

The Effect of Diphenhydramine on the Cognitive Function of Drosophila Melanogaster

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Introduction

With the increase of stress levels in modern society, the use of nontraditional forms of sleep medication have become more and more common. Benadryl, the generic form of diphenhydramine, which is intended to be used as an allergy product, has become increasingly popular as a sleep medication. This affects the brain's function in the form of cognitive behavior and basic motor functioning. By affecting the function of the brain and causing drowsiness, Benadryl has become a drug that you can be pulled over for driving while still under the influence of the effects of the medication.

Every year, driving while not fully functioning and being drowsy causes about 100,000 crashes, 71,000 injuries, and 1,550 fatalities, according to the National Safety Council (NSC). Drowsy driving contributes to an estimated 9.5% of all crashes, according to AAA.



Purpose of Study

The purpose of this study is to observe the effect that diphenhydramine will have on drosophilae in the way they are able to function and react to environmental stimuli.

Hypothesis

Diphenhydramine will have an effect on the motion function of the drosophilae, and will present in slower motion as well as a limited ability to react to environmental changes that the untreated drosophila will respond to faster. Those who are exposed to the medication will have slowed actions, and will have more difficulty reacting to stimuli and will just generally be less active than those not exposed to medication.

Methodology

Data Collection and Analysis

Data will be recorded in charts that will be separated by group and by trial numbers. Each trial will represent a different drosophila subject. Because identification is too difficult to keep each subject individually recorded, data will be averaged and categorized under the groups, which will also keep outliers from affecting the trial outcomes.

Methodology

Sampling

Drosophilae were chosen as the subject of study because of the similarity in their cognitive function to humans. Drosophilae are Carolina® Easy Fly® Drosophila Culture, Living, wild type (Oregon-R with HSY), Vial of 12-20. They have been purified and are all female drosophila to avoid possible extreme aggression and reproduction in order to keep the amount of drosophilae relatively constant throughout the experiment. Four vials will be purchased, with about 36-60 drosophilae used. There will be four experimental groups. The first group will not receive the excess proteins and will also not receive Benadryl. This will act as the complete control group. The second group will be given diphenhydramine in meals. This will act as a control for the general effects of Benadryl as a comparison for behavioral changes. The last two groups will be kept alive, one treated and one not, in order to have extra on hand, because of the short lifespan, and the inaccuracy in age of the drosophila when they arrive. Because there is no true knowledge of how old the drosophila are when arriving from Carolina®, having extra on hand would mean not being set back if some are to die. Drosophila are also checked under microscope upon receipt to confirm that they entire sample population is female to prevent reproduction.

Each group will remain in the vials they arrived in as to limit the possibility of the change in environment affecting results. These vials will be plugged with foam to allow air in for the drosophila, as well as to provide a simple way to keep and administer fly nap when necessary. Feeding vials will be used to keep living and food separate and to make footage easier to review. There will also be vials specifically for shake tests, so that one drosophila at a time can be tested and data can be recorded. Drosophila will always be kept with their groups when not being used for experimentation. When the drosophila arrive, they will undergo a brief acclimation period. Because drosophilae at best have around a sixty day life span, they need only a few days to adjust to the medication before being ready for experiments.

Drosophilae will be fed with the food they come with from Carolina®. Treatment will be administered through tiny holes in the top of the molasses food (pre ordered in tubes ready to be used in experimentation), with 5 squirts from a Benadryl Liqui-Gels Antihistamine Allergy Medicine & Cold Relief pill, a name brand version of diphenhydramine HCl. Using name brand instead of generic means factoring in effects of inactive ingredients (carnauba wax, croscarmellose sodium, D&C red no. 27 aluminum lake, dibasic calcium phosphate, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, titanium dioxide). The mentioned inactive ingredients will have no effect on experimentation. Using name brand is more similar to actual usage in humans because of what is more simply available and supplied. Food will be administered into the bottom of the feeding vials, and the drosophilae will be put to sleep using flynap or cold shock in order to transfer between living vial to feeding vial. Flynap is administered via instructions on the packaging, and cold shock is done by placing the drosophila in the freezer for fifteen minutes, then checking on them every two minutes until they can be confirmed to be asleep.

Experimentation

Cognitive and motor function can most easily be identified by poor motor coordination, confusion, loss of short-term or long-term memory, inability confusion, and impaired judgment. Neural signaling can be tested through environmental stimulation and reaction times.

The drosophilae will be filmed 24/7 to monitor behavior, and will be reviewed 2-3 times a week to study the differences in behavior between the drosophilae groups.

They will be tested twice a week with a shake test to see how fast they can fly from the bottom to the top to see how their motion and function has been affected. Times will be recorded and averaged to determine if the diphenhydramine is affecting their movement. A faster time will mean that the drosophilae have not been significantly effected by either the disease or treatment.

Data



Control Group: avg time: 28.86 seconds	Treatment Group: avg time: 31.77 seconds
data: 11.23 seconds 17.92 seconds 23.96 seconds 28.53 seconds 36.02 seconds 40.10 seconds 44.23 seconds	data: 10.27 seconds 13.43 seconds 29.01 seconds 35.83 seconds 40.27 seconds 45.63 seconds 47.90 seconds

The results confirmed the hypothesis, revealing that the motor function and reaction time of the drosophila in the treatment group was in fact longer than those who were untreated. Under observation, this only continued to be prevalent, as the untreated drosophila could typically be found flying around the vial, while the treated ones tended to stay towards the bottom and were much less active (see image B).

Limitations

A significant limitation in this process was figuring out how to keep the drosophila alive. Through multiple inexplicable deaths in isolated vials that should not have died, it was difficult to gather results with subjects that were not alive for consistency of trials. Because originally backup vials of drosophila were not used, if one test vial died, then the entire process would need to be restarted with a new assimilation period, because using drosophila at different stages of life would alter the results concluded. This limitation set back the experimentation several weeks as each week the flies died for a different reason. The first week, there was an overdose of flynap, which was solved by adding in cold shocking as an alternative form of putting the drosophila to sleep. The second week, the drosophila died due to an overdose of the benadryl, which was solved by changing the dosage that the drosophila were administered. The third week, an unknown death occurred. This was extremely odd and was difficult to figure out the cause and therefore solution to because it was one vial of the untreated drosophila groups, and it had food that had been prepared and made from the same batch as the food in the vial of the other untreated control group vials, but only one died. And the entire vial died unexpectedly, with no drosophilae left alive and no known cause to this. Both vials had the exact same food, had drosophila that had been randomized from the original vials that had been purchased, and had no known differences or had been effected or experimented on in any different way. The solution to this ended up being to change the type of food the drosophila were being given from a powdered mix to premade molasses food already in vials. The cause of the death of individual vials still remains unknown. This happened in two consecutive weeks with absolutely no drawn connection.

The other major limitation occurred during experimentation. The original plan had been to run two tests, using a stimulus test to test a different type of environmental change to see how the drosophila would react, however when actually doing a trial run of this test, the drosophila had no interest in the banana, which was used as the stimuli in trial because of the affinity drosophila have for it, and this proved this test to be rendered ineffective.

Conclusion

Based on the results, it is fair to conclude that the generic diphenhydramine significantly effected the functioning abilities of the drosophila, and should continue to be taken under advisement, and only used for intended purposes in order to prevent possible accidents and simply because it is not meant as a sleep medication.

Major References

Center for Drug Evaluation and Research. "Taking Too Much of the Allergy Medicine Benadryl Is Dangerous." U.S. Food and Drug Administration. FDA, 24 Sept. 2020. Web. 3 Nov. 2021.

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