer same slice into a drop of toluidine blue solution for 10 seconds
  - Wash in water
  - Transfer back on a slide for microccopy
  - Visitors see now precise structures and appreciate the importance of the histological staining procedure
  - At any given time, **one person** is enough to demonstrate the activity

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7) DEMONSTRATING ADH ACTIVITY AND REQUIREMENT

- Making up ADH staining solutions (from *O'Donnell et al., 1975, Genetics 79, 73ff.* - LINK)

Chemical Name	SigmaCatalogue No.	CAS-No.
Phenazine methosulfate (PMS)	P9625-500mg	299-11-6
Nitrotetrazolium Blue chloride (NBT)	N6639-50MG	298-83-9
β-Nicotinamide dinucleotide hydrate adenine (NAD)	N1511-250MG	53-84-9
2-Butanol	294810-100ML	78-92-2
sodium phosphate buffer (pH 7.5)		N/A

  - Personal protection equipment: Lab coats, gloves, lab safety specs, work in fume cupboard when preparing the ADH staining solution and NBT and PMS solutions.
  - 2 ml 0.05M sodium phosphate buffer (pH 7.5)
  - 0.5 ml nitro blue tetrazolium (NBT) (5mg/ml stock solution made up in water - can be stored in the fridge for 1-2 weeks)
  - 0.1 ml nicotinamide adenine dinucleotide (NAD) (50mg/ml stock solution made up in water)
  - 0.05 ml phenazine methosulfate (PMS) (2mg/ml stock solution made up in water, stable when frozen and protected from light for several months)
  - 0.1 ml 2-butanol
  - Use within a week (store in the fridge) and protect from light (wrap the tube in tin foil).
  - Disposal guidance:
    - Fly stocks are not genetically modified organisms; larvae should be frozen at -20°C for two days and disposed via biohazard waste, also standard litter is acceptable.
    - Dilutions of final solutions are high enough that they can be disposed via the sink; for ethanol, please follow your own local procedures for solvent disposal

- Fly stocks required: *white*^{1118} as control, *Adh*^{n1} null mutant flies
- Materials: 50 mm Petridishes, empty vials, food vials, cotton wool, 100% ethanol
- Carrying out the Adh staining experiment:
  - Pupils transfer two ~500 µl drops of staining solution on a petridish lid.
  - Transfer one control maggot into one drop and one *Adh*^{n1} null mutant maggot into the other.
  - Pupils use two forceps or dissection needles to rip open the two larvae (ideally on black surface to better see the maggots).

- Transfer petridish lid to white surface and observe colour reaction; only the control maggots should turn dark.

- Carrying out the alcohol intolerance experiment:
  - The afternoon/evening before the event, transfer ~10 *white*^{1118} control flies and ~10 *Adh*^{n1} null mutant flies into an empty vial and close with a ball of cotton wool.
  - Pipette 1 ml 100% ethanol onto the inner side of the cotton wool, but take care that the ethanol does not run down the sides of the vial.
  - incubate for 20-30 min, sufficient time for most flies to be sedated from the alcohol fumes.
  - Transfer the sedated flies on a fresh food vial and shut with fresh cotton wool.
  - Maintain flies at room temperature until next day: *Adh*^{n1} null mutant (red eyed) flies that have no Adh enzyme activity will have died from the alcohol exposure whereas the white eyed flies are alive and run around.

Appendix: Safety Summary of Stock Chemical Substances:

Ethanol:

Signal word: Danger; Hazard statement(s): H225 Highly flammable liquid and vapour, flash point: 14.0 °C; Precautionary statement(s): P210 Keep away from heat/sparks/open flames/hot surfaces. - No smoking.

UK. EH40 WEL – Workplace Exposure Limit; TWA (i.e. exposure permissible over 8 hours): 1,920 mg/m3

2-Butanol:

Signal word: Warning; Hazard statement(s): H226 Flammable liquid and vapour, flash point = 27 °C; H319 Causes serious eye irritation, H335 + H336 May cause respiratory irritation, and drowsiness or dizziness; Precautionary statement(s): P261 Avoid breathing vapours, P305 + P351 + P338 IF IN EYES: rinse cautiously with water for several minutes, remove contact lenses, if present and easy to do, continue rinsing.

UK. EH40 WEL – Workplace Exposure Limit:

TWA (i.e. exposure permissible over 8 hours) = 308 mg/m3

Nitrotetrazolium Blue chloride

Signal Word: Warning; Hazard statement(s): H302 Harmful if swallowed; Precautionary statement(s): None

Phenazine methosulfate

Signal word: Warning; Hazard statement(s): H315 Causes skin irritation, H319 Causes serious eye irritation, H335 May cause respiratory irritation; Precautionary statement(s): P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray, P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do; continue rinsing.

β-Nicotinamide adenine dinucleotide hydrate

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008

Sodium phosphate buffer (pH 7.5)

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008