Olfactory Conditioning Apparatus for Fruit Flies

May 6, 2009

Team:

Robert Bjerregaard-Team Leader

Graham Bousley- Communicator

Scott Carpenter- BWIG

Chuck Donaldson- BSAC

Client:

Jerry Yin, Ph.D.

Professor of Genetics and Psychiatry

Advisor:

Paul Thompson, Ph.D.

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 problem 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 accurate or reproducible. The client has previously attempted to resolve the problem without much success. 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.

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 air pressure and air flow 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 air pressure, air flow, and the need for 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 has been formulated. These factors showed up in the experimentation on memory. The researcher did some experiments related to pressure changes and airflow and realized they were factors left uncontrolled. Were these problems fixed it could potentially reduce variability of retrieved data and make that data much more significant.

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 middle. Flies are

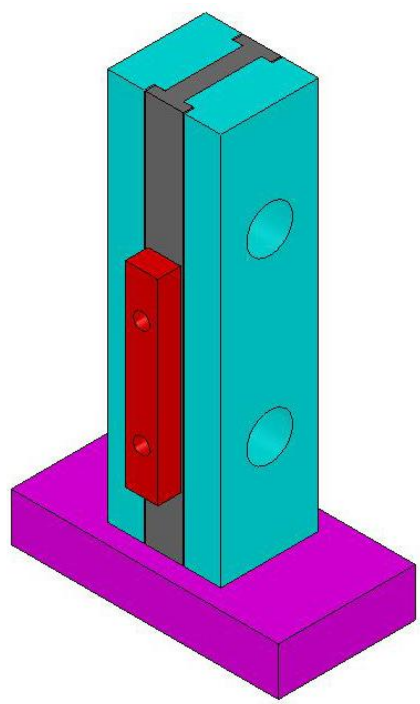

Figure 1 Body of current apparatus

transported from the training tube to the testing site via an elevator (center sliding piece), which is part of the body. Odors are introduced through tubes connected to the ends of the three tubes. Air is bubbled through mineral oil in order to introduce the odors into the system. Airflow is powered by a pump that sucks air out of the middle of the Lexan body. During training, it is sucked out of the end of the tube, while 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 reinforcement, multiple training sessions are sometimes carried out.

Testing

After the flies have been trained, they 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 opposite 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 at its 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 along the path of airflow will result in a drop in pressure in the tube. If the air is pushed from the odor source and out through the tube, the only point of pressure buildup will be the last point of resistance – diffusion holes 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.

A more permanent training tube (Figure 2) 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 after each use. If the tube is frequently cleaned, the flies should receive a consistent electric shock of 70 volts during training.

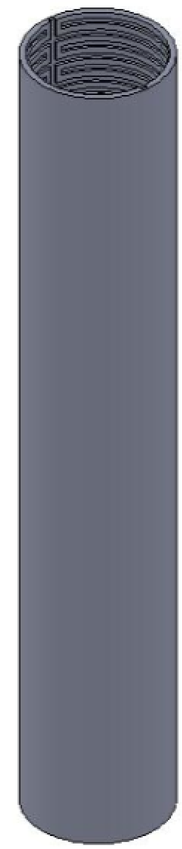

Figure 2 Permanent training tube

Even though improving the present device will solve the problem of differing air pressures, the procedure will remain 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 combing 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 multiple flies and would contain a 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 into 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 begin the testing stage.

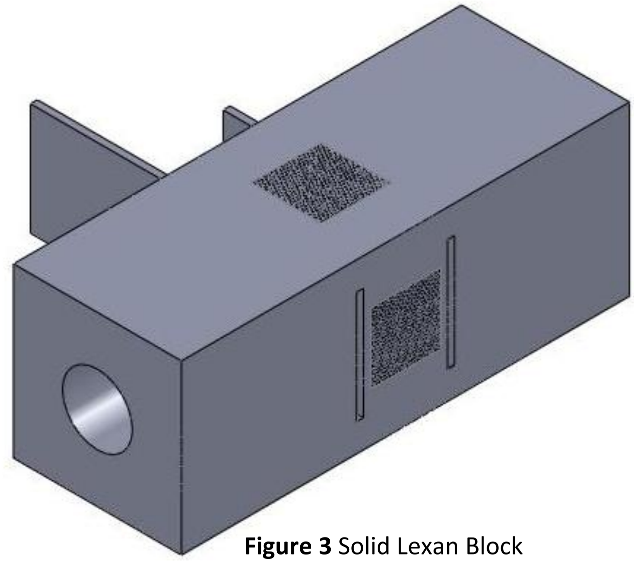
Figure 3 Solid Lexan Block

The device would work using a similar procedure to the first design. In the training phase, 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 during 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 aspect of this design is its durability. Because the electrical grid is etched into the sides of the training and testing tube, it would 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.

One of the major problems of this design is also its biggest disadvantage. With the electrical grid etched into the sides of the tube, it would be very hard to drill or place anything else into the sides of the tube. Because the diffusion holes and doors interfere with electrical grid in the middle of the tube, there is some concern that the constant and equal shock throughout the tube in order to achieve the best results would not be realized.

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 inner cylinder would contain the flies and would be able to move up, down, and rotate inside of the outer cylinder. The outer cylinder has two levels of holes. The top level would contain three holes. Each hole would be same diameter as that in 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

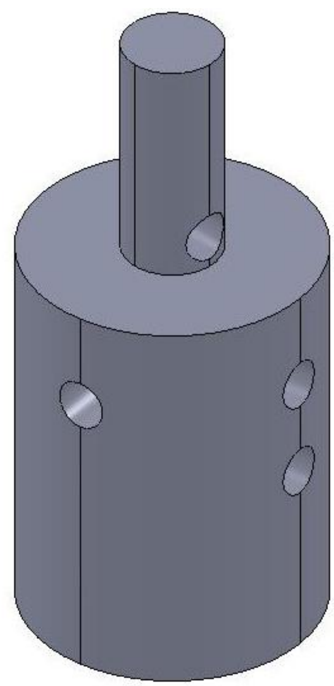

Figure 4 Cylindrical Selector

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 track the flies during testing.

The device would be used with a procedure similar 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 second odor without shock. 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 flies 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 the data to include the exact location and movements of each fly during testing. This would add a qualitative aspect to the data, as well as reduce uncontrolled variables of the experiment, such as crowding and the influence of other flies on one another. Testing could be done with a single fly that produces results more significant than the existing apparatus that uses one hundred flies.

The main and single disadvantage of this design is its complexity. 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 skill in order to make sure that it delivers a consistent shock. Because of these reasons, this design is not feasible for us to construct at this time.

Design Matrix

In order to compare 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 our designs by were safety, ergonomics, durability, cost, accuracy, and feasibility to construct. 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 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.

The infrared tracking system wouldn't be utilized in the other designs because they would use multiple flies in their respective experiments. 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 was 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 simple 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 less quality, we feel that making tangible improvements to the current apparatus would benefit our client 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

Final Design

Fabrication

The final design was created with the main priority being fixing the air pressure problem. Since air is pulled out of the old device at the choice point, 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 in the center of the training site. For the final design, the center sliding piece was modified (Figure 6) by drilling more

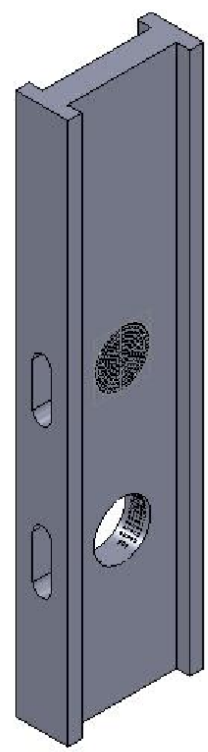

Figure 6 Modified center sliding piece

diffusion holes on the center piece (6", 1.5", 0.58"). Next, single-cylinder piston airbrush compressors (Model TC-20) were implemented to push air through the device. They have an output that ranges from 0-100 psi, which is especially useful because we want to limit air pressure through the device.

Our next priority was to simplify the experiment for the user because there were multiple steps and simultaneous processes involved. Two manifolds (3", 1 ¼", 1 ¼") containing 6 valves each were incorporated in order to select a specific odor rather than having the user switch the tubes on the device manually. Each manifold was connected to 2 jars (2 ½"tall, 2" wide) containing mineral oil, to a ball valve, and to the device with plastic tubing (outer diameter 3/8" and inner diameter ¼"). One needle valve was placed after 6" of tubing on each compressor to better control the amount of air flow. Ball valves were placed 3" after the needle valves to decrease the amount of pressure in the tubing system. The valves on the manifold assembly are either open or closed in the new design, which makes the fine-tuning the system much more straightforward compared to the variable valves on the old design. Finally, the two testing tubes on either side of the device were simplified to decrease resistance in the system.

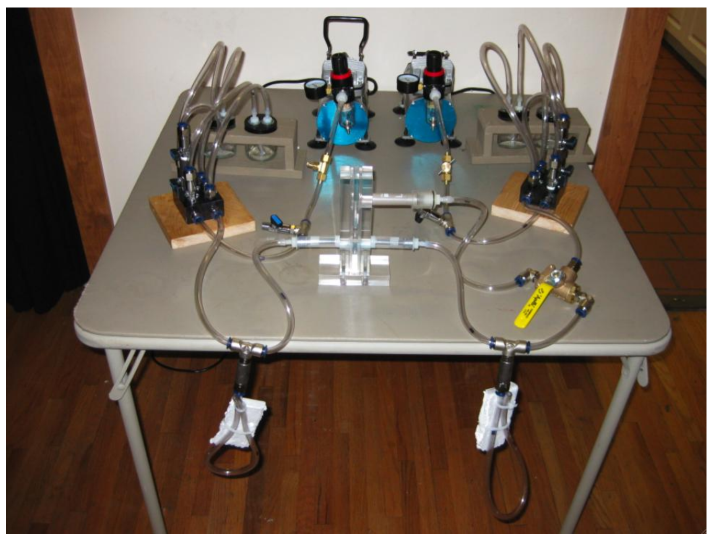

Figure 7 Final design set-up

Actuation

In the experiment, the compressors are turned on and set to deliver 1 psi. The air travels out of a pump, through a needle valve (where the pressure is dropped dramatically), through a ball valve, into the manifold, and into a jar containing mineral oil with a dissolved odor. The odor is picked up in the air and travels out of the jar, into the manifold, and into the device from one side. Both odors diffuse out of the diffusion holes at the center of the device, the choice point, with minimal mixing at the choice point and no mixing in either test tube.

Testing Procedure:

To test the airflow in the design, we pumped in different colored smoke and observed how the smoke mixed and diffused. Initially, the smoke filled the chambers rapidly and did not go to the opposing sides. After about thirty seconds, there was a pressure overload and the mineral oil bubblers started to backup. This was because the diffusion ports at the choice point became filled with deposits from the colored smoke. Sealing the smoke capturing devices was another problem with testing that gave us inconsistent results. To test the air pressure in the design, we incorporated two water-column pressure gauges. The difference in water height for each side was approximately 0.5 cm, which converts to an air pressure of approximately 0.0071 psi. We were unable to measure any differences in pressure between each side because each side gave essentially identical pressure readouts using our water-column pressure gauges.

Cost Analysis:

A breakdown of the total cost can be seen in Figure 8. Tubing and connectors include plastic barb adapters, couplers, and new mineral oil holders. Pressure valves used include needle valves for fine tune adjusting and ball valves for the manifold selector. The middle slider piece was the most expensive because it was ordered through a company and had to be specially made from scratch based on our Solidworks design.

Item	Cost
Manifold selectors, tubing and connectors, pressure valves, mineral oil containers	320.33
Middle Slider Piece	335.00
Pumps	219.96
Total	875.29

Figure 8 Breakdown of expenses

Conclusion

Future Work

We need to test the old device with smoke by hooking the two tubes up to smoke and using a vacuum to pull the smoke through. We could also compare air pressure results by measuring the changes in water column height between the old design and the new design. Another test on the new design could be to use a small object such as a small plastic ball and put it at the choice point. Similar to the smoke test, it would monitor the amount of airflow on both sides of the choice point. We could incorporate pressure transducers with real-time readouts as well, placing them at the edges of the testing tubes to equalize pressure in the testing area. Also, we could find a different manufacturer to create a good permanent shocking mechanism.

Delivery to Client

Once all testing is completed, the device can be delivered to the client. We need to be sure to follow proper design etiquette and make sure that the device functions exactly how we say it does. We cannot deliver a device that causes odors to mix in the testing chamber and gives inaccurate results in actual experiments with fruit flies. We also have to make sure that there is little to no risk of pressure backups and spilling of mineral oils, even if an inexperience user is operating the apparatus.

Once the device has passed all tests, it can be delivered to the client. The client needs to be instructed on how to operate everything, and also on how to properly control and monitor airflow and pressure. If the client cannot control airflow and pressure, there is little hope that the improved device will give better results in actual experiments. Experiments with fruit flies can then be run on the improved apparatus, and results from these experiments can be compared to results from the old apparatus. Results should be compared over a relatively long period of time (at least one month) because the flies' behavior changes for unknown reasons. With the old apparatus, sometimes the flies cooperate and the results are very promising. At other times results are very poor. Our client told us that the flies have these "waves" of good behavior, so in order for the improved device to be properly tested, experiments using the improved device should be run over the same period of time as experiments with the client's current device.

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 testing/training component must not occupy more space than one square foot, and must be portable. The entire device, including pumps, odor sources, and tube selectors, must fit on a 3 foot by 5 foot desktop.
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.