ESTABLISHING AN OPTOMOTOR EXPERIMENT FOR *DROSOPHILA*

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Bachelor's Thesis

Accomplished at

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Scientific Faculty III

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Submitted by

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Declaration of self-reliance

I, Maximilian von der Linde, hereby declare that the work submitted is my own and that all passages and ideas that are not mine have been fully and properly acknowledged.

Ich habe die Arbeit selbständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und bisher keiner anderen Prüfungsbehörde vorgelegt.

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Signature, Date

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Abstract

English

The optomotor response is a reflex of insects to turn in way to stabilize themselves against movement of the environment. So far the scientific standard has been a walking simulator including a styrofoam ball that is suspended on top of a constant stream of hot air. The fly is sitting stationary on top of the ball and moves it with its legs. Movement of the ball is being picked up by a camera. The complexity of this setup motivated to engineer a new optomotor assay that is easier to reproduce and delivers equal or better results. Here I will demonstrate a build derived from the works of Wolf et al. (1992) which makes use of a small platform out of tin that can be rotated by the fly as it turns and an optoelectrical sensor in the form of a lightgate. This composition comes with user friendly software for both the process of testing flys and the evaluation of the generated data.

German

Die optomotorische Reaktion ist ein Reflex von Insekten durch drehen eine rotierende Umwelt zu stabilisieren. Bisher war der wissenschaftliche Standard zum Testen dieser Reaktion ein Gerät, das aus einem Styroporball auf einem Strom Warmluft und einer Kamera besteht. Die Fliege sitzt dabei stationär auf dem Ball, der durch die Beine der Fliege bewegt wird. Diese Bewegungen werden von einer Kamera aufgenommen. Die Komplexität dieses Aufbaus motivierte zur Entwicklung eines neuen optomotorischen Assays, der einfacher zu reproduzieren ist und gleiche oder bessere Ergebnisse liefert. Hier stelle ich einen Bau aus den Werken von Wolf et al. (1992) dar, der eine kleine Plattform aus Zinn, die von der Fliege gedreht werden kann, und einen fotoelektrischen Sensor in Form einer Lichtschranke verwendete. Dieser Aufbau enthält eine benutzerfreundliche Software, die sowohl den Prozess des Testens von Flugzeugen als auch die Auswertung der erzeugten Daten erleichtert.

Introduction

Optomotor behaviour in *Drosophila Melanogaster* has always been a very robust behavioural observation. The optomotor response describes the innate behaviour of insects to correct their flying or walking path by adapting their own movement to maintain course stabilization (Heisenberg and Wolf 1984). Rotation of the visual environment is believed to be interpreted as self-rotation by the fly (Wolf et al. 1992). To counteract this, the insect will try to turn in the same direction as the environmental rotation. One difference from walking to flying that should be kept in mind, is the tarsal contact in walking. If orientation is derived from the contact, it is possible that this discourages optomotor behaviour (Wolf et al. 1992).

The optomotor response in walking behaviour has so far been mostly tested with a styrofoam ball suspended by an air cushion as described in the early works from Buchner (1976) or Götz and Wenking (1973). The fly is being tethered and suspended at the top of the ball (Figure 1). Movement of the ball is recorded by servo motors (Götz and Wenking 1973) or in newer versions by cameras (Seelig et al. 2010; Kohatsu and Yamamoto 2015) and evaluated with a computer.

This technique allows measuring several parameters such as rotation, sideslip and backwards or forwards movement and has been adapted and improved over the years. But results like the ones from Seelig (Figure 2) come from a very complicated and expensive setup. This level of complexity is a general trend in science and impedes a crucial part of research: Reproducibility (Begley and Ioannidis 2015; Benjamin et al. 2018). The easy questions have all been answered, so now every new discovery must be highly complex and get even more complex until the ground-

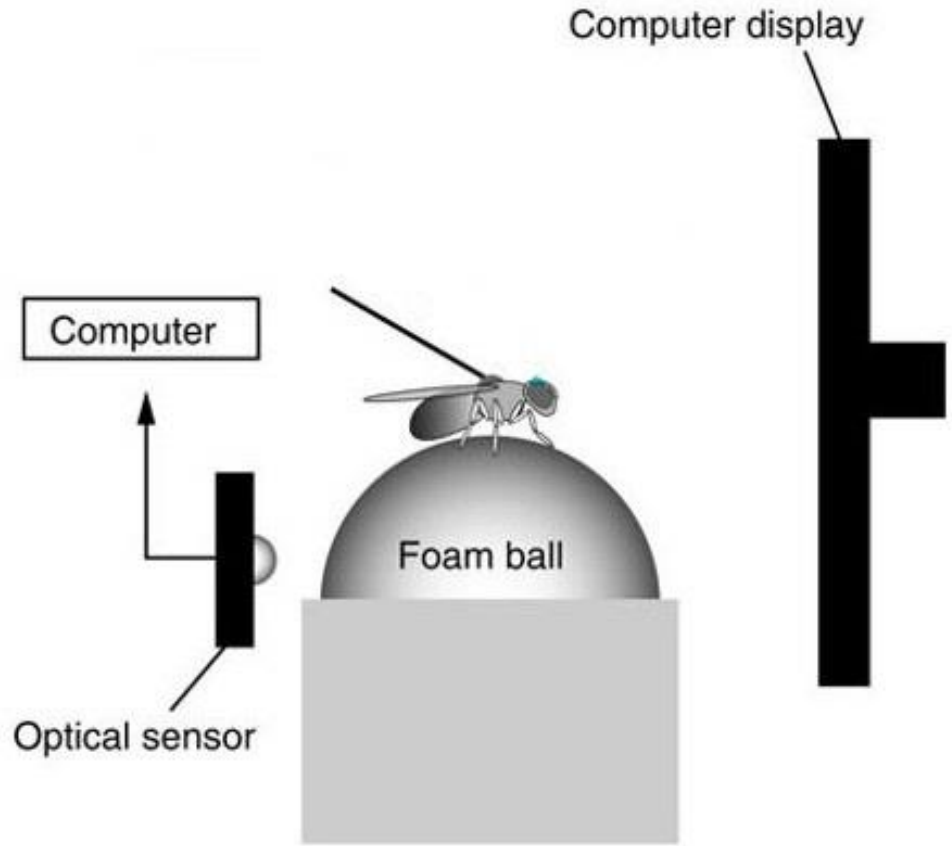


Figure 1: Exemplary design of a styrofoam ball walking simulator. The metal wire is fixed at the fly's thorax. A optical sensor detects the movement of the ball. Visual input is delivered through a computer display (Kohatsu and Yamamoto 2015).

breaking result that is being chased becomes statistically significant. This begs the question: Why not design a simpler walking simulator only responsible for detecting the rotational movement that is prevalently relevant for the optomotor response?

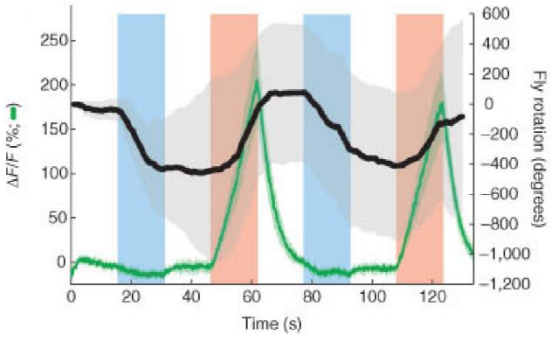


Figure 2: Optomotor response time traces generated by a styrofoam ball setup. The black line indicates the rotational movement of a fly (seven trials pooled) in the styrofoam ball setup. The coloured bars show the phases where environmental stimulus is being applied, blue being rotation to one direction, pink to the other (Seelig et al. 2010). The green line has no relevance for the topic discussed here.

Wolf et al. designed one like that in their paper "Can a Fly Ride a Bicycle?" (1992). This setup is derived from a classical flight simulator. The fly is tethered at its thorax instead of the hook. A small platform under the fly can be moved sideways by the fly against the force of a spring. This horizontal movement is being detected by an optoelectrical sensor positioned beneath the platform (Figure 3). Wolf et al. (1992) speculate that they are able to detect open-loop optomotor turning behaviour as a position histogram (Figure 4) shows a displacement to the positive side of the x axis. Positive bias would have been expected if displacement of the platform is considered a reaction coupled with optomotor response. Yet no actual turning of the fly is involved. Further testing entailed closed loop behaviour with normal (optomotor response helps the fly to stabilize the environment) and inverse (counter-intuitive for optomotor reflex) coupling.

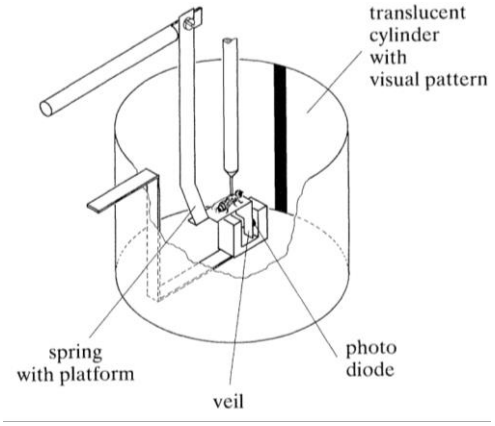


Figure 3: Adapted flight simulator for walking experiments (Wolf et al. 1992).

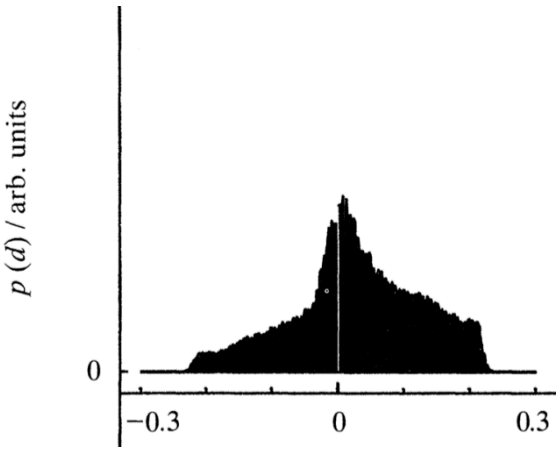


Figure 4: Histogram of open-loop optomotor test with the single stripe moving clockwise, which should be generating positive bias. A displacement to the positive side of the x axis can be seen (Wolf et al. 1992).

They find that it is possible to overwrite the open-loop response almost to the opposite when training the fly to inverse coupling. If only the reflex would be measured, this would be impossible. Thus, optomotor response can't be measured reliably with this setup.

Because styrofoam ball setups are overengineered for optomotor testing and there is no standardised blueprint for such a setup, in an effort towards simplicity and reproducibility this work will describe a walking optomotor experiment based upon the setup from Wolf et al. (1992).

Material & Methods

Fly Care

The experimental subjects were female *Drosophila Melanogaster* flies of the strain Wildtype Berlin (WTB) collected at 3 days after eclosion. They were kept at 25 °C, 60% humidity and a 12/12 light/dark cycle (Brembs 2008) and fed on standard cornmeal-agar medium supplemented with a small heap of fresh yeast.

Fly Tethering

To enable proper handling, the flies were tethered. First they were immobilised by cooling on a cooling station (Fryka-Kälteteschnik, Esslingen am Neckar, Germany) at -2 – 0°C. Next a fishing line with a length of ~25 mm and a diameter of 0.7 mm was glued to the thorax of the fly via a UV sensitive glue (Loctite, Sinfony Indirect Lab Composite; M ESPE, St. Paul, MN, USA). It should be noted that the head and wings were kept free of any glue and consequently freely movable. Anesthesia by CO2 is problematic as the fly can experience brain trauma in the process (Brembs 2008). For precise instructions of this process see attachment 1.1.

For transportation and storing of the flies a ring of thin foam rubber with notches cut to the inside of the ring was prepared. This ring with the same diameter as a petri dish was then glued to the inside of said petri bowl. The flies were slid into the notches by their leash so that the fly itself is either sitting on the bottom of the bowl or hanging in the air between the foam and the bottom.

Optomotor Experiment Setup

The light source for the whole experiment is a 100W, 12V light bulb with a filter for heat generating wavelengths. Approximately 125 transparent plastic tubes are responsible for the even distribution of the light to a cylindrical diffuser (diameter 20cm) with holes for the tubes. Inside this diffuser another cylinder, the arena (diameter 5.9cm), can be found (Figure 6). This one has thin plastic walls, a pattern (Figure 5) on the inside and is locked on top of a motor responsible for rotating the arena. The motor is controlled by a motor control unit, which respectively receives its signal from a Digital-Analog Converter (DAC) (USB-1208FS, measurement computing Inc.; Norton, MA, USA), which is connected to the computer. In order to achieve reversal clockwise and counterclockwise rotation of the arena the motor control unit needs positive and negative voltage input. As the USB-1208FS is not capable of generating negative voltage an additional device had to be designed which generates an offset of -2V and is interconnected between the DAC and the motor control unit. Consequently, the stable state is now when the output is at 2V and direction of rotation can be changed by adding

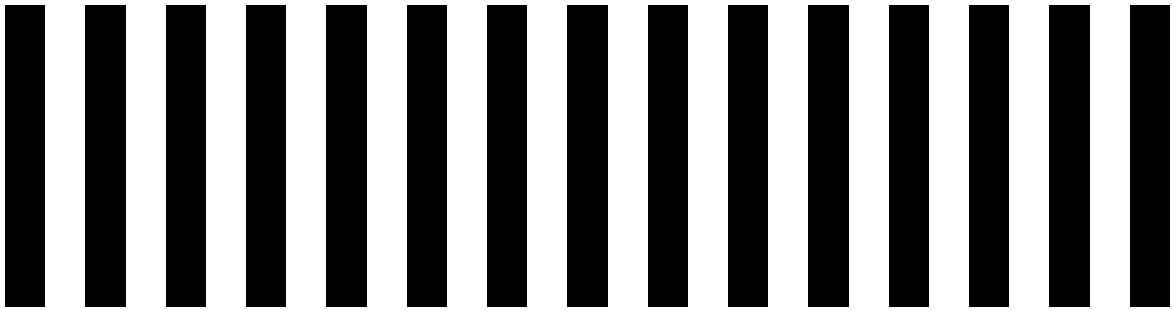


Figure 5: Pattern for the inside of the arena drum. The wavelength is 24° (i.e., 15 evenly spaced stripes), with 90° height.

and subtracting a value from the 2V. This value impacts the velocity of the arena.

USB-1208FS has multiple ports for both D/A converting as well as A/D converting. That is why the device does not only generate the output, but also takes in every input from the experiment, the first and most important signal being the platform. The platform is a small 5mm wide and 7mm long vertical plane at the end of a tin stripe which is fixed 10cm above the plane. 3cm below the point of fixation a small lateral extension reaches into a contraption containing a photoelectric sensor responsible for the detection of any movement of the tin arm. This sensor ultimately encodes clockwise and counterclockwise partial rotation of the platform as electrical impulses of opposite polarity. The fly's turning behaviour generates the platform's partial rotation, which is being picked up by the supporting arm as lateral displacement and finally moves the tin stripe in and out of the photosensor (Figure 6). Other inputs are arena speed (unused so far) and arena position from the motor control unit.

A clamp with a micromanipulator positioned above the arena and reaching inside towards the platform with its arm (not included in Figure 6) was used for positioning the fly onto the platform. The fly's position should be as close to its natural walking position as possible. While a tilt towards the back or front can be compensated by the fly, a tilt towards the sides can easily bias the fly's turning response.

Software

The program driving the experiment and controlling USB-1208FS, which can be used as an Analog-Digital Converter (ADC) as well, is a python-based script. The general

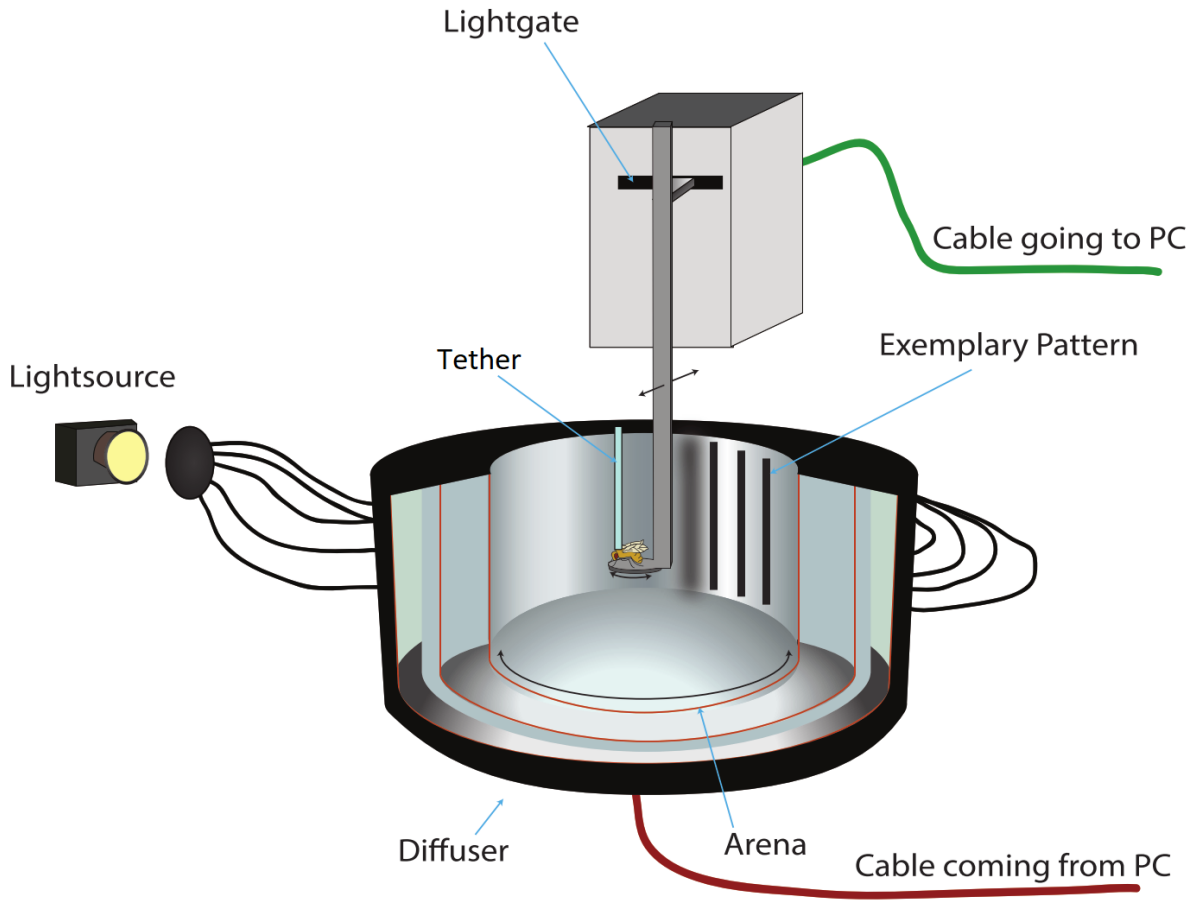


Figure 6: Schematic of the experiment setup. The black arrows indicate potential rotation or shift. For further details and description of the functionality see the text.

structure is based on an example from the Universal Library from Measurement Computing called “ULAIO01.py” and has been modified to fit the needs of this experiment. The platform signal is being read at 100 HZ, a live graph plotting the platform position and the arena position can be started at any time while the program is running and in the end all the data with its metadata is saved in an XML file. For further instructions on how to install the program see attachment 2 and on how to operate it see attachment 1.2.

Statistical Analysis

The program handling the statistical analysis is an R based script. It is suited to handle the data from different experiments in the laboratory and processes data from multiple tests at once. The main file controlling what the program evaluates is a YAML file and functioning as a sort of laboratory protocol where all the general metadata specifications and XML data files from each test are manually specified and collected. Ideally this should be done while conducting the experiments, every time a test is finished and the name of the XML file is known. If a new set of experiments is to be started, an existing YAML file can be copied and changed to fit the new test group.

The result is an evaluation page for every fly as well as one page that makes evaluations for groups of flies. If the metadata points to a platform optomotor experiment is at hand, the single fly test evaluation files contain graphs about the time trace, the distribution of data points, the pooled traces sorted by left and right rotation of the environment/arena and the power spectrum. The group evaluation page pools the data from all the flies in one group, generates the same graphs and makes comparisons across the groups.

Results

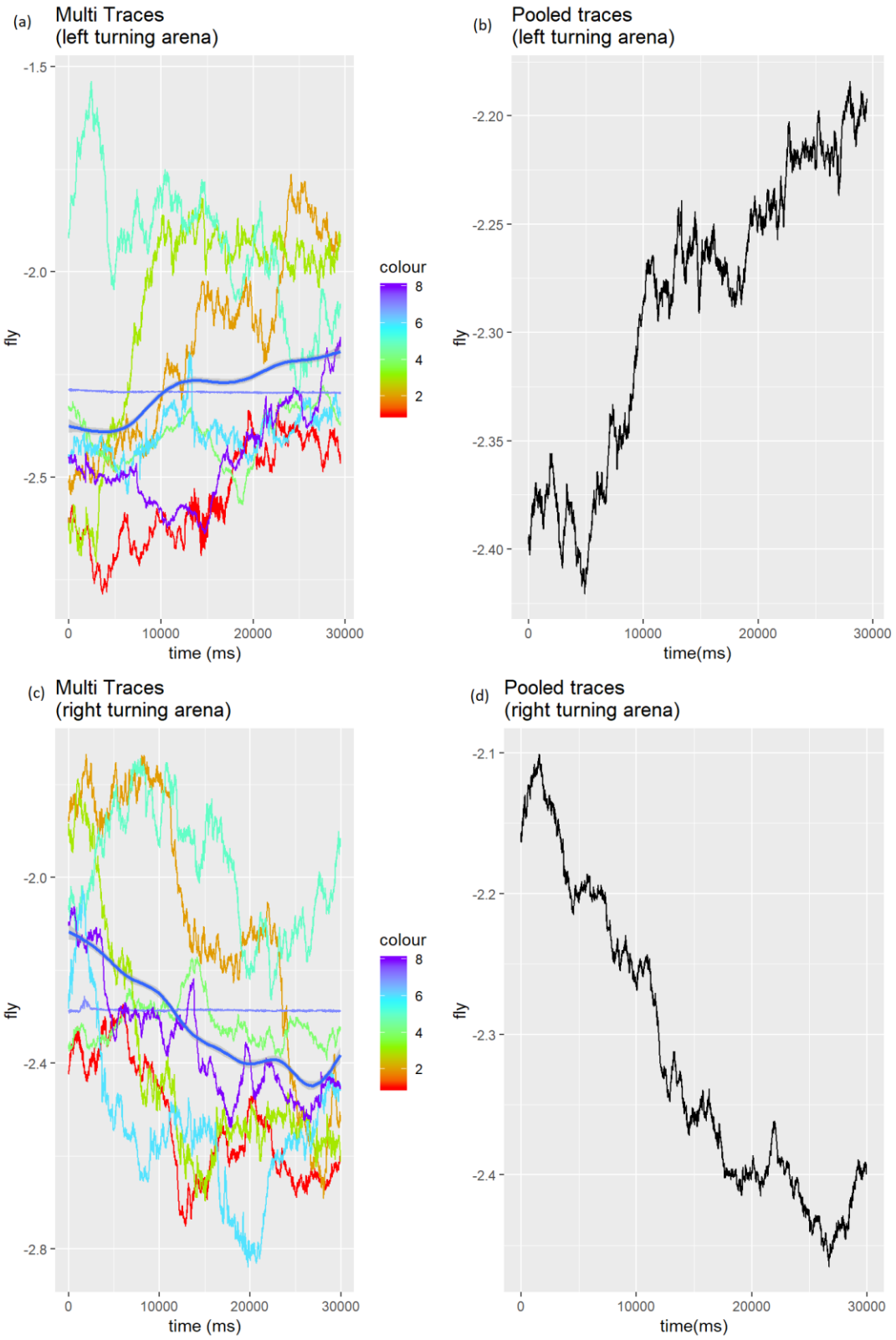


Figure 7: Time traces of all counterclockwise (a, b) or clockwise (c, d) periods pooled to generate one trace per period type ($N_{clockwise}/N_{counterclockwise}$ = 8) for each fly. These traces can be seen on the left (a, c), each coloured trace represents one fly (N_{flies} = 8) and the big blue line in a and c is a hint at the average trace but smoothed a lot. On the right (b, d) the actual average trace of all flies can be seen.

The new optimized optomotor assay allows for multiple improvements while still benefiting on the essential features of the original assay designed by Wolf et al. (1992). These improvements include a setup that allows for measurement of the naive optomotor reflex

The experiment is set to have a certain duration of each period and a certain number of periods. E. g. if each period is set to have 30s and 16 periods get measured, the overall experiment duration will be 8 minutes. With the first period the arena begins to turn clockwise, the following period reverses this rotation and so on for every period. The number of periods should be kept even to avoid measuring one more clockwise period than counterclockwise period.

To optimize the best possible signal and determining the best conditions for the fly optomotor assay the following factors were adjusted for: The scale of the fly's leash (diameter of the fishing line), and the fly's position on the platform. The best signal was detected by looking at the data from flies who were tethered with thicker fishing line pieces and who were placed at the front of the platform (Figure 7). All flies compared here were collected, tethered and tested on the same day.

The strong effect suggested by Figure 7 b and d can already be made out in the single fly traces on the left. An exception is the straight blue line at about y = -2.3. This fly is not showing any significant movement and is the only one whose wings were accidentally glued to the thorax.

Manual Stimulation

In order to make sure the data is processed correctly by every program and to compare the fly's movement with the expected motions manual stimulations were applied. In detail this means that the platform was moved into the direction it would be expected to be bend by the fly considering the direction of the arena. The collected data supports the tendencies from Figure 7.

Position Histograms

To compare the results to the data previously demonstrated by Wolf et al. (Figure 4), position histograms were generated (Figure 8). The data used is a simple collection of all data points per type of period. The big column at approximately -2.3 is the position at which the platform is at ease and consequently where the fly is at while it rests. While a general bias towards more negative voltage can be seen, no certain distinction can be made between the two period types (Figure 8, a & b).

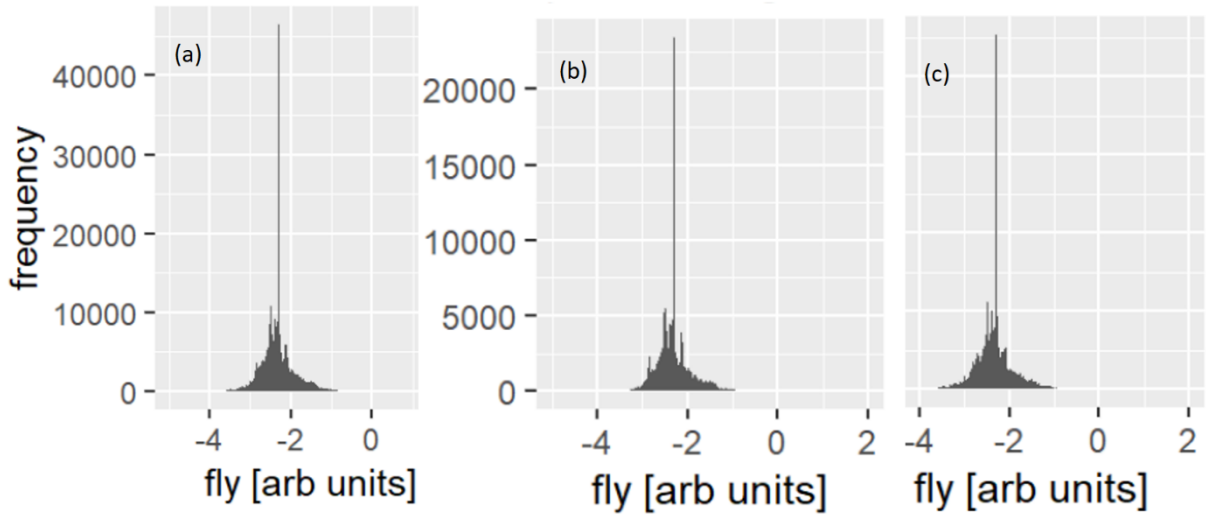


Figure 8: Position distribution across all flies and all periods (a); Position distribution of all counterclockwise periods (b); Position distribution of all clockwise periods (c).

Discussion

Comparison to previous setups

When comparing the generated histograms (Figure 8) to the results displayed by Wolf et al. (1992) (Figure 4) the obvious difference is that the displacement which motivated Wolf et al. (1992) to speculate having found the optomotor response is missing in the recent data. Yet this circumstance does not discourage the discussed effect as the two setups have a critical difference. While the flies in Wolf's platform experiment were always located at the resting position when introduced to the turning environment and would then make short efforts to the one side or the other, the flies in this experiment initially are not in a resting position. Instead they received input (rotation of the arena) encouraging torsion to the opposite direction. The rotation is being reversed abruptly with the onset of the new period. Thus, each period detects the process of changing directional behaviour from one extremum to the other (Figure 7). For the position histograms that miss the temporal factor this means that positions are equally over and under the resting point for each type of period and a difference between the two would not be expected. To better compare the two results one could add one period where the arena is resting between each period of left or right turning arena. Another possibility would be to simply increase the period duration and give the flies enough time to finish the process of traversing from one extremum to the other and then stay on the preferred side a little longer. Following this theory, the histograms (Figure 8) should be normally distributed, but instead they are left-slanted. A hint at this trend can be derived from Figure 7, too: The range of the pooled right turning traces (d) is ~1.5 times greater than the range of the pooled left turning traces (b). A logical conclusion would be that it is easier for the fly to move the platform to the right than to move it to the left. This would most likely be a result of imperfect design of the platform and its arm. Another possibility would be an imbalance in the sensitivity of the lightgate.

With the detector being located at the base of the platform arm, the angular movement is reduced and the signal somewhat smaller than when it is being detected right at the platform like in the build from Wolf et al. (1992). Regarding that the signal to noise ratio is not a problem, strengthening the signal becomes optional. This way one can do without the extra arm holding the lightgate (Figure 3) which would reduce accessibility and add another potentially disturbing factor.

As mentioned in the beginning the setup from Wolf et al. (1992) has one crucial difference: It can't be expected that naive animals like *Drosophila Melanogaster* are able generate an optomotor response in a complex environment. They cannot rely on the simple reflex but have to adapt their turning reflex to a lateral push (Figure 3). In the design presented here (Figure 6) the platform measures the simple naive reflex because the fly can actually turn. In this way it compares better against the styrofoam ball setup where the fly can turn as well.

Keeping in mind that optomotor responses recorded via a styrofoam ball experiment are considered reliable (Kohatsu and Yamamoto 2015; Seelig et al. 2010) and considering the similarity of the fly behaviour during optomotor reflex stimulation in Figure 2 (blue and pink phases) and Figure 7 (b and d), the new platform optomotor experiment can be called reliable as well. This applies despite the fact that the y-axes do not represent exactly the same things. In the styrofoam ball setup the y-axis can be read as "distance covered", while in the platform setup it can be read as "directional force applied". This is an important point because it allows for tests revolving around the force that a fly applies,

which could not be measured with the styrofoam ball setup so far.

Improvements

This setup will be used in teaching and is therefore designed to be user-friendly. This is being succeeded by automating a lot of steps and simplifying the build. As in all newly developed constructions there are some minor tweaks that might make the experiment even more accessible. In the current design a rod with the clamp on its end is fixed by a micromanipulator and reaching straight down onto the platform. This creates a conflict for space between the sensor box and the micromanipulator. A suggested improvement would be a hinge integrated into the micromanipulator that can be swung out to access the clamp with the fly. Such a modification would increase the fly placement accuracy and thereby reducing a potential source of error.

The second obvious simplification would be the use of a DAC that can generate Voltages ranging from -5 to 5V. While such a device is a little more expensive, costs can be saved as no offset generator would be necessary. The program should run with any A/D converter from Measurement Computing with small adjustments to the existing code. The code used for the data acquisition during the experiment already features many parameters which are easily adjusted via the user interface. Future updates could make the experiment more variable and enlarge the application range. The possibility to change the frequency at which the data is collected would be a nice feature to implement because a rate of 20 hz would be sufficient. This is further complicated as the code relies on a specific frequency of 100 hz for computing the time passed.

If a stronger signal is desired the first point of attack should be to find the ideal voltage for the arena output. When the arena is turning around at one hz the optimal stimulus for optomotor behaviour would be given (Buchner 1976). Another option would be to move the lightgate closer to the platform and increase the detected range of the fly's motion.

Conclusion

Tests have shown that the platform setup is a reliable tool to test optomotor behaviour in *Drosophila*. With the relatively easily acquirable components and the freely accessible software it succeeds in its aim to be easily reproducible. Furthermore it has possible applications for tests including the force a fly is able to exert.

Attachments

Attachment 1

Exact process of fly gluing

1. Make sure the cooling station is as cold as it gets
2. Prepare enough pieces of fishing line (length ~2.5cm)
3. Isolate a couple of flies (they should be cooled for 45 minutes at most) into the cooled vial with the net on the bottom by using a funnel
4. Make sure the flies fall asleep
5. Transfer them onto the metal surface
6. If only one gender should be tested, sort them now
7. Use the small brush to isolate and center the one fly that should be glued under the microscope so that it can be seen clearly
8. Use the weight fixate the fly in the correct position by placing the weight on the abdomen, all while looking through the microscope (thorax must stay accessible!)
9. Take the cross-lock forceps and pick up one of the fishing line pieces (= leashes)
10. Use the glue stick to apply a very small layer of glue to the bottom of the leash
11. Insert the forceps into the micromanipulator
12. Use the micromanipulator and the microscope to position the line on top of the fly's thorax (lightly touching, not pressing) and make sure everything is aligned perfectly
13. Take the UV light and illuminate the gluing spot from as closely as possible without touching anything
14. Remove the weight from the fly
15. Use the micromanipulator to slowly lift the fly into the air
16. Unhitch the forceps with the fly and slide the leash into one of the styrofoam slots of the transportation container
17. Repeat step 7 - 16 for all flies, remember not to keep them on the cooling station for too long

Start of the experiment

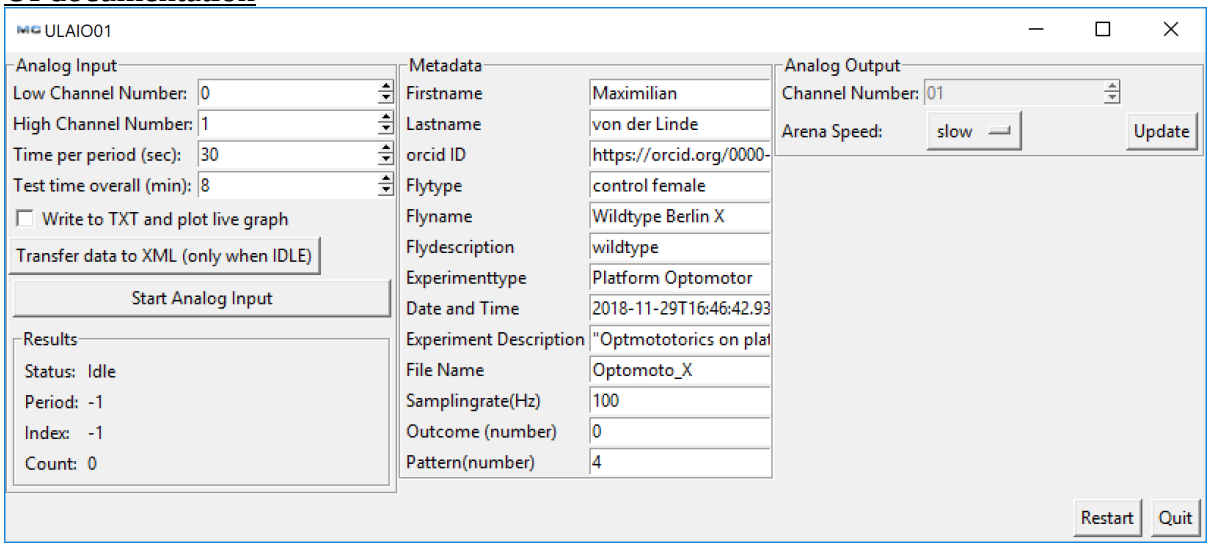
1. Start the Opt_Moto.py program and check the parameters (see attachment 2)
2. Plug in the lamp for the arena
3. Retract and position the arm responsible for holding the fly in a way that makes it easily accessible
4. Attach a fly to the clamp on the arm and make sure it is perfectly horizontal
5. Use the micromanipulator to lower the fly to the desired position (standard: central tip of the platform, facing away from the platform suspension)
6. Switch all light sources except the arena light in the room off
7. Switch the motor control unit on and press the "Start Analog Input" button in the program at the same time if possible
8. Once the program is completed check the metadata and press the button "Transfer data to XML (only when idle)"
9. To run another test, click the "Quit" button, restart the program and begin from step 1, only disregarding the motor control switch in step 7

Attachment 2

Installation of the experiment software

See "https://github.co"/InfiniteWhite/Optomotorics-Bachelor-Thesis.git" to download any software used and read the README file for instructions on how to install the programs.

UI documentation



Analog Input:

- **Low Channel Number** represents the first channel of the A/D converter that should be taken into account. In the existing setup Channel 0 is the fly's signal.
- **High Channel Number** represents the last channel of the A/D converter that should be taken into account. In the existing setup Channel 1 is the arena position signal. If Channel 2 would be added, the arena speed should be measured additionally.
- **Period Duration** defines the timespan of one period in seconds
- **Number of Periods** defines the amount of periods that should be measured. By multiplying "Period Duration" with "Number of Periods" the experiment duration can be derived.
- **Write to TXT and plot live graph** starts the live plotting ("live_graph.py") of all channels that are measured.
- **Start Analog Input** starts the actual experiment.
- **Status** can be either "idle" or "running".
- **Period** depicts which period has been reached in the time series.
- **Index** depicts number of data points that have been acquired so far.
- **Count** depicts number of data points that have been acquired so far.

Metadata:

- **Firstname** of the experimenter
- **Lastname** of the experimenter
- **Orcid ID** of the experimenter
- **Flytype**

- **Flyname**. An identifier string. Lower case characters with ., _, - and / are allowed. This is ideally a url-usable and human-readable name.
- **Flydescription** of the fly strain
- **Experimenttype** with the type of "joystick"
- **Date and Time** is being generated automatically but can be changed manually.
- **File Name** is the name for the XML file that will be generated.
- **Samplingrate(HZ)** shows the frequency at which data is being acquired.
- **Pattern(number)** is a documentation field for the used pattern. For the pattern classification see attachment 3, (3).

Data Evaluation

See "https://github.com/brembslab/DVisualisedons.git" to download the R script capable of evaluating data generated by Opt_moto.py if the specifications are met (Methods, Software).

If problems arise using the main branch, try using the scripts from a side branch called "InfiniteWhite-patch-3.1".

Attachment 3

The following file is a documentation of the variables relevant for the metadata and therefore evaluation of most of the experiments from the Laboratory under B. Brembs. For the newest version see the Google Document file:

https://docs.google.com/document/d/1AN1AaDx_QCwTGT3eXNvgVLIGefST_Jaa31iktVDaSc0/edit?ts=5bc70f33

Figure Index

Publication bibliography

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