

Olfactory Conditioning Apparatus for Fruit Flies

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Abstract

Current devices that utilize fruit flies for research for the memory genes are largely similar, both in their functionality and restrictions. These devices share the trait that air flow and air pressure are uncontrolled variables in the experiments done on the subjects. While the experiment produces data to interpret, the data retrieved might not necessarily be as accurate. The client has previously attempted to resolve the problem without much luck. This is the first time that the client has employed engineers for anything more than advice. The goal for this semester is to modify the current design or design a completely new apparatus that has control over the environment in which the fruit flies are trained without compromising data integrity.

Abstract.....	2
Problem Statement.....	4
Background.....	4
• Motivation.....	4
• Design Constraints.....	5
Current Apparatus.....	5
Current Procedure.....	6
Design Ideas.....	7
1. Improving current design.....	7
2. Solid Lexan Block.....	9
3. Cylindrical Selector.....	10
Design Matrix.....	12
Future Work.....	14
References.....	15
Product Design Specifications.....	16

Problem Statement

Our goal is to create an apparatus that trains and tests fruit flies' memory and ability to learn by means of electric shock and Pavlovian response. The apparatus must be able to control the pressure in the training and testing chambers, deliver a constant voltage across the flies in the training tube and have all elements encase the fruit flies in 5/8 inch diameter tubes.

Background

Motivation

Dr. Jerry Yin conducts research on the memory genes for the University of Wisconsin – Madison. Research on the subject has been going on since the 1970's, when the memory genes were identified. He and his colleagues came up with a design for an apparatus that would train fruit flies with odors, pairing one of the two with an electric shock and testing their ability to recall what they learned after the training was over. Those who designed this apparatus neglected to take into consideration effects of pressure and an electric grid that would deliver a consistent electric shock to the flies. While general information is known about fruit flies, nothing specific to their responses to changes in pressure and air flow are formulated. These factors showed up in the experimentation for memory. The researcher did some experiments

related to pressure changes and airflow and realized it was a factor left unchecked. Were these problems fixed it would potentially increase accuracy of retrieved data and make that data much more meaningful.

Design Constraints

The following requirements must be included in the design process:

1. The apparatus must be able to control and monitor air pressure during the experiment.
2. It must be able to deliver a known voltage across an electric grid consistently.
3. It must be able to be cleaned and maintained effectively with low risk of damaging the apparatus to ensure long life span.
4. It must achieve data of similar or better accuracy than the previous device.
5. Diameter of testing and training tubes must be $5/8$ inch or less.

Current Apparatus

The apparatus that is currently being used to test fruit flies' learning ability and memory consists of three separate tubes connected to the main Lexan body (Figure 1). The top plastic tube, called the training tube, contains an electric grid used to shock the fruit flies. This electric grid delivers a shock of 70 volts across each fly. The bottom two tubes are considered the testing site, with the choice point in the

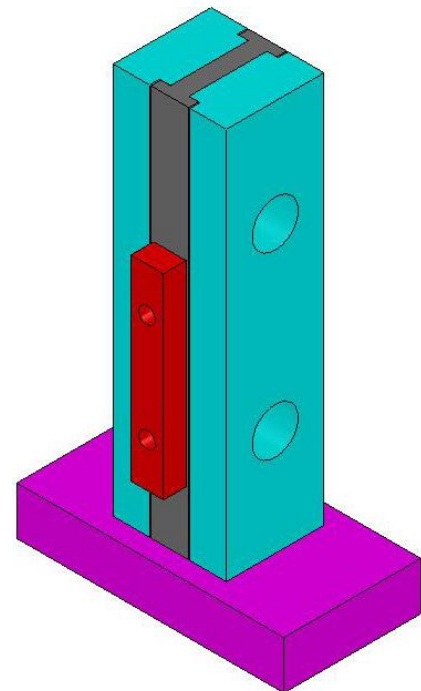


Figure 1: Current Apparatus

middle. Flies are transported from the training tube to the testing site via an elevator, which is part of the body. Odors are introduced through tubes connected to the ends of the three tubes. The odors are bubbled through mineral oil prior to being introduced to the tubes in order to humidify the air. This increases the flies' ability to smell the odors. Airflow is powered by a pump that sucks air out of the middle of the Lexan body, so during training it is sucked out of the end of the tube, and during testing it is sucked out through the choice point.

Current Procedure

Training

Training the fruit flies is a five step process. Approximately 100 flies are placed inside the training tube. First, air from the lab is passed by the flies for 45 seconds. Next, one odor is passed by the flies for one minute, and during this time the flies receive a shock of 70 volts every five seconds. The flies are then presented with air from the lab for 45 seconds. The second odor is then passed by the flies for one minute, but with no electric shock. Finally, the air from the lab is passed by the flies for 45 seconds. This is considered a single training session. For further training, multiple sessions are sometimes carried out.

Testing

After the flies have been trained, the flies are knocked into the elevator. The user does this by physically knocking on the side of the device. The flies are then released in the choice point, which is between the two testing tubes. One odor is presented in one testing tube, and

the other odor is presented in the second testing tube. After two minutes, the tubes are removed and capped. The user later counts the flies and records this data.

The current device has been obtaining data that supports the client's hypotheses, but he believes that a new device should be able to obtain better data. According to the client, approximately ninety percent of the flies choose the correct odor when the device is working best. This percent is lower when new tubes are used in the device, and also after tubes have been used for too long (more than a month). Tubes are replaced because they become dirty from the flies defecating in them. The electric grid is also replaced, but much less frequently.

The client believes that the current apparatus needs to be improved to obtain better quantitative data. Measurements of air pressure have been taken inside of the three tubes, and all three tubes have differing air pressures during the procedure. The client believes that these differences in air pressure are affecting the results of the experiment. The client also believes that the flies are not receiving a consistent 70 volt electric shock. From the standpoint of the user, the current device can be improved by simplifying the operations involved in training and testing. The user changes the tubes multiple times during training and testing, so the procedure becomes rather complex.

Design Ideas

Improving the current device

The current device can be improved in a number of ways. First of all, air will be pushed through instead of pulled through. Currently the pump sucks air out of the center of the device. Any restriction in airflow along the path from an odor to the tube will result in a drop in

pressure in the tube. If the air is pushed from the odor and out through the tube, the only point of pressure buildup will be the last point of resistance – diffusion holes at the end of the training tube and in the center of the training site. Another way to improve the current device will be to simplify the tubes by removing unnecessary connectors and incorporating valves (Figure 2).

A more permanent training tube will be incorporated into the improved device. This will be a Lexan tube with an electric grid etched into the inside. To do this, a grid will be cut into the inside of the tube. Conductive paint will then be painted onto this grid. Finally the inside will be sanded so only the etched grid will contain the conductive paint. This tube will be cleaned instead of discarded. If the tube is frequently cleaned, the flies should receive a consistent electric shock of 70 volts during training.

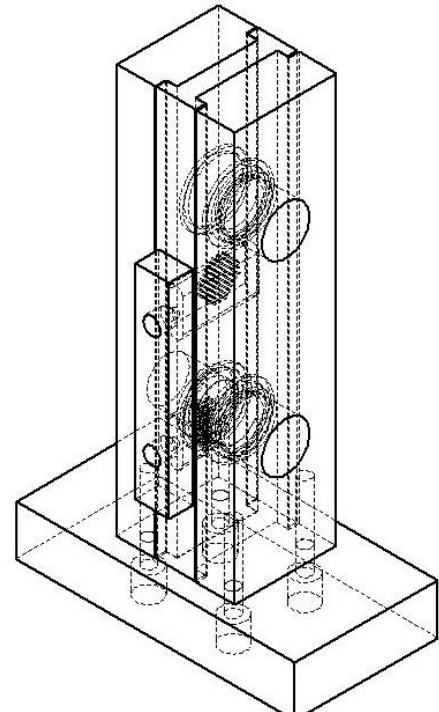


Figure 2: Improved device

Even though improving the current device will solve the problem of differing air pressures, the procedure will remain similarly complex. The flies will still need to be knocked into the elevator in order to transport them to the testing site. The procedure used for the current device will also be used in the improved device. Incorporating these new components into the improved device should yield more consistent data than the current device, but the procedure will remain somewhat complex.

Solid Lexan Block

The second design idea is called the solid Lexan block. It is a simplification of the first design by combining the testing and training into a single tube that would be drilled through the center of a rectangular Lexan block (Figure 3). The tube would be able to hold a single fly or multiple flies and would contain a

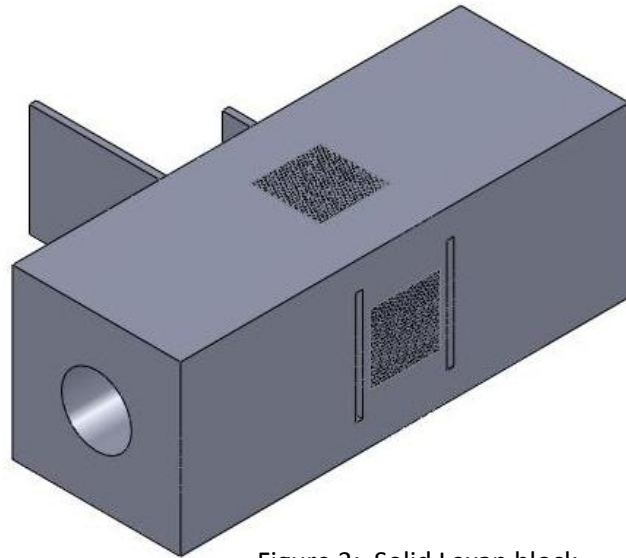


Figure 3: Solid Lexan block

permanent electrical grid etched into the sides of the tube. The air and odors would be pushed through using a pump and selected using a valve system. Diffusion holes would be drilled in to the center of the block allowing the odors to diffuse into the lab. To contain the flies and center them back in the middle between training and testing, doors would be placed on either side of the diffusion holes. This would allow for separation of odors before the flies enter the testing stage.

The device would work in a similar procedure to the first design. Flies are placed into the middle in between the doors and the first odor is pushed through both sides and paired with an electrical shock. The odor is cleared out using air, and the second odor is brought through. After this odor is cleared out, because the flies may have spread out during the training stage, one door is closed and the flies are knocked back to the middle. The other door is closed and two odors are pushed into the apparatus to do the testing stage.

One of the major advantages of this design is the fact that it combines both the training and testing into the same place. This not only makes the design more compact, but also simplifies the procedure a makes it much easier to operate. Another positive to this design is its durability. Because the electrical grid is etched into the sides of the training and testing tube, this would allow it to be much easier to clean. Also, this design does not need multiple test tubes or an elevator to hold the flies. This would allow for a much simpler cleaning process compared to the original apparatus. A

One of the major problems of this design is also its biggest disadvantage. With the electrical grid etched into the sides of the tube, this makes it very hard to drill or place anything else into the sides of the tube. Because of this, the diffusion holes and doors interfere with electrical grid in the middle of the tube. This causes some concern because there needs to be a constant and equal shock throughout the tube in order to achieve the best results.

Cylindrical Selector

Our third design is called the Lexan cylindrical selector. It is a cylinder inside of a cylinder design with two levels (Figure 4). The inside cylinder has a hole drilled through it that would be big enough to contain one to five flies at a time. Inside of this is an electrical grid that would be etched into the sides of the hole. This middle cylinder would contain the flies and would be able to move

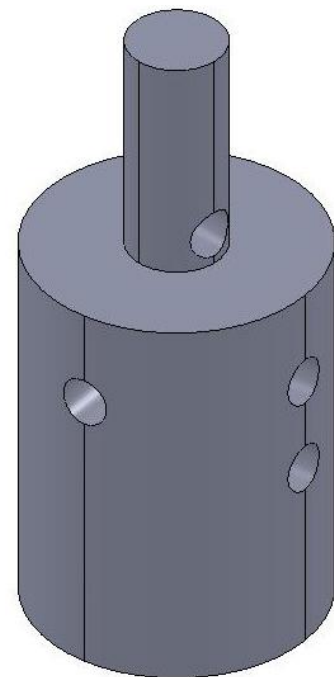


Figure 4: Cylindrical Selector

up, down, and rotate inside of the bigger cylinder. The outer cylinder has two levels of holes. The top level would contain three holes drilled into them. Each of them would be same size as the middle cylinder and none of them would be parallel to each other. The second level of holes would be lower than the first level. These holes would be drilled on opposite sides of the outer cylinder and would line up with each other along with the hole in the middle cylinder. Odors and air would also be pushed into the cylinders while they are simultaneously being pulled out through a vacuum. Also, an infrared tracking system would be able to be incorporated into this design. This would contain infrared lights, an infrared camera, and software to take the data during testing.

The device would follow a similar procedure to the previous two designs. A fly would be placed in the middle cylinder with electrical grid. It would be moved down to the first level of holes and rotated to line up with the hole that would deliver the first odor paired with a shock. After this, the middle cylinder is rotated to the hole with the air to clear the odor. The cylinder is then rotated again to the third hole which will expose the fly to the third odor. After this training session, the middle cylinder is pushed down to the second level and lined up with holes. The holes contain different odors and when the middle cylinder is rotated to line up with them, the fly will be able to choose which side to go to. The infrared camera will be placed above the apparatus to monitor the fly and take data during this stage.

One of the major advantages of this design is the incorporation of the infrared tracking and data system. This would allow for accurate meaningful data because of the exact location and movements of the fly during testing. This would add a qualitative aspect to the data along

with the already quantitative data. Also, because this design contains only a single fly, less testing would have to be done to obtain similar results compared to the existing apparatus that uses one hundred flies.

The main and single disadvantage this design has is its complexity and attention to detail. This design contains many moving parts and small holes that would have to be precise and contain little to no error. Also, because the holes are smaller to contain a single fly, the engraving of the electrical grid would take a significant amount of time and skill in order to make sure that it delivers a consistent shock. Because of these reasons, this design is not feasible at this time.

Design Matrix

In order to evaluate our designs, we used a design matrix with certain aspects of the design weighted more heavily than others (Figure 5). The criteria that were chosen to evaluate these designs were safety, ergonomics, feasibility, cost, accuracy, and durability. Feasibility was deemed the most important aspect of the design, followed by accuracy and ergonomics. Safety and cost were assigned the lowest point values because safety we would not make a device that was harmful to the user and our client was not concerned with the cost of the device.

The cylindrical selector design scored the lowest out of the three designs with a score of 65 on the design matrix. Its main shortcoming was feasibility because of its complexities. To be

able to create a device that had a testing and a training area, multiple pathways to dispense odor and air, and created a center tube that would have both a vacuum and an electric grid, would take much longer than a semester to have a working product. Also, infrared sensors would have to be set up in the small tube that would be the testing area and the whole device would have to be easy to clean. Data tracking software would have to be implemented as well, further complicating the design. It achieved a perfect score of 25 for accuracy because it would be a single-fly device and could possibly achieve slightly better data than the other two designs. It scored the highest on ergonomics because its selection system would make it easy for the user to run the experiment.

The solid Lexan block design scored a 73 on the design matrix, the second highest total out of the three designs. It achieved a high score of 22 for accuracy due to its similarities to the current model. It could be used for single flies and multiple flies and would have multiple diffusion holes to relieve air pressure from the tube. It would be very durable and relatively easy to use because it would have the testing and training area in the same tube and therefore would have fewer pieces to the overall design. But feasibility for a big concern for this design because there would be no good way to deliver a consistent and uniform shock to the flies because of the moving doors in the design. These doors interrupt the electric grid and there is no feasible way to create a consistent grid across the whole tube with the doors and the diffusion holes around the choice point.

Improving the current design scored an 82 on our design matrix and is the design we chose to pursue. It achieved a very high feasibility score because there is already a model in

place that we can work with and improve. Although it achieves data of slightly lesser quality, we feel that making tangible improvements to their current experiment would benefit them tremendously right now and would give a good product to show for our work by the end of the semester. The improvements would make the current device less difficult to work with and produce more consistently meaningful data.

Criteria		Possible Designs		
Considerations	Weight	Improved Current Device	Solid Lexan Block	Cylindrical Selector
Safety	5	5	5	5
Ease of Use/Ergonomics	20	10	12	15
Feasibility	35	32	20	12
Cost	5	5	4	2
Accuracy	25	22	22	25
Durability	10	8	10	6
Total	100	82	73	65

Figure 5: Design Matrix

Future Work

Testing the mixing and diffusion of the odors is the next step in the design process. Colored smoke will be used and we will record the testing with a video camera to document our observations. Once we determine how odors will diffuse and observe the airflow and mixing when the odors are pushed through the device, we will make the necessary adjustments to reduce the air pressure and take data from air pressure measurements on built-in gauges and

record the data on the computer. Afterwards, a valve system will be incorporated to make it easier on the user to switch between odors and air when running the experiment. Finally, we will go through final testing procedures and analyze the data to optimize the experiment.

References:

Dr. Jerry Yin, personal interview

Yin, Jerry. *Nature Neuroscience*. 5, 316-324 (2002).

Product Design Specifications for BME 201 Group 48: Olfactory Device

(February 20, 2009)

Group Members: Robert Bjerregaard, Graham Bousley, Charles Donaldson, and Scott Carpenter

Problem Statement:

The aim of this project is to improve or completely re-design a device that is currently used to test fruit flies' olfactory sense, memory, and ability to learn. The current device is producing some inaccurate results because the fruit flies experience changes in air pressure and airflow. Airflow and pressure change need to be kept at a minimum while odors are introduced and cleared from the device.

1. Design Requirements:

The device must meet all of the client requirements

- a. *Performance Requirements:* The device must be durable enough for cleaning and repeated usage for at least 10 years. It should test 100 flies at a time and achieve data of similar or better accuracy than current device.
- b. *Safety:* Electrical system should not kill flies or cause any harm to operators during standard procedures.
- c. *Accuracy and Reliability:* Airflow and air pressure should be monitored and controllable. Results should be reproducible and consistent with previous data.
- d. *Life in Service:* The device must have lifespan of at least ten years.
- e. *Shelf Life:* The device must have a shelf life of five years.
- f. *Operating Environment:* The device must withstand normal room temperature, slight pressure changes, multiple electric shocks, and fly feces.
- g. *Ergonomics:* The device should be able to be operated by a single person.
- h. *Size:* The diameter of the testing and training area should be five eighths of an inch. The device must not occupy more space than one square foot, and must be portable.
- i. *Weight:* The device must not weigh more than five pounds.
- j. *Materials:* The device must be composed of transparent materials that are able to withstand thorough cleaning and electric shock. All of the inside surface must be able to deliver a shock of 70 volts.
- k. *Aesthetics, Appearance, and Finish:* The device must be transparent and have a smooth finish.

2. Production Characteristics:

- a. *Quantity:* One reproducible working prototype is necessary.
- b. *Target Product Cost:* Unknown at this time

3. Miscellaneous:

- a. *Standards and Specifications:* Normal lab safety specifications for odors.

- b. *Customer:* The customer prefers a device that produces more accurate and reproducible results than the current model.
- c. *Patient-related Concerns:* None
- d. *Competition:* Current device is being used in lab and producing somewhat accurate results.