2018

AP CollegeBoard

AP Research Academic Paper

Sample Student Responses and Scoring Commentary

Inside:

Sample B

- ☑ **Scoring Guideline**
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2018 AP Research Academic Paper Rubric **v1.0**

The response…

Score of 1 **Report on Existing Knowledge**	**Score of 2** **Report on Existing Knowledge with Simplistic Use of a Research Method**	**Score of 3** **Ineffectual Argument for a New Understanding**	**Score of 4** **Well-Supported, Articulate Argument Conveying a New Understanding**	**Score of 5** **Rich Analysis of a New Understanding Addressing a Gap in the Research Base**
• Presents an overly broad topic of inquiry.	• Presents a topic of inquiry with narrowing scope or focus, that is NOT carried through either in the method or in the overall line of reasoning.	• Carries the focus or scope of a topic of inquiry through the method AND overall line of reasoning, even though the focus or scope might still be narrowing.	• Focuses a topic of inquiry with clear and narrow parameters, which are addressed through the method and the conclusion.	• Focuses a topic of inquiry with clear and narrow parameters, which are addressed through the method and the conclusion.
• Situates a topic of inquiry within a single perspective derived from scholarly works **OR** through a variety of perspectives derived from mostly non-scholarly works.	• Situates a topic of inquiry within a single perspective derived from scholarly works **OR** through a variety of perspectives derived from mostly non-scholarly works.	• Situates a topic of inquiry within relevant scholarly works of varying perspectives, although connections to some works may be unclear.	• Explicitly connects a topic of inquiry to relevant scholarly works of varying perspectives **AND** logically explains how the topic of inquiry addresses a gap.	• Explicitly connects a topic of inquiry to relevant scholarly works of varying perspectives **AND** logically explains how the topic of inquiry addresses a gap.
• Describes a search and report process.	• Describes a nonreplicable research method **OR** provides an oversimplified description of a method, with questionable alignment to the purpose of the inquiry.	• Describes a reasonably replicable research method, with questionable alignment to the purpose of the inquiry.	• Logically defends the alignment of a detailed, replicable research method to the purpose of the inquiry.	• Logically defends the alignment of a detailed, replicable research method to the purpose of the inquiry.
• Summarizes or reports existing knowledge in the field of understanding pertaining to the topic of inquiry.	• Summarizes or reports existing knowledge in the field of understanding pertaining to the topic of inquiry.	• Conveys a new understanding or conclusion, with an underdeveloped line of reasoning **OR** insufficient evidence.	• Supports a new understanding or conclusion through a logically organized line of reasoning **AND** sufficient evidence. The limitations and/or implications, if present, of the new understanding or conclusion are oversimplified.	• Justifies a new understanding or conclusion through a logical progression of inquiry choices, sufficient evidence, explanation of the limitations of the conclusion, and an explanation of the implications to the community of practice.
• Generally communicates the student's ideas, although errors in grammar, discipline-specific style, and organization distract or confuse the reader.	• Generally communicates the student's ideas, although errors in grammar, discipline-specific style, and organization distract or confuse the reader.	• Competently communicates the student's ideas, although there may be some errors in grammar, discipline-specific style, and organization.	• Competently communicates the student's ideas, although there may be some errors in grammar, discipline-specific style, and organization.	• Enhances the communication of the student's ideas through organization, use of design elements, conventions of grammar, style, mechanics, and word precision, with few to no errors.
• Cites **AND/OR** attributes sources (in bibliography/works cited and/or in-text), with multiple errors and/or an inconsistent use of a discipline-specific style.	• Cites **AND/OR** attributes sources (in bibliography/works cited and/or in-text), with multiple errors and/or an inconsistent use of a discipline-specific style.	• Cites **AND** attributes sources, using a discipline-specific style (in both bibliography/works cited AND in-text), with few errors or inconsistencies.	• Cites **AND** attributes sources, with a consistent use of an appropriate discipline-specific style (in both bibliography/works cited AND in-text), with few to no errors.	• Cites **AND** attributes sources, with a consistent use of an appropriate discipline-specific style (in both bibliography/works cited AND in-text), with few to no errors.

AP® RESEARCH
2018 SCORING COMMENTARY

Academic Paper

Overview

This performance task was intended to assess students' ability to conduct scholarly and responsible research and articulate an evidence-based argument that clearly communicates the conclusion, solution, or answer to their stated research question. More specifically, this performance task was intended to assess students' ability to:

- Generate a focused research question that is situated within or connected to a larger scholarly context or community;
- Explore relationships between and among multiple works representing multiple perspectives within the scholarly literature related to the topic of inquiry;
- Articulate what approach, method, or process they have chosen to use to address their research question, why they have chosen that approach to answering their question, and how they employed it;
- Develop and present their own argument, conclusion, or new understanding while acknowledging its limitations and discussing implications;
- Support their conclusion through the compilation, use, and synthesis of relevant and significant evidence generated by their research;
- Use organizational and design elements to effectively convey the paper's message;
- Consistently and accurately cite, attribute, and integrate the knowledge and work of others, while distinguishing between the student's voice and that of others;
- Generate a paper in which word choice and syntax enhance communication by adhering to established conventions of grammar, usage, and mechanics.

Drosophila Insulin Like Peptides: Causal Neuropeptide for Fly Aggression?

Word Count: 4287

Introduction

A high frequency of aggressive disorders in the population is one of many American health concerns. Today America is home to over 3.5 million diagnosed patients with schizophrenia, over 3 million with bipolar disorder, and over 8 million diagnoses of Post-Traumatic Stress Disorder (NAMI). The cost borne by society for the treatment of aggression borne out by these mental disorders can be sobering. For instance, the government funded Veterans Health Administration reports that treating PTSD in veterans returning from various theatres of conflict, costs four to six times more than unaffected veterans, totaling around $8,300 per PTSD affected veteran as opposed to $2,400 per unaffected veteran, assuming all other health conditions remain constant. (CBO, 2012) With nearly 1.3 million active duty soldiers in the armed forces, these costs put a significant drain on resources which can be better used elsewhere (DOD, 2016).

On the other hand, a large number of psychopathies, such as schizophrenia and bipolar disorder can exact a similar toll on the already extended healthcare system in the country. There is significant evidence that patients with these psychopathies have an increased risk for aggression and violent behavior, including homicide. Data for this relationship comes from a study carried out by Fazel and Grann, which indicated that 5.2% of severe acts of violence are committed by individuals with a major psychiatric disorder, most commonly schizophrenia (Fazel et al., 2006). A 10-year follow-up study by Soyka and Graz of 1662 former schizophrenic inpatients in Germany showed that 10.7% were convicted of a crime, 94 of which were violent in nature (Sokya et al., 2007). Although attempts have been made to address such conditions with cognitive based therapy as well as pharmacological treatments, these are either marginally effective or carry significant side-effect profiles. Genome-wide association studies for the

neurobiological basis of aggression are either lacking or currently being investigated by very few researchers.

Literature Review

In 2002, Young et al. found that a homozygous deletion in mice *NR2E1* gene resulted in hyper-aggression (with significantly increased violent tendencies). The deletion of this gene also caused various developmental deficiencies, such as hypoplasia of the cerebrum and olfactory lobes (Young et al., 2002). These mice, although noticeably smaller in size would aggressively attack siblings, unrelated mice, and even mating partners, resulting in serious injury or death. Additionally, an array of sensorimotor tests measuring the efficiency of neuronal sensory-response pathways, showed that the mutant mice took longer to turn around corners and alleys and exhibited less exploratory behavior with fewer arm stretches to feel their surroundings. The time taken by them to find hidden food sources was also of several magnitudes as compared to wild type mice. Dr. Young concluded that the *NR2E1* gene was likely causal for decreased sensorimotor control. Although the degree of aggression and developmental abnormalities might be at least partially dependent on genetic background, violent behavior and behavioral deficits were found in all phenotypes with a homozygous *NR2E1* gene deletion. This study however, failed to explore whether the developmental deficiencies could have been at least partially responsible for causing the aforementioned violent behavior and decreased sensorimotor skills.

Another approach to better understand the genetic source of violent psychopathy is to use the extensively studied Drosophila melanogaster, the common fruit fly. In this species, the tailless gene (abbreviated *tll*) plays an important role in brain and body size development, Dr.

Ruth T. Yu found that the mammalian *NR2E1* gene was similar to the tailless gene in *Drosophila melanogaster* (Yu et al., 1994) shown by in-vitro DNA-binding assays exhibiting similarity in function. Both proteins would regulate another set of genes in the organism's genome, and these regulated genes showed resemblance despite belonging to different organisms. The study confirmed this finding by replacing the tailless gene in Drosophila with the mammalian *NR2E1* gene, which functioned as the tailless gene and allowed for almost normal fly development (Yu et al., 1994).

A recent study of the analogous tailless genes in *Drosophila* also provides evidence that the tailless gene is responsible for regulating aggressive behavior (Dierick et al., 2014). Dr. Herman Dierick at Baylor College of Medicine found that when mRNA from the tailless gene was continuously broken down by RNA interference, fly aggression increased dramatically. This study sorted flies of each genotype into groups of two and placed them on opposite sides of a small cubic ring separated by a thin removable wall allowing for controlled interactions between the flies. These were recorded using high resolution video, analysis of which affirmed that the tailless gene, analogous to the mammalian NR2E1 gene, also had a high degree of correlation with aggression. Specifically, *tll* and *NR2E1* coded for transcription factors that prevent uncontrolled transcription of an aggression neuropeptide in the fruit fly and mice models. Dr. Dierick also hypothesized that the tailless gene interacted with corepressor molecules Scribbler and Atrophin. By comparing aggression between these three set of flies, namely those unable to produce Scribbler, those unable to produce Atrophin, and finally flies that lacked a functional tailless gene, he discovered that only Atrophin negative flies and tailless negative flies displayed increased aggression. This allowed him to conclude that the tailless gene interacted with Atrophin to regulate transcription. Furthermore, tagging the tailless gene with a fluorescent

protein showed that it was prolifically expressed in the region of the fly brain which is analogous to the mammalian hypothalamus. Thus, one could conclude that the hitherto unknown gene responsible for expression of the aggression neuropeptide was regulated by the tailless gene and was present in the *pars intercerebralis*.

To determine potential neuropeptides that could be the cause of aggression in *Drosophila melanogaster*, one can look to two major parameters: their location and associated symptoms. Since the tailless gene is expressed mostly in the in the *pars intercerebralis*¸ one can extrapolate that its regulated protein would also be found in the same area. One family of prominent neuropeptides that are often found in the *pars intercerebralis* neurons are the Drosophila Insulin Like Peptides or dILPs (specifically dILP 1, dILP 2, dILP 3, and dILP 4). A study published by Dr. Kavitha Kannan at the University of Connecticut-Storrs found a similar array of developmental deficiencies in the knockdown of dILPs as those with deficiencies of the tailless gene knockdown (Kannan et al., 2013). This, coupled with dILPs 1, 2, 3, and 4 localizations in the *pars intercerebralis* neurons of the brain, suggested that dILPs may be the unknown neuropeptides that cause aggression. Although the studies mentioned above and others proposed the significance of dlLPs, there remains a significant gap in the literature as far as decisive evidence of dILPs' role as the aggression neuropeptide.

These dILPs were the subject protein of my experiment, which examined their role in regulating aggression. I hypothesized that flies without the ability to produce dILPs would have lower quantifiable aggression than those that could produce dILPs. I tested my hypothesis by examining and directly comparing flies that did not have the ability to produce dILPs to flies that did have the ability to produce dILPs. This led to the development of my leading question: are the Drosophila Insulin Like Proteins (dILPs) 1 to 4 causal for aggression in *Drosophila*

melanogaster? The experiment that this paper will expound upon involved ordering *Drosophila* flies that had a mutation within each specific dILP gene which rendered them ineffective. The flies were then 'pitted against' flies from the same genotype to determine the level of aggression and thus the presence or absence of a neuropeptide responsible for aggression. Once this neuropeptide has been identified, it can be used to identify analogous mammalian proteins leading to potential advancements in identifying the etiology of aggression in human psychopathies.

Methods

Behavioral Observation

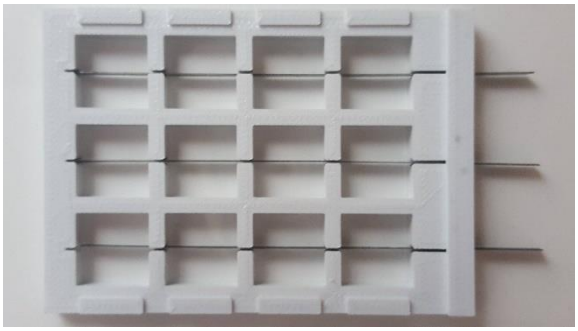
Drosophila melanogaster males are characterized by smaller size, a solid black coloration on the abdomen, and a tendency to "fight" under certain circumstances, including fighting for a mate, for territory and for dominance over a food source (Asahina et al., 2015). High intensity fights include exchanging blows with various appendages (mostly legs and wings) and grappling each other with limbs alone. These fights are rare but are found largely in territorial disputes or disputes over dominance of a food source. Using a high-resolution position tracking software JABBA developed by Dr. Kabra and Dr. Robie at the Howard Hughes Medical Institute, one can calculate the number of "lunges" that the flies attempt at each other (Kabra et al., 2012). The software tracks eleven essential positions on each fly's body: two wings, six legs, two sides of the abdomen and finally the head. The two sides and head are used to track each fly's unique position. Based on pre-defined parameters, the software starts detecting 'lunges' when these fly's are near each other. A 'lunge' occurs when a fly quickly moves its limbs or wings against the other. Using this method, the aggression of individual flies (in the number of lunges) can be

quantified or fighting frequency among large groups of flies with similar traits (in terms of percentages) can be determined (Kabra et al., 2012).

For my experiment, flies in the wildtype control group had no genetic mutations and were fully capable of producing the dILP neuropeptide. Conversely, the experimental group consisted of flies with the mutated dILP gene, lacking the dILP neuropeptide. In order to ensure that all the flies in the control group had the same genotype, I used a breed of genetically identical flies called Canton Special, abbreviated Canton-S. Although these flies are not truly "wild", most researchers use them in their control groups because they tend to exhibit characteristics one would expect in flies collected from the wild. On the other hand, the experimental group consisted of four genotypes: each with an inability to produce dILP 1, dILP 2, dILP 3, and dILP 4 (notated *dILP 1 negative*, *dILP 2 negative*, *dILP 3 negative* and *dILP 4 negative* respectively). Each of these strains were ordered online from the Indiana University Bloomington Drosophila Stock Center via FlyBase (a database of *Drosophila* genes and labs that produce several different strains) Each strain of *Drosophila* was unable to produce only one type of dILP while adequately producing all the others.

The observed behavioral aspect of my experiment comprised of three steps. First, I placed a parental generation of flies into a test tube, waited for them to produce offspring, and then removed the offspring immediately after hatching. I did so by first administering carbon dioxide into the sealed tubes to anesthetize the flies. Then I used a brush to move flies onto a plate, and separated 30 male flies of each genotype into two separate smaller tubes (totaling two tubes of 15 flies each). This was done to ensure that the flies would have no interactions with each other prior to their first contact in the ring. I did not want the flies to recognize each other during the recorded fight nor did I want the flies to fight before the recording was initiated. This

was followed by a waiting period of four days which allowed the flies to mature in their tubes. The flies that would eventually fight were seven days old since it has been well established that flies become more aggressive as they attain maturity. I then took a fly from each of the two tubes of a single genotype and placed them into separated compartments of a square ring, 14 millimeters on each side and 3 millimeters deep. This ring was bisected by a removable sheath of plastic. After being placed in their respective compartments, the flies were allowed to acclimate to their new surroundings and explore the ring for three days, so that any individual exploratory behavior would not interfere with the aggressive interactions being tracked.



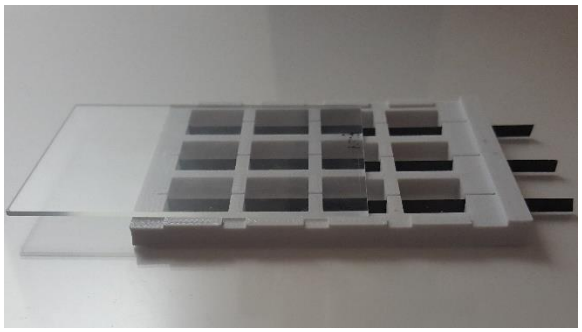


Figure 1a (left) and 1b (right). Images of sixteen fly rings, bisected by removable black plastic dividers. Figure 1a depicts an aerial view of the ring, while Figure 1b depicts a diagonal view with glass cover.

Finally, both compartments of the ring were placed under a high-definition camera which recorded 20 minutes of fly interactions immediately after the compartmental divider was removed. The camera was programmed to record images in the Tagged Image File Format (.tiff files) at 20 frames per second. Because the camera's field of vision was quite large, up to sixteen rings could be recorded simultaneously. Following the recordings, the images were converted to Audio Video Interleave (.avi files) video format via a custom MATLAB script so that they could be played back to identify lunges.

Figure 2. Image of behavioral observation setup, featuring fly rings positioned under an HD camera and the computer used to record and store the images.

Data Analysis

The video was then analyzed by the software program JABBA, (Janelia Automatic Animal Behavior Annotator), coded in MATLAB. JABBA used measurements known as classifiers to identify important aspects of each image; two important ones being distance between flies and limb velocity. Using these, JABBA identifies that flies which are close together and have high forelimb velocity have lunged at each other. After detecting the number of lunges in each video using JABBA, I reviewed each video and confirmed that each automatically detected lunge was indeed a lunge and not a computer measurement error. The total number of lunges per pair over a 20-minute period was calculated and transferred to an Excel document. This document contained details about the genotype, number of lunges and pair

ID (numbering system used to keep track of each pair in a genotype). We repeated this procedure for 25 pairs of flies per genotype for a total of 125 pairs of flies.

Although my flies should have been genetically identical, I expected some pairs of flies to exhibit high lunge numbers as a result of random mutation or chance. However, these outliers had to be eliminated to accurately compare the effect of *dILP negative* mutations on fly aggression. In order to do this, I first found the interquartile range of my data set (the upper quartile value subtracted from the lower quartile value) and then multiplied this value by three. This new value was added to the upper quartile and subtracted from the lower quartile to calculate the outer fence range for my data set. Finally, I eliminated all the data points that exceeded the outer fences as major outliers.

After eliminating the outliers, I performed three statistical analyses to identify the average, median, and p-value generated by a T-test for significance. The T-test is a statistical tool used to decide whether the differences in values are significant between two groups. Since my experiment called for comparing each dILP to the wildtype genotype, I performed T-tests between each dILP and the Canton-S stock, rather than T-tests between different types of dILP. Furthermore, I used a two-tailed distribution because I was unsure of which data set would be higher and used a two-sample equal variance because I was comparing two similar variance sets. If the p-value generated was below 0.05, I could consider the difference between sets significant. However, if this value was above 0.05, then my results would be regarded as insignificant. Following this analysis, I created a histogram comparing the average number of lunges for each genotype over twenty minutes of exposure to another fly.

With this methodology, I could form my final hypothesis concerning the role of different dILPs in fly aggression: *Drosophila melanogaster* that are incapable of producing the dILP

protein will have fewer lunges over a twenty-minute period than that of wildtype flies which can produce an effective dILP protein.

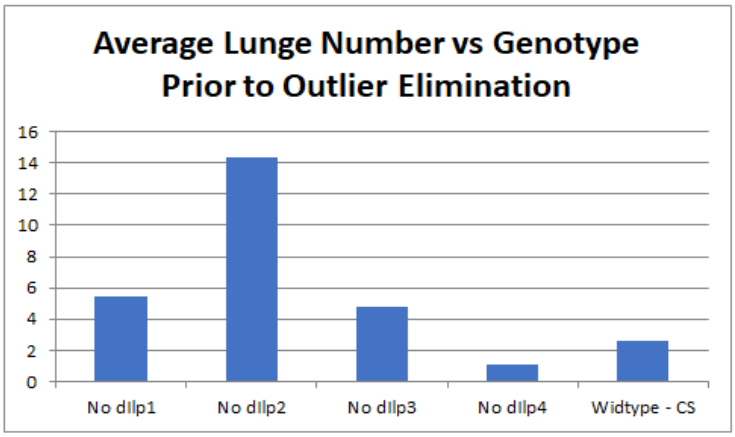
The methods that I used to answer my research question align with my project goal because they directly compared quantitative aggression in flies without an effective dILP protein to flies with a functioning dILP protein. Due to an absence of other confounding factors between the control group and the experimental group, my experiment provides a side-by-side comparison of the effect of dILPs on aggression in *Drosophila melanogaster*. Conclusive evidence that this experiment could provide can be used to identify the analogous mammalian protein that is responsible for aggression in rodent models, leading to potential advancements in identifying the etiology of aggression in human psychopathies.

Results

After obtaining the lunge number for wildtype Canton-S flies, as well as those for mutant flies unable to produce each type of dILP these were plotted on a histogram and analyzed in detail. Prior to eliminating outliers, flies unable to produce dILP 1 had an average lunge number of 5.48 over the twenty-minute period, those unable to produce dILP 2 had an average of 14.29, those unable to produce dILP 3 had an average of 4.77, and those unable to produce dILP 4 had an average lunge number of 1.08. On the other hand, the Canton-S stock with an intact capacity to produce dILP had an average lunge number of 2.60. Flies without the ability to produce either dILPs 1, 2, or 3 actually tended to have a higher lunge number than the wildtype, but *dILP 4* had a lower lunge number.

Table 1. The table depicts statistical analyses, including mean value, median, first (1st Q) and third (3rd Q) quartile, and outer fences, of lunge number over the 20-minute interaction period. All genotypes were compared to the same wildtype control group using T-tests; P-value is the result of each test. Pair ID denotes the identification number given to each pair of flies.

	No dIlp1	No dIlp2	No dIlp3	No dIlp4	Widtype - CS
Pair ID	Lunge Number per 20 minute Interaction Period				
1	Outlier	1	0	0	0
2	2	12	10	2	2
3	8	1	15	1	3
4	1	6	1	0	6
5	3	5	0	2	3
6	3	Outlier	10	0	0
7	Outlier	1	1	1	2
8	2	2	0	2	6
9	0	0	3	2	0
10	0	6	13	0	2
11	3	0	5	3	0
12	0	3	Outlier	0	3
13	0	3	1	0	Outlier
14	Outlier	1	0	1	0
15	5	3	1	0	0
16	2	1	0	2	0
17	1	Outlier	4	0	1
18	3	4	4	2	3
19	4	Outlier	0	4	2
20	2	0	2	3	0
21	3	2	2	0	2
22	0	19	0	2	1
23	2	0	NaN	0	5
24	1	18	NaN	0	2
25	2	NaN	NaN	0	0
Average	2.14	4.19	3.43	1.08	1.79
Median	2.00	2.00	1.00	1.00	2.00
1st Q	1	1	0	0	0
3rd Q	3	5	4	2	3
Interquartile Range	6	12	12	6	9
Outer Fences	0 to 9	0 to 27	0 to 19	0 to 8	0 to 12
Outliers	24, 27, 39	160, 28, 67	33	None	22
P-value	0.54158844	0.05222738	0.11798863	0.12242311	



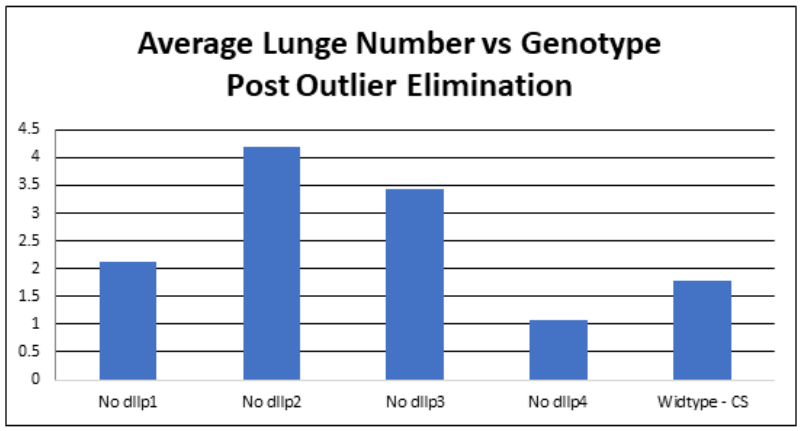


Figure 3a (left) and 3b (right). Average lunge number per genotype prior to elimination of outliers (Fig. 2a) and post-elimination (Fig. 2b) in mutated dILP gene 1, mutated dILP gene 2, mutated dILP gene 3, mutated dILP gene 4, and wildtype *Drosophila melanogaster*. Y-axis represents average lunge number per 20-minute period.

During my analysis, I found that experimental genotypes *dILP 1 negative* and *dILP 2 negative* had three outliers each while genotype *dILP 3 negative* and the wildtype control group each had one outlier in their data set, and experimental genotype *dILP 4* had no outliers whatsoever. However, after eliminating outliers, the range of averages decreased dramatically.

Flies incapable of producing dILP 1 had an average lunge number of 2.14, those unable to produce dILP 2 had an average of 4.19, those unable to produce dILP 3 had an average of 3.43, and those unable to produce dILP 4 remained at an average of 1.08. Furthermore, the Canton-S stock exhibited a lunge number of 1.79.

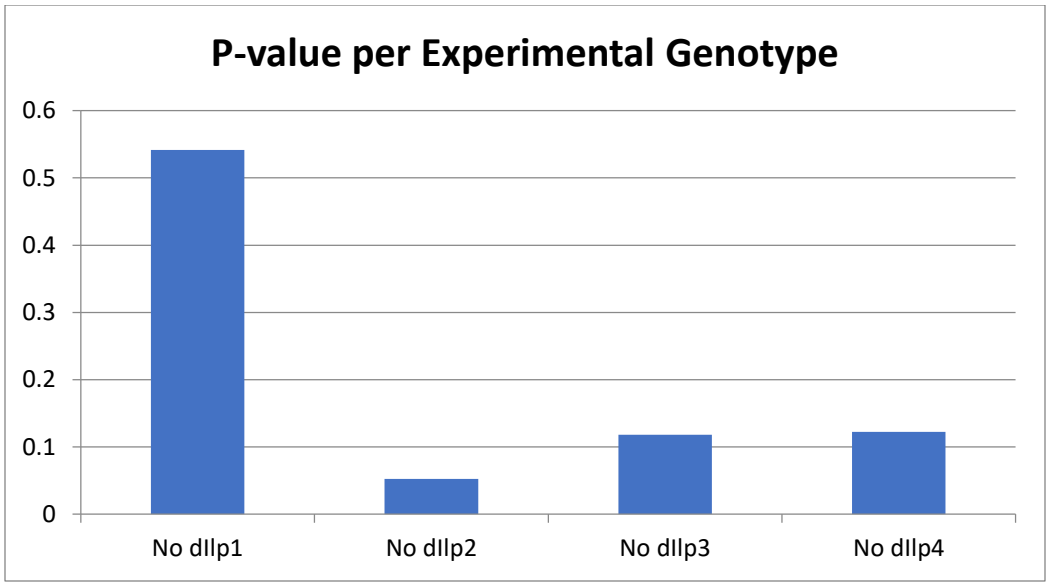


Figure 4. Statistical P-value (result of T-test) per mutated dILP genotype data sets compared to wildtype genotype data set post elimination of outliers (Fig. 2b).

I then performed four T-tests to determine the significance of my results after eliminating outliers. The p-value for the experimental groups *dILP 1 negative*, *dILP 2 negative*, *dILP 3 negative*, and *dILP 4 negative* as opposed to the wildtype data set were 0.542, 0.052, 0.118, and 0.122 respectively. From this, I was able to conclude that the differences in lunge number between each data set were insignificant and that the flies which were unable to produce dILP did not have a significant difference in lunge number from the wildtype flies with an intact ability to produce dILPs.

Discussion

As seen in Figures 3a and 3b, *dILP 1 negative*, *dILP 2 negative*, and *dILP 3 negative* feature pairs that have an average lunge number higher than that of the wildtype flies over the twenty-minute period. Although this may suggest that these dILPs could be another type of transcription factor for aggression, further analysis revealed that the p-values for these data sets

are higher than 0.05, deeming that the averages were insignificantly different from that of the wildtype flies, that could produce functional versions of the same dILPs (Fig. 4). However, the p-value for flies that could not produce dILP 2 approached 0.05, which might be due to the fact that dILP 2 may be another transcription factor for the unknown gene producing the aggression neuropeptide. On the other hand, *dILP 4 negative* appears to show a lower average lunge number over the twenty-minute period when compared to the wildtype lunge number. However, the T-test (p-value greater than 0.05) between them reveals that the difference is insignificant.

Thus, because the difference between wildtype flies capable of producing dILPs and experimental mutants that are incapable of doing so, is insignificant, I rejected my hypothesis: flies with the inability to produce dILPs have a lower lunge number than those of flies capable of producing dILPs. Due to this, it is unlikely that dILP 1, dILP 2, dILP 3, or dILP 4 are individually causal for aggression in *Drosophila melanogaster*. However, it is possible that multiple dILPs are causal for aggression, in which case a decrease in aggression would only be present if all dILPs were mutated. Moreover, further study into flies without dILP 4 (those that exhibited decreased lunge number in my experiment) might reveal a significant decrease in aggression if a similar experiment is performed in the background of more aggressive flies.

Having understood that dILPs are unlikely to be the cause of aggression in *Drosophila melanogaster*, we can now discuss the implications of this finding. First and foremost, looking back at Dr. Dierick's research of the tailless gene as a transcription factor, it is doubtful that the dILP genes 1 to 4 are the target genes of the tailless gene, because the tailless gene should regulate the aggression neuropeptide producing gene (Dierick, 2014). Next, it is improbable that the mammalian analogues of dILPs are responsible for aggression in mice models or humans. Because Dr. Ruth T. Yu's research found that the tailless gene and the analogous *NR2E1* gene

share target genes, Dr. Yu concluded that the target genes that produce the aggression peptides share a similar base pair sequence in both mice models and *Drosophila melanogaster* (Yu et al., 1994). One can then extrapolate that the protein produced by the unknown target gene should be similar between both species. Thus, because dILPs are unlikely to cause aggression in *Drosophila melanogaster*, its analogues are unlikely to cause aggression in mice models or humans. Knowing this, further study into possible causal aggression peptides in *Drosophila melanogaster* models or mice models should exclude dILPs and their mammalian analogues as potential candidates. Rather, researchers in the field should focus on other possible aggression neuropeptides in the PI region of the brain (where the tailless gene is expressed the most) that may be regulated by the tailless gene. These neuropeptides should also have analogous mammalian proteins that are regulated by NR2E1. By following these guidelines and by excluding dILPs, researchers would be more likely to isolate neuropeptides that cause aggression in *Drosophila melanogaster* and eventually those in mammals.

Limitations

In light of rejecting my hypothesis, there are certain limitations to the experiment that need to be mentioned. It is possible that multiple neuropeptides are causal for aggression, which would explain why *dILP 4 negative* exhibited a slight decrease in quantifiable aggression. Conversely, it is also possible that dILP 2 is another transcription factor for the unknown gene that produces the aggression neuropeptide. This would support the hypothesis that aggression in *Drosophila melanogaster* is caused by multiple neuropeptides and regulated by multiple transcription factors (such as the tailless gene). However, although the tailless gene has co-repressors (such as the Atrophin molecule), Dr. Herman Dierick proved that the tailless gene is

the principal transcription factor by finding that Atrophin works with the tailless gene to regulate transcription. Moreover, it is unlikely that dILP 2 alone is a transcription factor; one could infer that the peptides in the dILP family share similar functions. Thus, the biggest limitation of this experiment would be the background in which it was performed. The experiment was conducted with flies that had functional tailless genes. These may have suppressed the transcription of dILPs in the control group Canton-S flies (Dierick et al., 2014). Therefore, it is possible that all flies had minimal dILP present in the brain to begin with, and this, in turn, caused insignificant differences between the data sets. However, this limitation is mitigated by the fact that one would expect to see some difference between flies that have transcription factors that simply regulate the production of a potential neuropeptide and flies that cannot produce the potential neuropeptide whatsoever. Regulation of genes via transcription factors depend on the concentration of the transcription factors and the concentration of the DNA it regulates; it is possible – even probable – that some neuropeptide is produced, evidenced by the fact that wildtype flies with functional tailless genes must produce aggression neuropeptides when fighting. On the other hand, flies without the DNA to even begin transcription of an aggression neuropeptide would have almost no chance to produce any neuropeptide at all. Nevertheless, it is important to note that differences between data sets were considered insignificant in this paper and might have been caused by this discrepancy. Performing a similar experiment with flies that are more aggressive (specifically those that have a knocked down tailless gene) may reveal a profound difference in aggression phenotypes, especially for *dILP 4* that exhibited some decrease in aggression. Such an experiment would eliminate the possibility that the tailless gene was hindering significant production of aggression neuropeptides, solving for this potential limitation.

Future Research

Further research that should be performed in the future could address these limitations by testing flies that are incapable of producing multiple dILPs (as compared to an inability to produce only a single dILP) as well as by using flies that display a higher level of aggression. Future testing will focus on recreating this experiment in the presence of a tailless gene knockdown because differences in aggression between flies that cannot produce aggression neuropeptides at all versus flies produce the same neuropeptides in significantly increased quantities would be more obvious.

In continuance of this research, I plan to use the RNA interference system (RNAi) to nullify mRNA produced by the tailless gene (Dierick et al., 2014). By crossing flies with RNA interference of the tailless gene and flies that cannot produce dILPs, I should be able to compare the differences in aggression and subvert the limitations that my original experiment encountered. Nullifying the tailless gene would allow me to conduct my experiment with flies having a higher level of aggression, and in doing so, any decrease in aggressive behavior would be more profound and readily observable. Furthermore, to test the possibility that aggression is the result of multiple proteins, this experiment will also test more *dILP negative* genotypes and *dILP negative* genotype combinations, including *dILP 1 negative, dILP 2 negative, dILP 3 negative, dILP 4 negative, dILP 5 negative, dILP 6 negative, dILP 7 negative, dILP 1-4 negative,* and *dILP 5-7 negative* (these are the only combinations available on FlyBase, the commercial site where mutated flies are bought). This experiment would then be able to conclusively

determine if proteins dILPs 1 to 4 are individually causal or whether they are collectively contributory towards aggression.

By knocking down the tailless gene in flies that are incapable of producing these neuropeptides, one should not see an increase in aggression. This would prove that the said neuropeptides are those that the tailless gene regulates. The goal of such future research would then be to answer the question whether Drosophila Insulin Like Proteins (dILPs) 1 to 4 cause increased fighting frequency in the simultaneous presence of a ‘knocked down’ tailless gene.

References

Chyb, S., & Gompel N. (2013). *Atlas of Drosophila Morphology: Wild-Type and Classical Mutants*. Academic Press.

Dierick, H., Davis, S., Thomas, A., &Nomie, K. (2014). *Tailless and Atrophin Control Drosophila Aggression by Regulating Neuropeptide Signalling in the Pars Intercerebralis*. Nature Communications, 5. doi:10.1038/ncomms4177.

Duffy, J. B. (2002). *GAL4 system in Drosophila: A fly geneticist's swiss army knife*. Genesis, 34(1-2), 1-15. doi:10.1002/gene.10150

Fazel, S. (2006). *The Population Impact of Severe Mental Illness on Violent Crime*. American Journal of Psychiatry, 163(8), 1397. doi:10.1176/appi.ajp.163.8.1397

Jones, W. D. (2009). *The expanding reach of the GAL4/UAS system into the behavioral neurobiology of Drosophila*. BMB Reports, 42(11), 705-712. doi:10.5483/bmbrep.2009.42.11.705

Kabra, M., Robie, A. A., Rivera-Alba, M., Branson, S., & Branson, K. (2012). *JAABA: Interactive machine learning for automatic annotation of animal behavior*. Nature Methods, 10(1), 64-67. doi:10.1038/nmeth.2281

Kannan, K., & Fridell, Y. C. (2013). *Functional implications of Drosophila insulin-like peptides in metabolism, aging, and dietary restriction*. Frontiers in Physiology, 4. doi:10.3389/fphys.2013.00288

Soyka, M., Graz, C., Bottlender, R., Dirschedl, P., & Schoech, H. (2007). *Clinical correlates of later violence and criminal offences in schizophrenia*. Schizophrenia Research, 94(1-3), 89-98. doi:10.1016/j.schres.2007.03.027

Thomas, A. L., Davis, S. M., & Dierick, H. A. (2015). *Of Fighting Flies, Mice, and Men: Are Some of the Molecular and Neuronal Mechanisms of Aggression Universal in the Animal Kingdom?* PLOS Genetics, 11(8). doi:10.1371/journal.pgen.1005416

United States Department of Defense. (2004, July). Department of Defense (DoD) Releases Fiscal Year 2017 President's Budget Proposal. Retrieved from US DoD website https://www.defense.gov/News/News-Releases/News-Release-View/Article/652687/department-of-defense-dod-releases-fiscal-year-2017-presidents-budget-proposal/

Congressional Budget Office. (2012). *The Veterans Health Administration's Treatment of PTSD and Traumatic Brain Injury among Recent Combat Veterans*. Washington, DC.

Wilson, J. A., Jayasena, S., Khvorova, A., Sabatinos, S., Rodrigue-Gervais, I. G., Arya, S., … Richardson, C. D. (2003). *RNA interference blocks gene expression and RNA synthesis*

from hepatitis C replicons propagated in human liver cells. Proceedings of the National Academy of Sciences, 100(5), 2783-2788. doi:10.1073/pnas.252758799

Young, K. A., Berry, M. L., Mahaffey, C. L., Saionz, J. R., Hawes, N. L., Chang, B., … Simpson, E. M. (2002). *Fierce: a new mouse deletion of Nr2e1; violent behaviour and ocular abnormalities are background-dependent*. Behavioural Brain Research, 132(2), 145–158.

Yu, R. T., Mckeown, M., Evans, R. M., & Umesono, K. (1994). *Relationship between Drosophila gap gene tailless and a vertebrate nuclear receptor Tlx*. Nature, 370(6488), 375-379. doi:10.1038/370375a0

AP® RESEARCH
2018 SCORING COMMENTARY

Academic Paper

Sample: B
Score: 5

The paper earned a score of 5 because it establishes a focused topic of inquiry, clearly indicating the relevance of its question (page 2, paragraphs 1–2). The paper also identifies a research gap within its superior review of literature (see page 2/3 "Genome wide …" and page 5, paragraph 2: "… there remains a significant gap …") that leads to the question on pages 5 and 6. The paper carefully identifies its conclusion via a presentation of its evidence (pages 11–14) while also noting limitations of its study, including possible explanations for the null hypothesis (pages 16–18). The writing is elegant: The student does an excellent job of making complex terminology accessible to nonexperts and in defining the meaning and import of statistical terms and data. The paper also uses visuals that enhance communication of the method (e.g., page 7).

The paper did not score a 4 because it clearly conveys an understanding of the importance of this study in relation to a larger context and carefully reflects upon its research process in order to promote further study.