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A Search for the Punction of Corazorim in Drosophila Mecanoguster

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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

Name: Sean C. Slusher

College: BCMB Arts & Sciences Department: BCMB

Faculty Mentor: Dr. Jae Park

PROJECT TITLE: A search for the Function of Corazonin in *Drosophila Melanogaster*

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: Jae Park, Faculty Mentor

Date: May 3, 2004

Comments (Optional):

A Search for the Function of Corazonin in *Drosophila melanogaster*

Sean Slusher

Senior Honors Project

May 3, 2004

Faculty Mentor: Dr. Jae Park

Abstract:

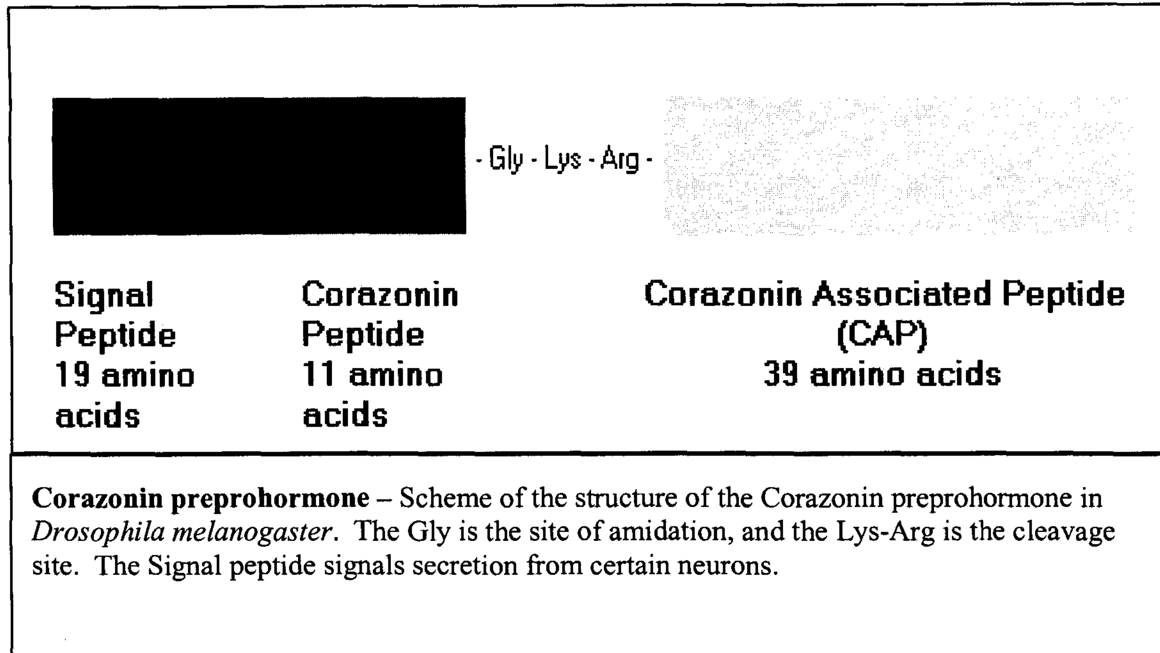
The function of the neuropeptide Corazonin in *Drosophila melanogaster* is currently unknown. Corazonin has been found in many different insect species, and the neuropeptide's amino acid sequence is highly conserved throughout these organisms. This high sequence homology would suggest that the function of the protein is important in these organisms and that the function is also highly conserved between them. However, current research has not supported this hypothesis. This project was an attempt to use previously developed tests and to develop new tests that would help elucidate the function of Corazonin in the model species *Drosophila melanogaster.* There were two types of tests used and developed. The first part of the project consisted of testing the larval motility of Corazonin-deficient, 3rd instar larvae. The second part of the project consisted of developing a feeding assay that could accurately measure the amount of food that adult flies were ingesting during a set-feeding period. Once developed and validated, this assay will allow for the testing of the feeding behavior of Corazonin-deficient flies as a further method of determining the neuropeptide's function.

Introduction:

To date, Corazonin has been found in a variety of different insect species in one of two isoforms. In 1989, [Arg7]-Corazonin was identified from the American Cockroach, *Periplaneta americana.* The structure was discovered to be Glp-Thr-Phe-Gln-Tyr-Ser-Arg-Gly-Trp-Thr-Asn-amide and the cardiopeptide was shown to affect heart rate, hence the name Corazonin from Spanish for heart (Veenstra, 1989). In 1991, it was also found in the cockroach *Nauphoeta cinerea*, the locust *Schistocerca americana*, and the hawkmoth *Manduca Sexta.* Veenstra noticed that the Corazonin in

the Schistocerca species was actually a different isoform called [His7]-corazonin. Its sequence was identical except that it had a histidine instead of an arginine in the seventh spot of its amino acid sequence. Neither [His7] or [Arg7] was shown to affect heart rate in these insects (Veenstra, 1991). Later, the Corazonin encoding gene was found and sequenced in *Drosophila melanogaster* (Veenstra, 1994). In 1999, [His7]-Corazonin was isolated as a dark-inducing peptide in the locust, *Locusta migratoria* (Tawfik *et. al.,* 1999). In 2000, [Arg7]-Corazonin was also found in the silkworm, *Bombyx mori,* and in a cricket, *Gryllus bimaculatus* (Hua et al., 2000). In 2001, it was found and sequenced in the waxmoth, *Galleria mellonella* (Hansen et al., 2001). In 2002, [Arg7]-Corazonin was shown, by injection, to affect the spinning rates of the silkworm, *Bombyx mori* (Tanaka et al., 2002). Due to colocalization with PER protein in brain neurons, Corazonin has also been suggested to have a possible role in circadian pacemaking (Wise et al., 2002). The combination of these findings suggests that Corazonin is a widespread neuropeptide throughout the insect world (Hansen et al., 2001). "Corazonin is produced by lateral brain neurosecretory cells projecting to the corpora cardiaca–corpora allata complex and in neurons of the ventral nerve cord." Spatial expression is conserved between species, and this, along with the sequence conservation, suggests that function should be conserved as well (Kim et al., 2004). Obviously, this has not yet been found.

In *Drosophila melanogaster,* the *Corazonin* gene has been sequenced and the structure of a preprohormone has been discovered (see figure on next page). A very similar preprohormone has also been discovered in the waxmoth, *Galleria mellonella.* The *Drosophila melanogaster* preprohormone consist of a 19 amino acid signal peptide, the 11 amino acid Corazonin sequence, a glycine residue that aids in amidation, a Lys-



Corazonin preprohormone – Scheme of the structure of the Corazonin preprohormone in *Drosophila melanogaster*. The Gly is the site of amidation, and the Lys-Arg is the cleavage site. The Signal peptide signals secretion from certain neurons.

Arg sequence that acts as a cleavage site, and a 39 amino acid Corazonin Precursor-related peptide (CPRP) (Veenstra, 1994). The waxmoth has the same 19 amino acid signal peptide, the same 11 amino acid Corazonin peptide, the same amidation and lysing sequences, but it has an eighty amino acid CPRP instead of 39 (Hansen et al., 2001). This is an example of the similarities of sequence between species.

In relation to expression in *Drosophila Melanogaster*, it has been shown (in unpublished data from Dr. Jae Park's Laboratory) that Corazonin is expressed in Drosophila through the 3rd instar larval stage of development. At this time, the Corazonin expressing neurons undergo apoptosis. This suggests that the hormone might have an effect on larval development. Therefore, in this project, I tested whether or not Corazonin deficiency affected larval motility. CCAP deficient flies were also tested in the same manner. This was done both as a control for the Corazonin experiments and for the attempted revelation of the function of CCAP as well.

The second section of this project concerned the development of a feeding assay in order to test all the neuropeptide deficient mutants in Dr. Park's laboratory, including Corazonin mutants. The assay that was developed was modified from the two-choice feeding assay proposed by Teiichi Tanimura in 1982 (Tanimura, 1982). The key to this part of the project was to develop an easy way to determine the amount of food that a population of flies ingests after a short starvation period. The system developed and its similarities to and differences from the Tanimura system are explained in the methods section. Developing a system was not as easy as expected because a large number of factors that affected feeding became apparent during development. Even though this made the development of a system of feeding much more difficult, it also created some interesting new research avenues to explore.

Methods:

Larval Motility

Larval Motility was tested using third-instar larvae of *Drosophila melanogaster.* Each test began with a single larva being placed in a petri dish. These petri dishes were 8.5 cm in diameter, and each dish had its bottom coated with a volume of 5-7 ml of 1% agarose. Graph paper with grids measuring 2.5 mm for the *Corazonin* tests and changed to 4.0 mm for the *CCAP* tests, to assist in ease of counting, was placed under the agarose-filled petri dish before the tests began. Each larva was tested for 5 minutes in total after being placed in the dish with a count taken every minute of the number of times the posterior end of the larva had crossed through a grid. Five larvae were tested, one after another, on a petri dish before that dish was discarded and a new dish was made. Fifteen larvae of each genotype were tested. Due to a low humidity in the lab during the *CCAP*

test and a perceived possible sensitivity of the larva to that low humidity, those tests were performed with the petri dishes located in a sealable, transparent plastic box, containing a wet Kim-wipe in order to maintain a high and consistent humidity. The humidity and temperature in the room for the Corazonin tests and in the box for the *CCAP* tests was measured during each run. Tests were stopped and the data discarded if the larva crawled onto the roof of the petri dish or if the larva burrowed itself into the agarose layer in the bottom of the petri dish.

Scribbler Quantification

Similar procedures were used in the attempt to quantify the scribbler behavior of the Corazonin Knockout larvae. Petri dishes were prepared in the same manner as the motility tests (i.e. 7 ml of 1% agarose in an 8.5 cm petri dish). Three 3rd instar larvae were placed on one petri dish at a time and allowed to move around for a five-minute time interval. The three larvae were then removed after the five minutes, and their tracts left on the agarose were examined. The larva were scored as having either scribbler, mild scribbler, or normal movement (see figure 2). The number of larva tested for each genotype depended on the number of larva available at the time.

Feeding Assay

The feeding assay developed was a modified version of the two-choice feeding assay proposed by Tanimura et al. (1982). In his method, he used a micro-well plate to give flies the choice of two different foods or a choice of the same food at two different concentrations. Each of the different foods or concentrations had a different color dye added to it. An example of such a set up is having red dye added to a 5% solution of sucrose while adding blue dye to a 10% solution of sucrose. The two dyes that Tanimura

used were sulforhodamine B (acid red) and erioglaucine sodium salt (brilliant blue FCF). He added the two (dye/food) solutions in a checkerboard fashion among the wells in increments of 10 μl per well. Each of these solutions contained 0.5 % agarose for solidification purposes. He fed 50 flies 0.1 M sucrose for 2 hours prior to a 20-hour starvation period when only water was provided. The 50 starved flies were then fed on the micro-plate in total darkness for two hours. They were then killed in a –20° freezer. The food that the flies ate during the 2-hour feeding period was visible through their bodies. A count was then made of each of the flies' colors. The flies were then added to Potassium Phosphate buffer, in 50% ethanol, homogenized and centrifuged. The absorbance of the resulting supernatants was taken using a photo-spectrometer reading at 565 nm for the red dye and at 630 nm for the blue dye. This allowed for the quantification how much of each food each population ate and what preference they had between the foods.

I made many modifications to this procedure. First and foremost, because I was only interested in determining the amount of food eaten during the starvation tests and not preference, I limited my test to only one dye and one type of food. In attempts to replicate Tanimura's method, I had encountered difficulties during many of the stages of the procedure. This led to many of the changes that eventually were made. The number of flies used per dish was reduced to 25. Also, only male flies were used. I disliked the use of the micro-well plate because it seemed to limit the mobility of the flies and was difficult and time-consuming to prepare. I also disliked the use of the agarose-based food because it was much too difficult to prepare as well. Therefore, I decided to not use the micro-well plates or agarose-based food for my method. I replaced the micro-well plates

with 8.5 cm petri dishes and replaced the agarose-based food with a yeast paste. Due to difficulty in seeing small amounts of the blue dye in fly abdomens, I chose the red dye as a feeding marker. Trial and error led to the following composition of food for the tests: 1 mg/ml acid red dye, 0.56 g/ml of yeast dust, 5% sucrose. A square piece of Parafilm about 3 cm by 3 cm was placed in the bottom of each petri dish. A drop of food was then added onto the top of this, and a second and smaller piece of Parafilm was then placed on top of this drop of food and squashed down until the food was pushed out from its edges. This was done in order both to increase the number of locations from which the tested flies could eat and to prevent the tested flies from becoming stuck in the food when placed in the petri dishes. I learned that it was unwise to use CO_2 for the transfer of test flies from the starvation vials to the petri dishes, since the starved flies are much too weak and may not wake up from CO_2-mediated anesthesia. As an alternative, I used an aspirator for this transfer. Because it was much more difficult to transfer 50 flies using this method, I decided to use only 25. I also found that pre-feeding the test flies with 0.1 M sucrose was likely to cause more damage than good. Many of the flies would be killed in the process of feeding by either sticking to the paper on which the sucrose was added or not eating it at all. Not eating during this stage prolonged the starvation period and prevented some of them from surviving until the feeding period. This sucrose feeding stage was replaced with a procedure in which the test flies are added to a food vial with extra yeast dust the night before. The feeding period itself was modified from 2 hours to 1.5 hours, since 2 hours could be so long that food was being excreted before the end of the test. The only change that was made in the homogenization process was to change buffers from Potassium Phosphate, in 50% ethanol to 1X PBS, containing 50 % ethanol.

This change did not seem to affect the absorbance readings. The length of the starvation period was also varied, but because a final decision on this portion of the experiment has not yet been made, it will be discussed in greater detail in the results and conclusion sections.

Results:

Larval motility (Corazonin / *scribbler* behavior):

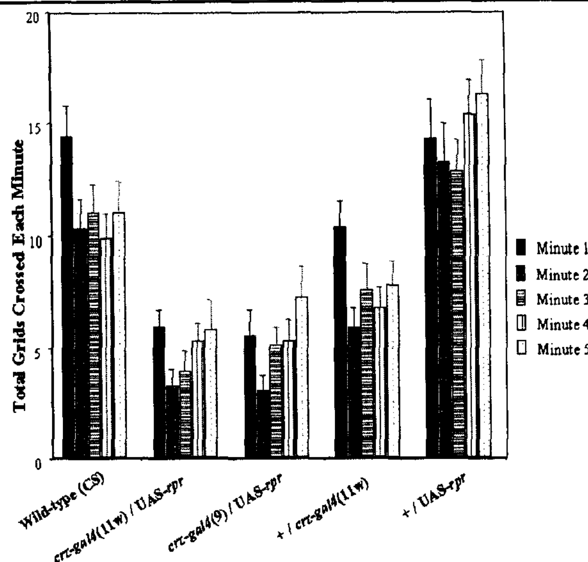
The genotypes tested during the Corazonin larval motility tests were UAS-*rpr X crz-gal4*(11w), *UAS-rpr X crz-gal4*(9), UAS-*rpr X CS*, CS X *crz-gal4*(11w), and wild type (CS). Crossing virgin female flies of the first genotype to male flies of the second genotype led to these offspring. The UAS-*rpr X crz-gal4*(11w) and *UAS-rpr X crz-gal4*(9) larva had the neuropeptide Corazonin knocked out through cell ablation. This was done through Gal4 driven production of Reaper in Corazonin expressing cells. Reaper is a protein that induces apoptosis. Therefore, all cells that produce Corazonin in these larva undergo cell death. UAS-*rpr X* CS, CS *X crz-gal4*(11w), and wild type (CS) were used as controls. The first two were heterozygous controls used to show whether there was any genetic background effect caused by the insertion of the expression system.

As a result, larval motility was found to be significantly lower for Corazonin cell-ablation larvae (the knockouts) when compared to the motility of both wild type (CS) and genetic control larvae (See figure 1). UAS-*rpr X crz-gal4*(11w) and *UAS-rpr X crz-gal4*(9) averaged 24.53 and 26.47 grids crossed, respectively, during their 5-minute tests. This was much lower than wild type (CS) larvae, which averaged 56.8 and 72.13 grids crossed respectively in their 5-minute runs. It was noticed that the lower larval motility seen in the Corazonin cell-deficient larvae was caused by a greater frequency of stopping

and changing directions. This behavior is similar to a phenotype for a Drosophila gene called *scribbler (sbb)* described by Yang et al. (2000). Wild type and control larvae seemed to generally move in continuous straight lines across the agar surface, while the knockout larvae moved with a tract that resembled a scribble in the agar (figure 2). The penetrance of the scribbler phenotype is shown in figure 3. The data seems to suggest a large difference between the cell-ablated larva and the controls in terms of scribbler

Figure 1a – *Corazonin* Larval Motility Results

Each bar shows the average number of grids crossed during that minute (n=15) for that particular genotype. Note that motility was significantly lower in the two *Corazonin* knockout lines as compared to the motility of wild type (CS) and heterozygous genetic controls.



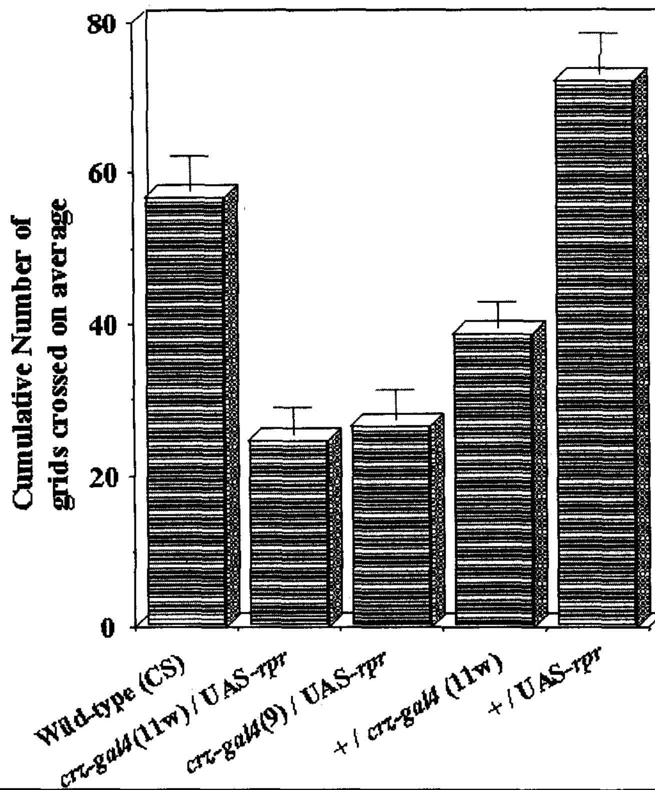
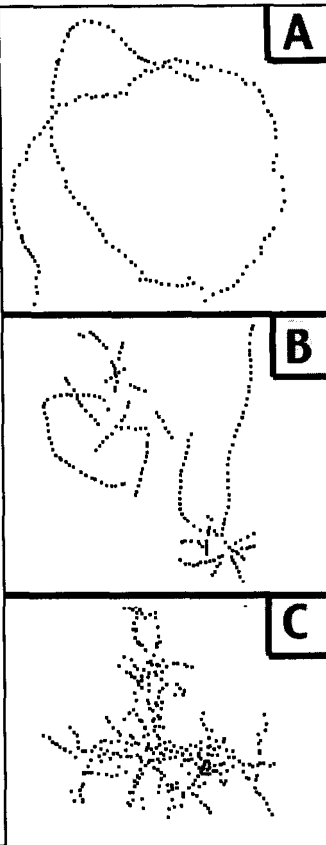


Figure 1b – Cumulative Results

Each bar shows the average number of grids crossed during the entire five minute run for that particular genotype (n=15). The difference between genotypes, moreover the lower motilities for the knockout lines, is again clear as it was in figure 1a.

Figure 2 – Scribbler Phenotype defined
A, B, and C are outlines of larval tracts left on agar plates after a five-minute run by a single larva. The different phenotypes are described as follows:
A.) Normal phenotype - Notice there are no sudden changes in direction. Movement is constant and straight.
B.) Mild Scribbler Phenotype – While there are large sections when the larva moved in a normal manner, there are intermittent spans of sudden changes in direction.
C.) Scribbler Phenotype – There are few if any stretches of straight, normal movement. Stoppages and directional changes are frequent which greatly reduces distance traveled.



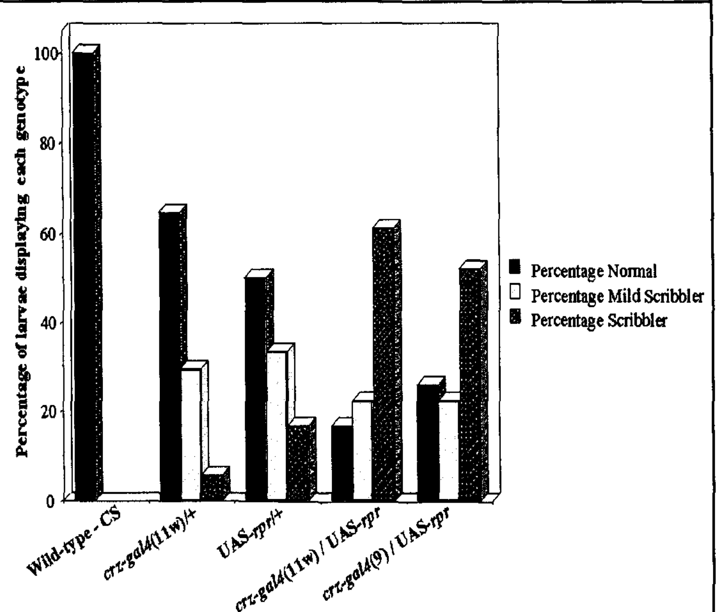


Figure 3 – Preliminary Scribbler Data
This is preliminary data from tests that used the same agar dishes as in the motility test in order to visually determine which type of larval tract (see figure 2) each genotype predominately left.

behavior, and therefore, it suggests the *Crz* gene has a role for the larval locomotion. However, there were problems associated with this test that have not yet been solved. Only data from the first attempt of this test is shown because subsequent attempts at the test yielded similar results. The only difference noticed was a slightly higher percentage of mild scribblers in the wildtype larva. During this first test, the humidity in the room was low. At the time, it was assumed that this caused the high percentage of scribbler behavior in the horizontal controls of the test. This led to the use of the humidity box mentioned in the "methods" section above in the subsequent tests. However, the

humidity box did not prevent the high percentage of scribbler behavior in the horizontal controls. Why the horizontal controls have a higher value of scribbler behavior than the wildtype larva is still unknown. It is possible that there was a genetic background effect from the expression system causing thist phenotype; therefore, it was decided to test another neuropeptide, CCAP, in order to determine whether the expression system was the cause.

Larval Motility (CCAP):

The genotypes used for this tests were UAS-*rpr* X *ccap-gal4*(16), UAS-*rpr* X *ccap-gal4*(18), UAS-*rpr* X *yw*, *yw* X *ccap-gal4*(18), and *yw*. The UAS-*rpr* X *ccap-gal4*(16), UAS-*rpr* X *ccap-gal4*(18) larva were devoid of CCAP-expressing neurons. Larval motility for CCAP cell-ablation larvae was normal, if not

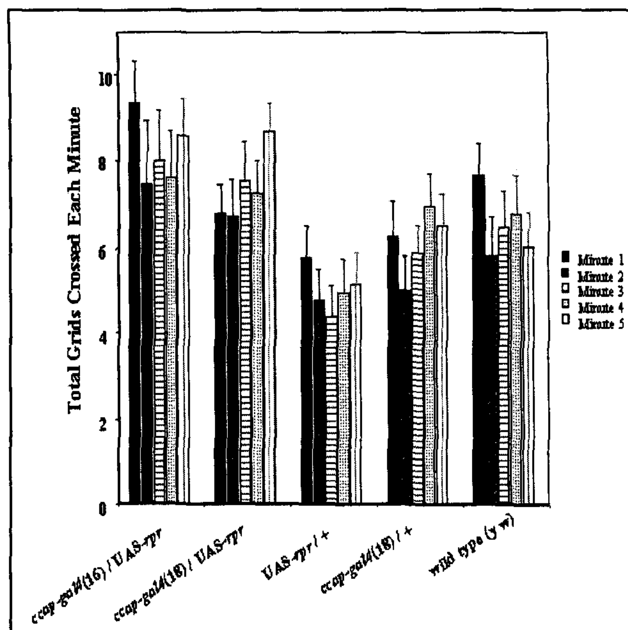


Figure 4a – By Minute
Each bar shows the average number of grids crossed that minute (n=15) for that particular genotype of larvae. Note that motility was slightly higher in the two *CCAP* knockout lines as compared to the motility of wild type (*y w*) and heterozygous genetic controls. This difference is not as significant as before with *Corazonin* (figure 1).

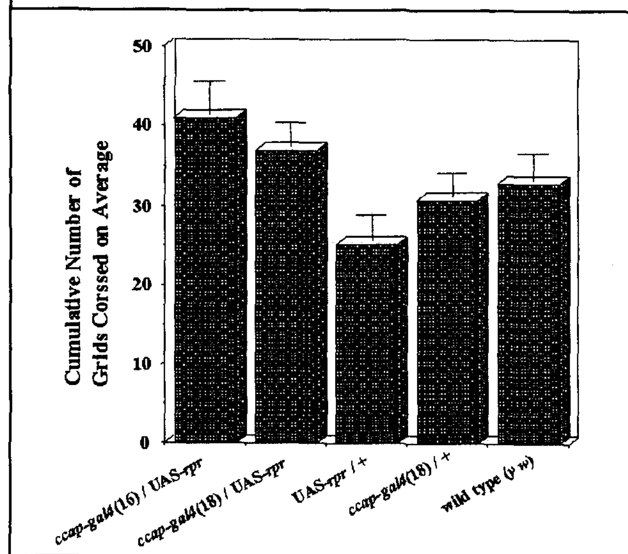


Figure 4b – Cumulative Results
Each bar shows the average number of grids crossed during the entire five minute run for that particular genotype (n=15). The difference between genotypes, moreover the lower motilities for the knockout lines, is again clear as it was in figure 4a.

hyperactive, when compared to the larval motility for wild type and genetic controls (see figures 4a and 4b). This suggests that the lower motility of the Corazonin cell-ablation larvae results from the lack of *corazonin* expression in select larval neurons. Unlike during the previous tests using Corazonin cell-ablation larvae, the differences between the knockout and wild type / genetic controls in this experiment did not seem to be caused by the presence of the scribbler phenotype. CCAP cell-ablation larvae were just moving faster than the control larvae. The presence of possible hyperactivity in the larval motility of CCAP cell-ablation larvae was surprising. Previous observation of CCAP cell-ablation pupa indicated possible faster heartbeats (unpublished data from Dr. Park's lab), but how this could be connected to the possible hyperactivity in third-instar larvae is unknown.

Feeding Assay:

The feeding assays began with a plan to validate various aspects of the developed test. The first aspect to validate was whether the presence of dye in the food made a significant difference in absorption values from any background readings that flies would give after being fed food not containing the dye. The results of this test are shown in Table 1 under Test 1. Flies that ate food that included dye had a significantly higher absorption value than those flies that ate the same type of food only absent the dye. This test showed that *yw* flies give a small background effect, but it is not great enough to prevent seeing which flies ate the food with the dye and absorbance readings.

The second aspect of the test to validate was whether the starvation effect was present. To answer this question, flies that were fed up until the test feeding time had

their absorbances compared to flies that were starved for 20 hours before the test feeding time. The results of this test are also in Table 1 below, and they were significant as well.

TABLE 1

Feeding Assay - Dye and Starvation Tests	Type of Population of *yw*	Absorbance reading (1 mL buffer - 20 flies) @ 565 nm
TEST 1	Dyed #1	0.143
Dye present in food versus	Dyed #2	0.131
no dye present in food	No dye #1	0.026
Both sets starved	No dye #2	0.047
TEST 2	Starved #1	0.130
Starved flies versus	Not Starved	0.023
flies not starved	Not Starved	0.060
(All food contained dye)	Not Starved	0.040

The third aspect of this validation process was to see if genetic background was significant. To test this, I compared *yw* (fed) flies versus CS (fed) flies. The results of this test are listed in Table 2 (page 15). There was a significant difference between the two. The most likely cause of this is the higher amount of pigment in CS flies. The CS flies must have a higher background absorbance reading. This means that data cannot be directly compared between flies of different backgrounds, i.e. *y+ versus yw* flies. There is a possibility that this background effect could be subtracted out by testing them without dye, but this would increase the error present in the test readings and is undesirable.

When trying to repeat this past test with *yw* (starved) versus CS (starved) flies, odd things happened. A large percentage of the flies did not eat and were not colored.

This was unusual in comparison to earlier attempts. The only difference in procedure was the addition of more water in the vials in which the flies were starved in the form of

Table 2

Feeding Assay – CS (fed) versus *yw* (fed)	Absorbance reading (10 flies - 0.5 ml buffer) @ 565 nm
***yw* (fed) #1**	0.016
***yw* (fed) #2**	0.018
***yw* (fed) #3**	0.021
***yw* (fed) #4**	0.020
CS (fed) #1	0.040
CS (fed) #2	0.066
CS (fed) #3	0.040
CS (fed) #4	0.045

an agarose coating on the bottom of the vial and a wet cotton plug. The absorbances of these flies were not measured, but a new phenomenon was noticed. It seemed that the larger flies had eaten and the smaller flies had not. In order to quantify this observation, the flies were scored on size and on the amount of food that they ingested, determined by the amount of dye visible through their bodies. The results of this test are in Table 3.

Table 3

Genotype	Size Group	Size Distribution		Feeding Status	
			Full	*Partial*	*None*
CS (starved)	Large	10 %	100 %	0 %	0 %
n = 51	Medium	59 %	43 %	17 %	40 %
	Small	31 %	0 %	13 %	87 %
yw* (starved) *n=40	Medium	100 %	27.5 %	17.5 %	55 %

There were two hypotheses developed to explain these results. One possibility is that the flies are acting in a territorial manner. The larger flies are fighting the smaller flies to keep them away from the food. Territorial behavior has been observed in *Drosophila melanogaster*. The second hypothesis is that the smaller flies, created by being raised in an over-crowed vial, are more resistant to starvation and do not need to eat as much. This would be surprising as well because it would be logical to think that the larger the fly, the more food and energy that can be stored in preparation for starvation. Which hypothesis is correct will be determined through further testing. Flies of specific size can be breed and tested to determine if size affects durability to starvation, and the territorial behavior can be tested by observing and recording the flies during one of the tests to see if the larger flies are fighting off the smaller flies.

The size differential was not the only odd thing noticed during these tests. The tests performed up till this time had all shown the importance of having water present in the vials during starvation. At first, not enough water had been present, and many of the flies had died due to desiccation. In order to prevent these deaths, more and more water was added during subsequent tests. It was noticed that the flies no longer ate as much food when provided with this larger amount of water. This shows that there are two effects at play here if one is not careful. If enough water is not added, a thirst effect causes the flies to eat in order to get liquid nourishment. When water is added in ample amounts, a hunger effect takes over. When testing future flies, it will be important to separate these two effects. They can be separated either by adding ample water and extending the starvation period to see the hunger effect or by adding no water and shorting the starvation time to see the thirst effect. A test was run to determine whether

these two effects could be separated and quantified both by counting the percentage of flies that ate and by running the absorbance of the fly homogenate. The results of this test are in Table 4 below and figure 5 on the next page. Flies were starved 4 hours.

Table 4

Dry Versus Wet conditions *Genotype*	1st Trial *Total n*	*# Eating*	*# No food*	*% Eating*	*% No food*
yw (dry) #1	24	16	8		
yw (dry) #2	19	13	6		
yw (dry) #3	15	9	6		
yw (dry) #4	24	20	4		
TOTAL	**82**	**58**	**24**	**70.7**	**29.3**
yw (wet) #1	25	7	18		
yw (wet) #2	23	4	19		
yw (wet) #3	18	4	14		
yw (wet) #4	20	6	14		
TOTAL	**89**	**24**	**65**	**27.0**	**73**
Dry Versus Wet conditions *Genotype*	**2nd Trial** *Total n*	*# Eating*	*# No food*	*% Eating*	*% No food*
yw (wet) #1	25	21	4		
yw (wet) #2	20	15	5		
yw (wet) #3	18	8	10		
TOTAL	**63**	**44**	**19**	**69.8**	**30.2**
yw (dry) #1	25	20	5		
yw (dry) #2	*16*	16	0		
yw (dry) #3	*25*	24	1		
TOTAL	**66**	**60**	**6**	**90.9**	**9.1**

The results between trial 1 and trial 2 are slightly different. The flies during the second trial ate more, but overall the difference was still evident from the absorption values present in the figure. Natural variations between populations and a slightly low concentration of dye in the first trial explain any differences. The absorption values are higher for trial 2 because for the previous reasons and because only flies that ate any food were homogenized. This shows that even though the percentages became closer in the second trail the difference in the amount of food eaten did not change.

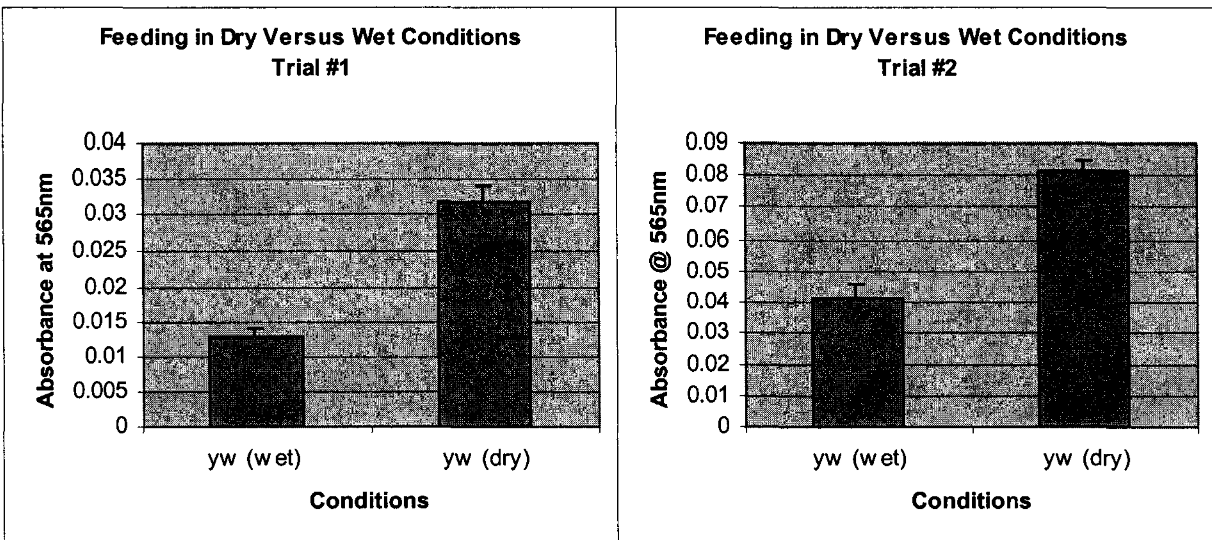


Figure 5 – Absorbance readings from trial 1 and 2 of the dry versus wet conditions tests.
These absorbance were taken at 565 nm. 10 flies / .5 ml of buffer. In trial 1, 80 flies were tested for each type. For trial 2, 40 flies were tested for the wet conditions and 60 for the dry conditions.

Conclusion / Future Works:

Larval Motility Tests

Overall, Corazonin cell-ablation larvae were found to have a significantly lower overall larval motility. This was seemingly caused in part or in its entirety by an increased frequency of scribbler behavior. More tests are needed to confirm and quantify this as the cause of the lower motility, but the initial indications are strong. Before these additional tests, however, new tests need to be run to determine how sensitive the larva are to changes in humidity and the moistness of the agarose. I expect them to be quite sensitive to such changes. Therefore, a test needs to be developed in which these conditions are kept more stable. In most organisms, behavior can change based on the conditions of the environment. For example, humans become irritable in intense heat. Changes in humidity or moistness of the agarose layer could induce the scribbler phenotype. It was noticed through non-scientific observation that Wild type

larva in dry conditions showed some scribbler behavior. Normal behavior was recovered in larva from the same population after a more moist and humid plate was made. The fact that Corazonin-cell ablated larva still showed, and have shown consistently before, the same phenotype, almost all scribbler, in no matter what conditions of moistness seems to indicate that Corazonin might play a role in the detection of either the humidity or the moistness of the medium on which the larva moves. If these new tests are successful in quantifying the scribbler behavior, then there are many tests that can be run in the future to further investigate this hypothesis. Through genetic techniques, *corazonin* can be over-expressed in larva. Similar techniques can force *corazonin* expression in adult flies where it is normally not present. Ectopic expression of *corazonin* in other neurons can also be forced. These flies will be used to further explore the effect of the *corazonin* gene on motility, locomotion behavior of adults, and development.

Developing Feeding assay

Once it is determined whether or not feeding is affected by size or territorial features, once the proper time for starvation is set, and once the background issue is taken care of by putting all of the knockout flies into the same genetic background, this test will be ready to use on all of the knockout flies in Dr. Parks laboratory, including Corazonin. Although nothing can quite be concluded about the issues this new feeding assay has brought to light, the fact that they are present is exciting in its own right. Hopefully, these issues can be solved and anyone will be able to use the methods elucidated to accurately measure feeding behavior.

Notes:

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