# Journal of Visualized Experiments

## Measurement of Larval Activity in the Drosophila Activity Monitor

--Manuscript Draft--

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| **Abstract:** | Drosophila larvae are used in many behavioral studies, yet a simple device for measuring basic parameters of larval activity has not been available. This protocol repurposes an instrument often used to measure adult activity, the TriKinetics Drosophila activity monitor (MB5 Multi-Beam Activity Monitor) to study larval activity. The instrument can monitor the movements of animals in 16 individual 8 cm glass assay tubes, using 17 infrared detection beams per tube. Logging software automatically saves data to a computer, recording parameters such as number of moves, times sensors were triggered, and animals' positions within the tubes. The data can then be analyzed to represent overall locomotion and/or position preference as well as other measurements. All data is easily accessible and compatible with basic graphing and data manipulation software. This protocol will discuss how to use the apparatus, how to operate the software and how to run a larval activity assay from start to finish. |
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November 17, 2014

Dear *Journal of Visualized Experiments* Editor Dr. Nam Nguyen:

Please find attached our revised manuscript, "Measurement of Larval Activity in the *Drosophila* Activity Monitor".

We thank the five anonymous reviewers very much for their thoughtful comments, and we have accordingly prepared this revised manuscript. As you will see, we have accepted nearly every suggestion made by the five reviewers. We look forward to your reactions to our improvements!

Sincerely yours,

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**TITLE:**
Measurement of Larval Activity in the *Drosophila* Activity Monitor

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**KEYWORDS:**
Neuroscience; *Drosophila melanogaster;* Fruit Flies; Larvae; Life Science; Behavioral Sciences; Locomotion; TriKinetics; Activity; Fly Behavior

**SHORT ABSTRACT:**
This report describes a method for measuring *Drosophila* larval activity using the TriKinetics *Drosophila* Activity Monitor. The device employs infrared beams to detect movements of up to 16 individual animals. Data can be analyzed to represent motion parameters including rates and the positions of the animals within the assay chambers.

**LONG ABSTRACT:**
*Drosophila* larvae are used in many behavioral studies, yet a simple device for measuring basic parameters of larval activity has not been available. This protocol repurposes an instrument often used to measure adult activity, the TriKinetics *Drosophila* activity monitor (MB5 Multi-Beam Activity Monitor) to study larval activity. The instrument can monitor the movements of animals in 16 individual 8 cm glass assay tubes, using 17 infrared detection beams per tube. Logging software automatically saves data to a computer, recording parameters such as number of moves, times sensors were triggered, and animals' positions within the tubes. The data can then be analyzed to represent overall locomotion and/or position preference as well as other measurements. All data are easily accessible and compatible with basic graphing and data

manipulation software. This protocol will discuss how to use the apparatus, how to operate the software and how to run a larval activity assay from start to finish.

**INTRODUCTION:**

The use of *Drosophila* as a genetic tool has transformed scientific knowledge of biological systems. *Drosophila* larvae have been used in a variety of studies including nociception[1], development[2] and as a model for the study of human disease genes[3]. *Drosophila* activity encompasses a range of behaviors that vary under different conditions including temperatures[2], exposure to drugs[4] and amongst different genotypes. Yet, despite the significant use of the larva as a model organism, a simple, standardized method to analyze larval activity has not been available. Presently, many larval locomotion studies employ sophisticated video analysis software[5]. While powerful, the complexity of such automated tools may discourage labs that are not already equipped to study locomotion from including analysis of informative activity parameters in their studies. In other current non-automated methods, such as the grid crawling analysis, motion is scored by a human observer, which introduces the possibility of subjectivity and limits throughput to one animal at a time[6-7]. A similar study used a 5-lane crawling assay, which measured the time it took larvae to travel a certain linear distance[8]. In such non-automated assays, displacement is measured but this does not account for non-linear travel between the start and end points. As discussed below, the method described here accounts for more of the actual larval movement, is objective, easy to operate, and offers robust throughput.

To easily study larval activity behavior without the compromise of accuracy, efficiency, or cost, this method employs the TriKinetics *Drosophila* Activity Monitor (DAM), a device often used to study adult activity. Using one device to study both adult and larval activity is cost-effective, and allows direct comparison of motion by animals at these two life stages. The system, featuring the manufacturer's highest level of resolution, makes use of 17 infrared detection beams per assay tube, which record larval activity when sensor beams are broken within the 16 individual tubes. The system then automatically saves recorded information to a computer, making it available for manipulation with basic graphing software. The data obtained represents the beams that were broken by individual larvae (which can be converted into a rate), movement when a larva stays within a detector beam and the position of the animals within the assay chambers during a recording period (allowing one to calculate position preference). The system is efficient and relatively simple to operate, and brings highly reproducible basic activity analysis within the reach of any laboratory studying *Drosophila* larvae.

To demonstrate the power of this assay, data are presented that show its use to verify differences in activity resulting from varying ambient temperatures, as well as through the comparison of a mutant previously described as hypoactive ($iav^{1}$)[9] with a widely analyzed control ($w^{1118}$).

**PROTOCOL:**

1. Preparation of Larvae

1.1) To analyze a desired larva for locomotion or position preference, grow larvae under standard conditions to the desired age to assay[10] using standard fly food[11].

1.2) Make a mesh filter by stretching silk-screen grade nylon mesh over a funnel. Secure the mesh at the funnel neck with rubber band. Place the funnel in a beaker.

1.3) To collect larvae for analysis, scoop a spatula-full of food containing foraging larvae from the culture bottle and wash with room temperature tap water over the mesh filter, collecting individual larvae directly from the mesh with a paintbrush[10].

## 2. Preparation of Assay Tubes

2.1) To prepare to plug the assay tubes, carefully boil a 4% agar gel and pour into a petri dish to a depth of 1.5 cm. This will provide a plug for either side of the tube when animal is enclosed. The 4% solution is sufficiently dense to prevent larvae from penetrating the plugs.

2.2) Ensure that tubes are clean and clear prior to insertion of larva. If they are not, this may block movements from being recorded.

2.3) To ensure larvae have sufficient moisture for movement, prepare a squeeze bottle containing a small amount of hot water. Invert the bottle, carefully orient the outlet toward a sink and squeeze gently to expel water from the pickup tube.

2.3.1) Insert the bottle outlet into the assay tube. Squeeze the bottle to deliver water vapor into the assay tube until a thin layer of condensation appears on the wall of the tube. Video 1 shows a larva moving in a tube with an appropriate amount of moisture.

2.4) To seal the larva in the tube, remove the 1.5 cm thick agar gel from the petri dish and place over a mesh surface to allow airflow beneath the agar so that an agar plug can be inserted into the tube. Press one end of the assay tube into the gel twice to insert two plugs of gel into one end.

2.5) With a paintbrush, place one larva into the tube approximately 1.5 inches deep and seal the tube by pressing the end nearest the larva into the agar gel. The resulting pressure will force out the second plug of agar gel from the opposite end and seal the animal within the tube.

2.6) Place tubes in the MB5 Multi-Beam *Drosophila* Activity Monitor (DAM) device, and adjust the tubes' positions so that the animals cannot move beyond the range of the sensors.

2.7) To ensure that assay tubes do not slide out of the device's infrared reading frame during transport, hold the tube in place by fastening a ring of putty around each tube where it contacts the recording device.

## 3. Measuring Activity

3.1) Record activity in an incubator to prevent inaccurate readings that may occur due to shadows, fluorescent lights or to temperature variations in the lab. See Figure 5 for a suggested arrangement of the system.

3.2) Set an incubator to 20 °C (see Figure 1). To avoid false recordings due to interference from incandescent light sources during recording, turn off fluorescent incubator lights and use a separate LED light source while recording in incubator. Perform a trial without any animals to ensure that light conditions do not aberrantly trigger sensors. There should be no recorded movement data after this test.

3.3) Allow larvae to acclimate to the 20°C incubator settings for 5 minutes prior to start of assay.

3.4) To set the DAM System to desired recording intervals (e.g., 1 minute), ensure DAM system recording software is downloaded to host computer[12] and open the DAM System file prior to connecting the recording chamber to the PSIU interface.

3.5) To set the desired recording frequency at which data will be saved, select **preferences** and then click above or below the **reading interval** option to select different time frames in which data will be stored. For example, select **reading interval** times from 1 second to 1 hour.

3.6) To select varying parameter for recording data, choose **preferences** and then select the corresponding boxes under output data type (counts, moves, positions and dwell, see Table 1). Each parameter provides a unique analysis of the larval activity within the tube.

3.7) To begin recording, connect the monitor to the Power Supply Interface Unit (PSIU) using CAB6 telephone cable. Connect the PSIU to a power outlet. A green light will indicate appropriate connection.

3.8) Connect the PSIU through a Universal Serial Bus (USB) cable to a Macintosh or Windows PC for data recording. Open the DAM SystemMB1v6x program on the computer to automatically start data recording.

3.9) After the desired collection time is complete, select **Quit**, and then **Quit now** on the DAM activity screen; data will then be automatically saved under DAM System Data. Take this data file (e.g., monitor 1) and drag into a separate folder. This will store the raw data from the DAM software so that they can later be processed.

4. Preparing Data for Processing (DAM FileScan)

4.1) To process the raw data into an understandable format open the DAM FileScan application and select **Select Input Data Folder**. Then choose the data folder and the desired file, and select the **scan** option. Lastly select the **bin length** to the desired reading period. This will organize the raw data into parameters set by the operator and the program will report the data collected in that particular range.

4.2) Select the **output data type** to analyze the desired motion setting (monitor counts, moves, dwell). Under the **extra readings** menu, select **sum into bin** (this is if the bin length is greater than the selected system reading interval).

4.3) Name the file appropriately and save. The file will be stored in the same location as the folder from step 3.9.

5. Accessing data for analysis

5.1) In order to collect average moves, process the raw data generated from step 4. To generate a table in a spreadsheet program, open the file that was previously saved (step 4.3), and when the text import wizard window appears, select **finish** to open the data files in a spreadsheet format.

Note: Depending on the data type analyzed, the spreadsheet will be read differently. Typically to measure monitor moves or counts, the time period of the study will be recorded numerically, while each individual reading tube will receive a designated letter. When viewing the data in the spreadsheet, columns K-Z represent the slots for assay tubes 1-16 respectively. The rows represent the data points collected.

5.2) For example, if moves were collected for 20 minutes in one-minute intervals, average the 20 rows to calculate an average number of moves/minute and other measurements.

**REPRESENTATIVE RESULTS:**

Figure 1 shows the results from a temperature response study of control third instar larvae, $w^{1118}$, using the monitoring device to detect differences in larval locomotion at seven different temperatures. Larvae were washed and placed into the DAM activity device as described above, and placed in an incubator set to the desired temperature. The apparatus was then allowed to acclimate to the environment for five minutes before recording began. Each larva was individually analyzed for locomotion over a 20-minute period and the average number of moves per minute was calculated for every animal and averaged for each set of 32 animals. Data were analyzed and graphed using a spreadsheet program. Larvae exhibited significantly increasing activity as temperature increased correspondingly from 5-35 °C in 5-degree increments, except for a break in this trend at 20 °C and 25 °C.

To verify that differences could be detected between a control and a mutant previously described as hypoactive, *inactive* larvae ($iav^1$) were tested. Data were analyzed as moves/minute for each of the 32 animals and an average was then calculated. As shown in Figure 2, the analysis indicates that *inactive* larvae were significantly less mobile than a control. While they are much smaller than third instar larvae, activity of first and second instar larvae was also measurable, as shown in Figure 3. Activity of third instar larvae in each minute of the 20-minute assay was shown to remain relatively consistent throughout the period (Figure 4).

Figure 1: $w^{1118}$ larval locomotion was recorded at temperatures varying from 5 °C – 35 °C. Each column represents the average motion of 32 third instar animals with individual moves per minute averaged amongst the set. *All averages are significantly different from each other except 10 °C and 15 °C (p=0.116) (Different letters indicate a significant difference, Student t-test).

Figure 2: Third instar control larvae compared to *inactive* ($iav^1$) larvae at 20 °C. $iav^1$ larvae exhibit significantly less mobility compared to control.

Figure 3. Measurement of activity of first, second and third instar larvae over a twenty-minute recording interval at 20 °C. N=32 for each larval stage.

Figure 4: Histogram displaying average number of moves occurring in each minute of the twenty-minute recording interval. 32 third instar larvae were assayed at 20 °C. The red line represents the number of moves per minute averaged for the entire twenty-minute interval.

Figure 5. Diagram illustrating the *Drosophila* Activity Monitor set-up and its connectivity to the PSIU interface unit and a desktop computer. The inside of the incubator is depicted, but during recording the incubator is to be shut.

Table 1: Description of measurement parameters (Counts, Moves, Dwell and Positions).

Video 1: DAM system assay tube prior to insertion into operating device. Moisture is provided by condensation from breath. This level is sufficient to maintain larval locomotion throughout the study period without causing the animal to float or swim.

**DISCUSSION:**

Activity of *Drosophila* larvae is influenced by a variety of factors including genotype[8], age[13] and ambient temperature[2]. Although powerful videographic methods capable of highly detailed analysis have been developed by those who study locomotion[5], this level of detail may be superfluous for those who wish to determine basic parameters of activity. The method described here employs a device that is available in many laboratories, is easy to operate, generates highly reproducible results, and is manageable even for those whose primary research focus is not locomotion. The example results demonstrate that this assay can be used to detect significant changes in motility in larvae subjected to different temperatures (Figure 1) and of different genotypes (Figure 2).

When larvae were measured at temperatures ranging from 5 °C – 35 °C, their activity increased with temperature, except for a break in the trend between 20 °C and 25 °C (Figure 1). It has been shown by Ainsley and coworkers that foraging early third instar larvae prefer temperatures within +/- 2 °C of the typical 25 °C culture temperature. However, when larvae enter wandering mid-third instar phase they prefer somewhat cooler temperatures[2]. That finding is consistent with the observation that locomotor activity for third-instar larvae is greater at 20 °C than 25 °C, suggesting that some portion of animals assayed were in the wandering stage and more active at the cooler temperatures, and less so at the normal culture temperature of 25 °C.

This method offers simplicity, objectivity and robust throughput, but there are limitations. The applications described above represent assays occurring over a relatively short time frame, in part because the current set-up does not provide larvae with a food source. Ensuring adequate nutrition would be necessary to study changes in activity over extended periods of time or to measure circadian rhythm in the longer term. The 4% agar plugs may restrict gas exchange between the chamber and the external environment, which could result in the larvae experiencing hypoxic conditions. However this does not appear to affect activity within a 20-minute assay period, because when average moves per minute of larvae during each minute of the period were

analyzed, larvae did not appear to show any change in activity over the recording period (Figure 4).

Because the device records position continuously it represents an improvement in capturing more motion compared to the non-automated methods cited, however some motion does escape detection. Very small movements of animals might not trigger a response from this device, and larvae can move in a circumferential fashion within one infrared beam without breaking neighboring beams, resulting in inaccurately low readings. However, since this type of error would be expected to occur in all treatment groups, it is unlikely to cause misleading results. Although third instar larvae are the primary focus of this analysis, the device is capable of measuring the motion of the much smaller first and second instar larvae as well (Figure 3). As expected, the number of moves recorded per minute in the younger animals is lower than that of the larger third instar animals.

Although the full range of uses for this device has yet to be demonstrated, there are a variety of other adaptations that could diversify the uses of this apparatus for studies involving larvae. For instance, the device allows a 'dwell' measurement, which represents time spent in a determined region of the tube. This may provide valuable information when employed in various *Drosophila* larval taxis assays. By placing the apparatus on its side so that the tubes are oriented vertically instead of horizontally, one could measure larval geotaxis. To measure phototaxis, a light gradient could be established in the tubes, testing whether larvae have a preference for light or its absence. To study chemotaxis, a test chemical could be placed on one of the agar plugs and the position of the larvae might then reveal determine their preference for or avoidance of the chemical.

The monitor system allows the analysis of various motion parameters, summarized in Table 1. By selecting all parameters during the pre-assay setup (see step 3.6), the experimenter can choose which parameter to analyze after the assay. However, if any setting is not selected, that data will not be available for *post hoc* analysis. It should be noted that after each selected period of time, data are frozen at current counts and saved to the host computer. Data collection then resets to zero after this period and begins again, providing a series of time interval data points. One must manually quit to end data recording.

Future studies involving this method will focus on the use of the dwell parameter and its various applications. Also it may be possible to develop a protocol that would allow for studies to occur over a longer period of time, such as circadian studies, by providing food and exchanging agar plugs for a more gas-permeable material[14]. Moisture levels would need to be controlled as well, as dry conditions inhibit locomotion[15]. Currently, this protocol provides an accurate, simple, cost-effective method to evaluate basic parameters of larval activity under a variety of experimental conditions.

**DISCLOSURES:**
The authors declare that they have no competing financial interests.

**ACKNOWLEDGEMENTS:**
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Figure

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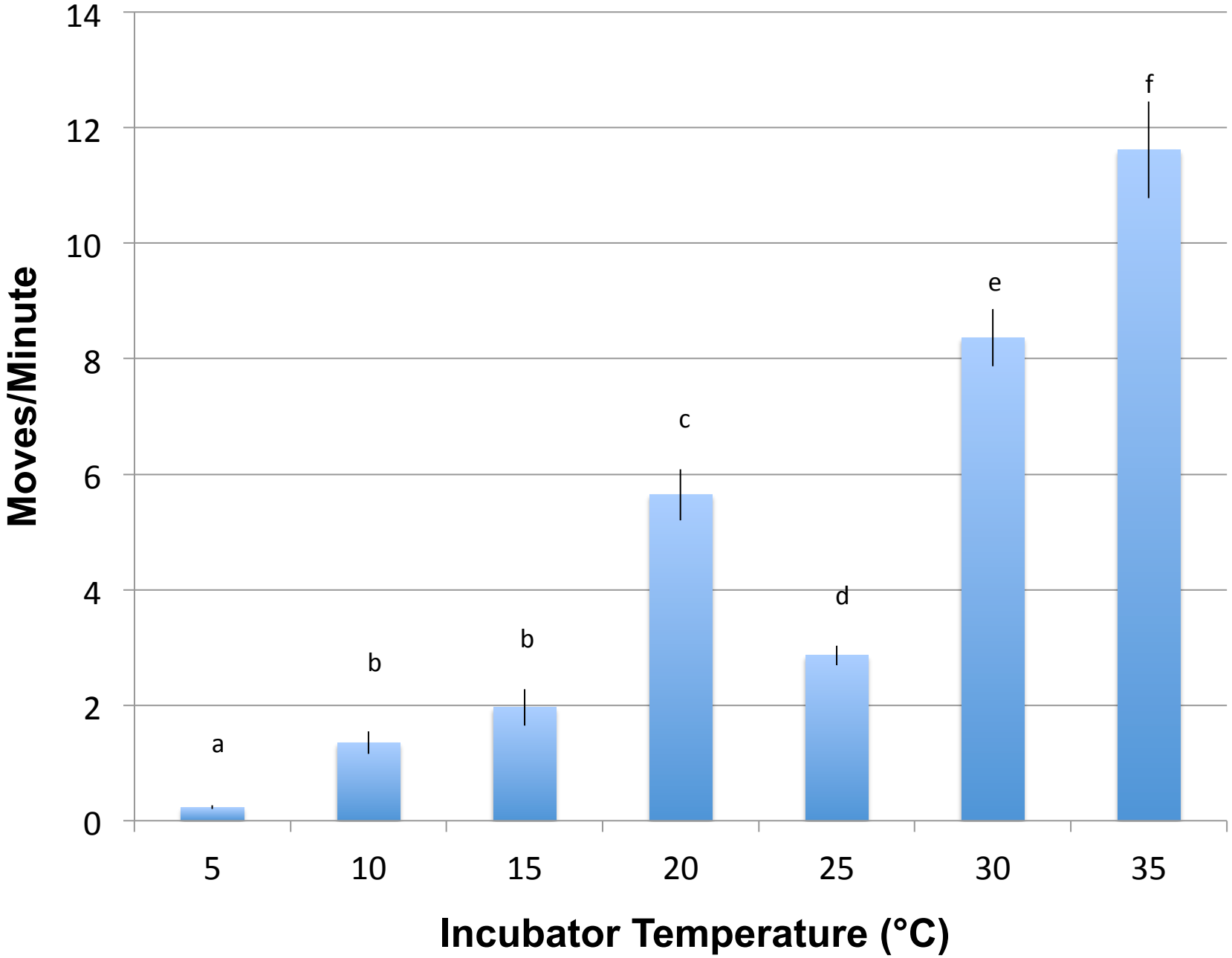

Figure 2

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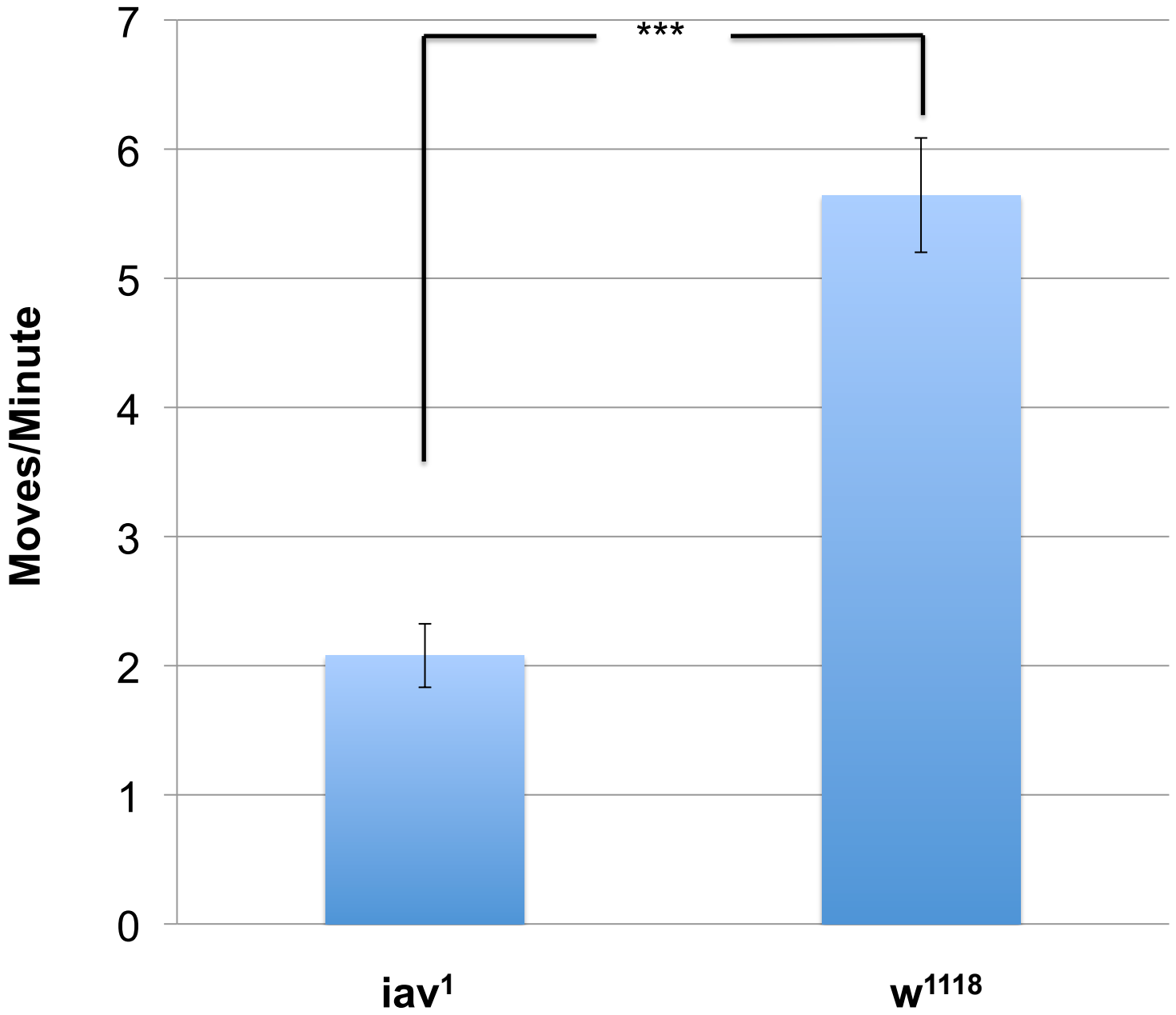

Figure 3
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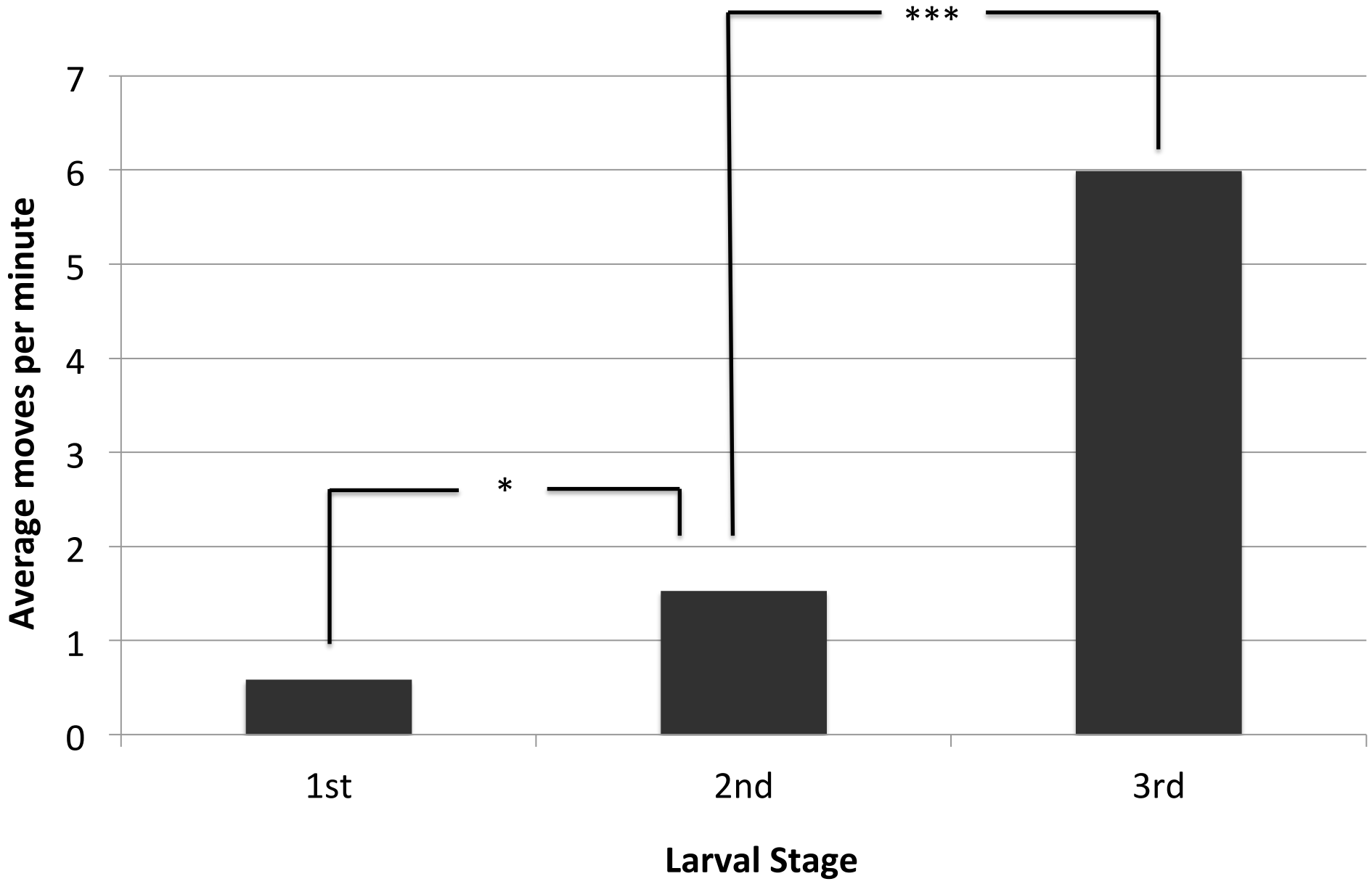

Figure 4

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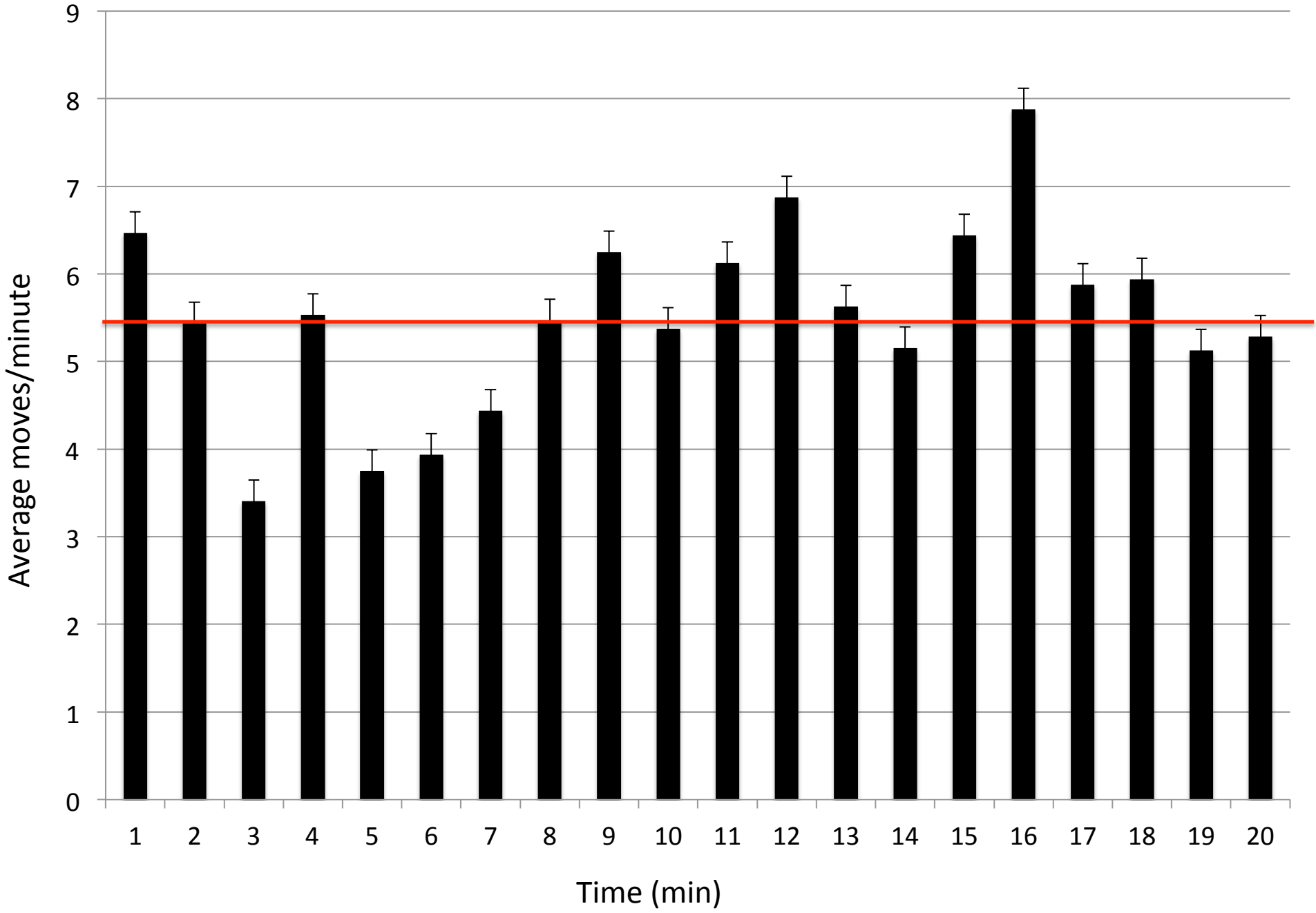

Figure 5

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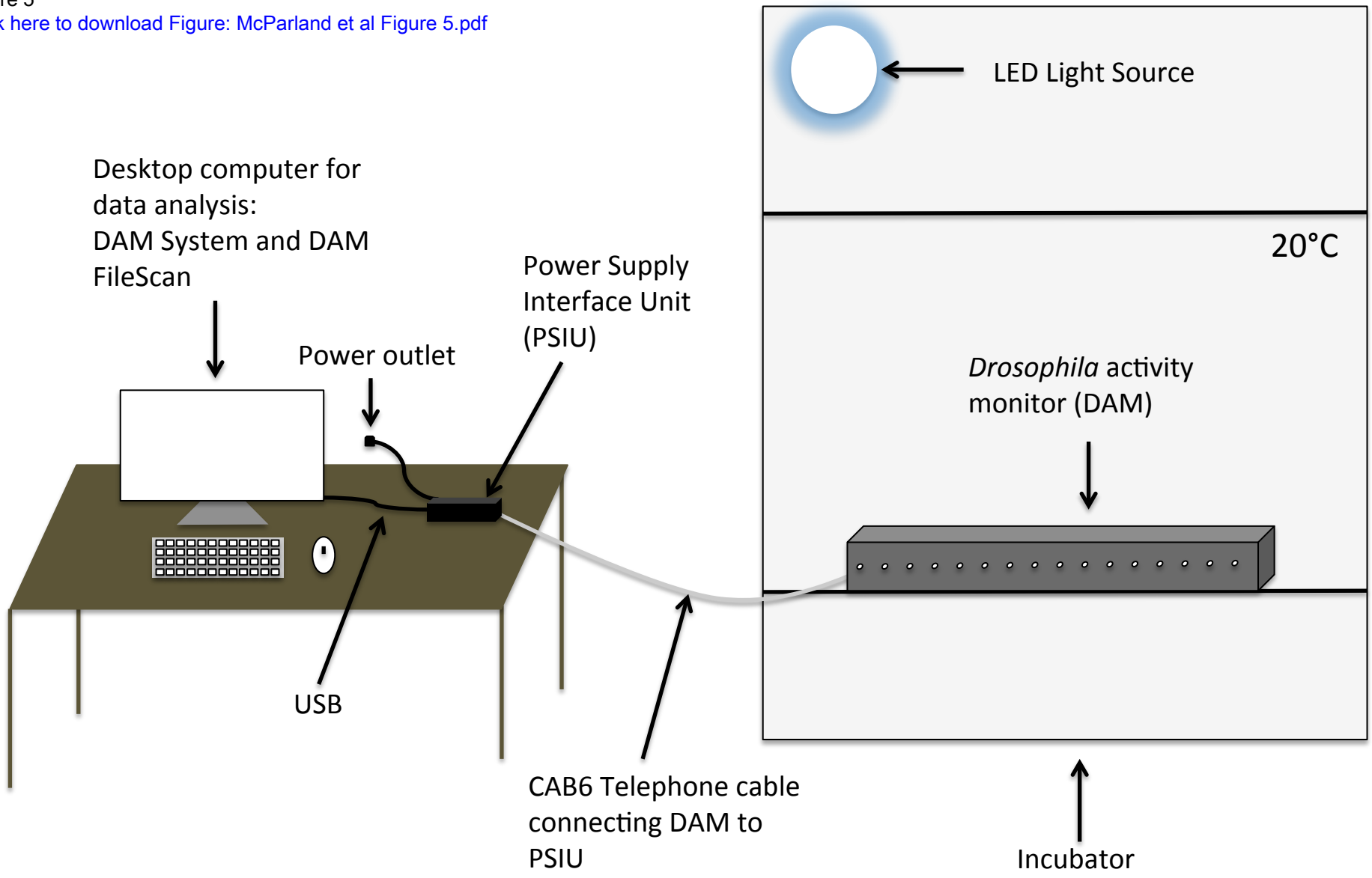

Excel Spreadsheet- Table of Materials/Equipment

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| Name of Reagent/ Equipment | Company | Catalog Number | Comments/Description | | |
|---|---|---|---|---|---|
| *Drosophila* Activity Monitor, Multibeam, 16 tubes, including wires | TriKinetics Inc. | MB5 | | | |
| Power Supply Interface for Activity Monitor | TriKinetics Inc. | PSIU24 | | | |
| Glass 80 x 5 mm tubes for Activity Monitor (100) | TriKinetics Inc. | PGT 5x80 | | | |
| DAMsystemMB1v6x Data Acquisitions Software for Macintonsh OSX (Intel) | www.trikinetics.com | | free download | | |
| DAMFileScan 108x software for Macintosh | www.trikinetics.com | | free download | | |
| USB software (PSIUdrivers.zip). | www.trikinetics.com | | free download | | |
| DAMSystem Notes 308 | www.trikinetics.com | | free download | | |
| Zeiss Stemi 2000C- Stereo Microscope | Spectra Services | SP-STEMI2000C-BS | | | |
| Carbon Dioxide | Maine Oxy | | anaesthesia | | |
| Fly Pad | Genesee | 59-114 | surface for sorting anaesthetized flies | | |
| Small paint brush | Winsor & Newton | #2 ROUND | or similar, used for sorting anaesthetized flies | | |
| Silk Screen Printing Mesh (160) | msj-gallery.com | SM160W63-3YD | pore sized used in this protocol was ~ 0.1 mm | | |
| Tegosept | Genesee | 20-258 | preservative | | |
| Ethanol (190proof) | Pharmco | 111000190 | used to dissolve Tegosept | | |
| 6 oz Square Bottom Bottle (PP) | Genesee | 32-130 | | | |
| "Flugs" for Plastic Fly bottles | Genesee | 49-100 | | | |
| *Drosophila* Vials, Wide (PS) | Genesee | 32-117 | | | |
| Flugs for wide plastic vials | Genesee | 49-101 | | | |
| Yellow Degerminated Corn Meal | Gold Medal | | | | |
| *Drosophila* agar | LabScientific | FLY 8020 | | | |
| Baker's Yeast - Red Star | King Arthur Flour | 1270 | | | |
| Granulated Sugar - Extra Fine | Domino | | | | |

Animated Figure (video and/or .ai figure files)

Click here to download Animated Figure (video and/or .ai figure files): McParland larva moving in moist assay tube

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Signature: Geoffrey Gunter Date: September 5, 2014

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***Reviewer #1:***

*Major Concerns:*
*1. The w1118 line contains a mutation in the white gene and should not be considered as wild-type. The authors could name it as "control" instead of "wild-type".*

***Changed to "control" in all instances.***

*2. What stages of larva (1st, 2nd, or 3rd instars) did the authors use in their experiment? Can the system detect all 3 stages or just the largest 3rd instar larvae?*

***We present additional data from first and second instars.***

*3. The larva is trapped insider the monitor tube that is sealed with agar in both ends. Does the lack of aeration affect the behavior of the larva? Does the larva show any changes in behavior during the 20min assay?*

***Activity over the 20 minutes was analyzed in one-minute bins, and shows no change. This will be presented as a separate figure.***

*4. Figure 1 and 2 seems switched in the pdf file.*

***Corrected*.**

*Minor Concerns:*
*1. Line 142: need to define what PSIU is.*

***Term defined.***

*2. Any speculation why there is a sudden reduction in larval activity at 25oC, which should be the usual culturing temperature for Drosophila.*

***Conjecture is offered.***

*3. Table of materials: "Flugs for plastic fly bottle" - typo "Plugs"? Materials*

*used or general fly husbandry (e.g. vials, corn meal, Tegosept… etc) may not need in this section since they are not specific to this experimental setup.*

*Spelling is correct for the product name "flugs", husbandry materials left to editor's discretion*

***Reviewer #2:***

*Major Concerns:*
*1) In Figure 1, the move/minute data for w1118 larvae at temperatures of 5°C-35°C show a consistent upward trend with the exception of either the 20°C or 25°C data point. It is surprising that steadily increasing temperature would cause larval activity to fluctuate in this manner. Is this pattern consistent between experimental runs? Is it possible that the 20°C and 25°C data were reversed or that some anomaly during the assay resulted in an abnormal reading?*

*Issue discussed. This is indeed what we actually observed.*

*2) If, as speculated above, either the 20°C or 25°C data point shown in Figure 1 is incorrect, is the 20°C moves/minute reading for w1118 still significantly different than that for iav1 shown in Figure 2?*

*Issue discussed.*

*3) Does this assay system provide as meaningful a representation of movement rates as other available methods? Video 1 shows an unidentified larva in an assay tube. A visual count of body wall contractions (a common low cost method used to quantify larval movement) during this video indicates ~12 in a 22 second period. This can be extrapolated to a rate of approximately 33 moves/minute, a rate nearly three times that of the highest rate shown in Figure 1. The two assay methods are obviously not directly comparable in terms of raw counts but this does reveal that the authors' assay system involves a significantly higher threshold in order to score a movement. The authors also note that their assay method may be unable to detect circumferential movements that do not involve breaking a*

*beam. They feel that this is not a serious limitation since it would affect all test samples equally, however, combined with the issue of a higher movement threshold, it suggests that this method could miss small but significant differences in movement rate between test groups or decrease the apparent significance of larger differences.*

***We admit that the device may not be sensitive to small-scale movements.***

*Minor Concerns:*
*1) The labels on Figures 1 and 2 are reversed compared to the figure legends.*

***Corrected.***

*2) Italics are generally used for both complete names of Drosophila genes and their abbreviations.*

***All instances italicized.***

*3) Formatting of Materials/Equipment Table could be improved to fit on one page and improve readability.*

***Now fits on one page and striped for readability.***

*4) The method used to humidify the assay tubes prior to larval insertion should be improved. Breathing into each tube introduces variability in the degree of condensation achieved based on differences between operators and environmental conditions. It also increases the possibility of sample contamination.*

***Great comment. The breathing method has been replaced with a method using a hot water bottle.***

*5) Which components of the assay system were located inside the incubator during testing (activity monitor, power supply, power supply interface, etc)? Are there any potential concerns with the stability of incubator temperature due to heat generation by the power supply (if it is located inside the*

*incubator) or the necessity of running cords out of the incubator (if power supply is located outside)? A diagram describing the experimental setup would be helpful.*

***A diagram has been supplied as Figure 5.***

***Reviewer #3:***

*For what time frame is the assay useful for?*

***Some analysis and discussion of the time frame used, as well as conjecture about how to increase the length of an assay, is provided***

*Do wildtype animals show changes since they are put in the assay? This is a key criterion since adults show no change in activity and thus the assay can be well used to monitor activity/sleep/rhythm.*

***We are not sure to what situation we are being asked to compare larval activity?***

*The proposed method uses Agar plugs at both sides of the tube. This would not allow a regular gas exchange to the chamber and thus could rapidly result in hypoxic conditions. This should be addressed by using an alternative plug at one side. Similarly the lack of food will affect the behavior of animals.*

***Behavior was analyzed over the 20-minute period and no change in locomotion was observed. See Figure 4.***

*Since both larvae and adults show circadian rhythms it could be tested if this assay can be used to monitor circadian activity.*

***We have offered discussion of this possibility, which would require modifications that allow for feeding and probably increased gas exchange.***

***Reviewer #4:***

*Minor Concerns:*
*I have one minor concern: The term "data" is plural and not singular (datum is the singular and is generally not used). I would urge the authors to go through the manuscript and change sentences in which the word data is used to reflect that the term is plural.*

*Good point. Done.*

***Reviewer #5:***

*Minor Concerns:*
*1. As the company Trikinetics provides a wide variety of monitoring devices, a brief discussion (in the introduction or discussion sections) on why the MB5 MultiBeam Activity Monitor was chosen over their other lines of products could be useful. That way, investigators unfamiliar with these devices know that the high number of IR beams in the MB5 (17) (and other benefits) versus their other products (DAM2 or LAM10 with 9 IR beams) provide the necessary resolution and special software to detect the small motions by the larvae.*

*We have added a phrase that makes this comparison.*

*2. A graphical representation (an actogram) of the larval activity over time of day would be a helpful addition. While the larvae are not in the devices for a long time, it would be an easy way to visualize high or low activity points over the course of the experiment, rather than only investigating the total movement counts at the end. The Trikinetics website provides free activity analysis software from other sources.*

*This has been added.*

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