AN ABSTRACT OF THE THESIS OF

Whitney M. Humphrey for the degree of Honors Baccalaureate of Science in Biology presented on June 3, 2010. Title: The Interaction Between the *fruitless* Gene and Complex Behavior in *Drosophila*

Abstract approved: _____
Barbara J. Taylor

CantonS wild-type flies were used to determine sex-specific differences in the daily locomotor activity patterns in males and females. Under light entrainment, when flies are kept in a 12:12 light-dark condition, both males and females showed bimodal activity with a morning peak around lights-on and an evening peak around lights off. In addition, distinct sex-specific characteristics of the activity pattern were found as had been reported in previous studies. Males were much less active in the middle of the day and had greater level of activity in anticipation of lights on compared to female flies. Females had a greater overall level of activity that was due to being more active during the day (lights on period). In order to determine if the genes involved in sex-determination were responsible for the sex-specific differences in activity, a total of thirteen genotypes of male *fruitless* mutants, two genotypes of female *fruitless* mutants, and six genotypes of female-masculinized mutants were analyzed for their locomotor activity. Males that were mutant for the male-specific products of *fru*, were found to have an overall decrease in activity levels and to have less anticipation of lights on whereas females genetically masculinized for Dsx^M and Fru^M did not show male specific activity patterns.

Key words: sex-specific behaviors, activity levels, chaining, *doublesex*, *fruitless*

Corresponding email: humphrwh@onid.orst.edu

The Interaction Between the *fruitless* Gene and Complex Behavior in *Drosophila*

by

Whitney M. Humphrey

A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology (Honors Scholar)

Presented June 3, 2010
Commencement June 2010

Honors Baccalaureate of Science in Biology project of Whitney M. Humphrey presented on June 3, 2010.

APPROVED:

_____

Mentor, representing Zoology

_____

Committee Member, representing Zoology

_____

Committee Member, representing Veterinary Medicine

_____

Chair, Department of Zoology

_____

Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Whitney M. Humphrey, Author

ACKNOWLEDGEMENT

I would like to Dr. Jaga Giebultowicz for allowing me to use her lab's Drosophila Activity Monitor. I thank Eileen Chow from the Giebultowicz lab for technical assistance and assisting me in data collection for these experiments and the other members of the Giebultowicz laboratory. I would also like to thank Dr. Kevin Ahern. I thank Will Gibson from the Taylor laboratory for his assistance in my experiments. Lastly, I am grateful for my mentor, Dr. Barbara Taylor for all her time, guidance and support over this last year. This work was supported by a summer Fellowship grant from HHMI and NIH808515 GM to BJT.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

The Interaction Between the *fruitless* Gene and Complex Behavior in *Drosophila*

INTRODUCTION

A captivating aspect of neurogenetics is the study of complex behaviors and the genes that control them. The *fruitless (fru)* gene is an example of an individual gene controlling or greatly influencing known male-specific complex behaviors associated with reproduction, such as aggression and courtship (Baker et al. 2001). A single gene, like *fru,* must exert its effect by interacting with other genes to produce changes in the way neurons develop and connect to other cells and/or function. The male-specific functions of *fru* have been shown to depend on the expression of male-specific protein isoforms of *fru* (Fru^M) in only in a small subset of neurons in males (Manoli et al. 2005). Males and females express other behaviors, such as walking or feeding, that may also show sex-specific differences.

Thus, it may be that *fru* has a role in many more complex behaviors, including a behavior that it has never been associated with in the past, locomotor activity. The daily activity cycle, which includes periods of rest or sleep and activity, is very important for the health of *Drosophila*, and the same is trur for other organisms, even humans (Harbison and Seghal 2008).

Drosophila is an ideal model organism to study these questions for several reasons. First, flies have extremely short generation times – about 10 days. This allows the study of many generations in a short period of time. Second, many strains and mutants exist that can test the roles of genes by loss of function and gain of function genotypes.

1.1 Sex-specific differences in *Drosophila* sleep and activity cycles

Flies like all other organisms have a regular pattern of activity and periods of rest during a twenty-four hour or circadian cycle. When the activity of individual flies is measured under conditions of a 12h light and 12h of dark (12:12 LD) cycle, both male and female flies have a bimodal activity pattern with the first peak of activity occurring around lights on (the morning peak) and a second more pronounced peak around lights off (the evening peak) (e.g. Helfrich-Förster 2000).

Thus far, few candidate genes that alter the rest and activity phenotypes in flies have been identified. The genes that control the molecular mechanism of the circadian clock have been found to be very important for the morning and evening peaks of activity as shown by the loss of elements of the activity rhythms in *period*, *timeless*, *cycle* and *clock* gene mutants (Helfrich-Forster, 2001; Grima et al, 2004; Stoleru et al 2004). The expression of these clock genes is needed in certain subsets of lateral clock neurons for the morning and evening peaks of activity. For example, flies that have a mutation in the molecular circadian clock genes *cycle* and *clock* sleep less in a 12h:12hr LD cycle (Hendricks et al. 2003). In addition, certain neurotransmitters have been found to promote either sleeping or waking. Serotonin promotes sleep through the d5-HT1A receptor, while increased dopamine lowers the arousal threshold and promotes waking (Kume et al. 2005; Yuan et al. 2006). Alterations in cAMP signaling in a specific region of the fly brain affect sleep duration (Hendricks et al. 2001).

Although the bimodal pattern of rest and activity is very similar in males and females, distinct sex-specific characteristics in the activity level of male and female flies have

been described. First, male flies are active significantly earlier than females before lights on, which is interpreted as evidence that they anticipate lights on (Helfrich-Förster 2000). Second, males are much less active during the middle of the day until the rise in activity preceding the lights off peak of activity in the late afternoon (Helfrich-Förster 2000). This period of little to no activity male flies display has been referred to as a 'siesta'. Few mutations are known that alter the sex-specific phenotypes of the rest-activity cycle. However, heterozygous null mutations in the *Relish* gene were found to reduce sleep during the day and night in females but only night sleep in males (Williams et al 2007).

1.2 *fruitless* Gene in Sex Determination Hierarchy

A well-known genetic cascade controls the development of sex differences in Drosophila (Sosnowski et al. 1989). Two genes, *fru* and *doublesex* (*dsx*) are found near the bottom of the sex determination hierarchy while the *Sex-lethal* gene is at the top of the pathway. *Sex-lethal* is only functional in females and acts as a splicing regulator that controls the female-specific expression of the *transformer* (*tra*) primary transcript into the mature mRNA, which can be translated into a functional Tra (Tra^F) protein, itself a splicing regulator. Tra^F can now interact with the Transformer-2 protein, another splicing regulator. Together, Tra^F and Tra-2 regulate the splicing of the *dsx* transcript into the female *dsx* mRNA, which is translated into the female-specific Dsx^F protein. Since *Sex lethal* does not function in males, these mRNAs remain unspliced and, in turn, RNAs downstream also remain unspliced (Sosnowski et al.1989). Therefore, if Tra^F proteins are present, Dsx^F dependent female developmental fate ensues. However, if Tra^F proteins are absent, a Dsx^M dependent male developmental fate occurs. A subset of transcripts from the *fru* gene are also spliced by Tra^F/Tra-2 in females, resulting in *fru* mRNAs that

are not translated; in males, the splicing reaction results in *fru* mRNAs that can be translated into male specific forms of Fru protein, Fru^M. Thus, the absence of Tra^F in males leads to the default splicing of both *dsx* and *fru* RNAs and to the production of male-specific Dsx^M and Fru^M proteins.

1.3 Courtship and Chaining Behavior in *fru* and *dsx* mutants

The sex-specific roles of the *fru* and *dsx* genes have been determined by examining the behavioral phenotypes of male and female mutant flies (Villella and Hall 1996). There is a range of loss-of-function mutations that have been used to study sex-specific phenotypes. The first *fru* allele to be discovered was called fru^1 and these male flies court both male and female flies, fail to produce a normal courtship song and do not copulate with females. These mutant flies that actively court male flies form long chains of courting male flies, a behavior known as chaining. The *fru* mutants fru^3 and fru^4 are very strong loss-of-function alleles that show a variety of male-specific behavioral defects, such as reduced courtship of females, no production of a component of the courtship song and display, and significantly less chaining behavior than fru^1 males (Anand et al 2001). The hierarchy of courtship responses by *fru* males directed toward females versus males, when presented with both sexes simultaneously, is that fru^1 males perform vigorous and indiscriminant courtship directed at either sex; fru^4 males court in a similarly gender indiscriminant fashion, but have courtship levels lower than fru^1 males; fru^3 males show a courtship bias toward males; and fru^3 and fru^4 males essentially lack a sex-specific muscle, the Muscle of Lawrence (MOL). To remove all of the male-specific *fru* function, it is necessary to combine two types of deficiency chromosomes, *Df(3R)*

fru^{440} and *Df(3R) fru*^{sat15} or *Df(3R) P14* (Anand et al. 2001). In these *fru* deficiency genotypes, no male-specific Fru^{M} protein is made. Even though these mutations cause loss of male-specific behaviors, *fru* mutant females are fertile and have normal female-specific behaviors (Anand et al. 2001).

In females, only Dsx^{F} acts to direct female-specific functions from the sex-determination hierarchy. It has been shown that females can be masculinized by misexpressing Dsx^{M} and Fru^{M} proteins. Females that have only a gain-of-function alleles of *dsx*, XX; *dsx*^{M}/*dsx*^{1}, are masculinized for Dsx^{M} dependent features and so are physically transformed into a male fly (Baker and Ridge 1980). Females that mis-express Fru^{M} transgenes under the control of a GAL4 driver inserted into the *fru* gene (*fruGAL4*; *UAS-fru*^{M}) are partly masculinized for Fru^{M} behavioral phenotypes and have been shown to court females (Manoli et al. 2005).

1.4 Other sex-specific behaviors in Drosophila influenced by *fruitless*

It has been shown that the *fruitless* gene influences the sexually dimorphic behavioral patterns (modules) in aggression in *Drosophila* (Lee and Hall, 2000; Nilsen et al. 2004). Certain components of aggression like "fencing," "approach," and "retreat" occur during fights by both sexes. However, there are other behaviors that appear to be sex-specific. For example, males perform "lunges" during fights where they raise up on their hind legs and snap down on the opponent. Females perform more horizontal shoves and "headbutt" movements during fights. A tight correspondence was seen between Fru^{M} expression in several subgroups of neurons usually expressing these proteins and the way flies fight. In general, it was found that when expression of Fru^{M} was absent,

male flies fought like females and Fru^M expression was present when flies fought like males (Chan and Kravitz 2007).

1.5 Thesis Statement and Hypothesis:

If Fru^M is responsible for the male activity pattern, then removing *fru* function should result in the loss of male pattern and perhaps the gain of the female pattern of activity in male flies. With similar logic, mis-expressing Fru^M should cause a gain of male-specific activity patterns in females. The research conducted for my thesis has broad implications for the understanding of the interaction between the central nervous system and behavior. It is known that there are substantial physical, personality, and behavioral consequences of mutating a single gene in both *Drosophila* and humans. The genes that will be studied in this project have counterparts in the human genome that are not well understood. By gaining a better understand of these genes in *Drosophila*, a better understanding of the human genome could be achieved.

MATERIALS AND METHODS

2.1 Fly Strains and Crosses

Wild-type *CantonS* (CS) flies and twenty *fru* mutant genotypes (Table 2.1) were used to measure the locomotor patterns of adult *Drosophila*. Three third chromosome balancers were used in our crosses. The first was *In(3LR)TM3*, marked with the dominant mutation *Stubble* (Sb). The second was *In(3LR)TM6*, marked with the dominant mutation *Tubby* (*Tb*). The third was *In(3LR)MKRS* also marked with the dominant mutation *Stubble*. Because these balancer chromosomes have visible dominant alleles and are homozygous lethal, they can be used to ensure the correct genotype is generated and can be selected for analysis.

All stocks were maintained at 25°C in 12h:12h light-dark (LD) cycles. The flies were raised on a sucrose-cornmeal-yeast medium with added mold inhibitor (Methyl Paraben, Sigma-Aldrich). Adults were cleared from vials every five days to avoid overcrowding (which prevents small flies that could behave differently). Virgin males and females were collected from the stocks three times during the day: once immediately after lights on, once in the middle of the day, and once an hour before lights off under light carbon-dioxide anesthesia. These virgins were kept in groups, separated by genotype and sex. Two to three days later, these virgins were crossed with another genotype (maintained in the same conditions) to produce the desired progeny. For example, a cross between and male $fru^{3}/TM3$ and a female $fru^{4}/TM6$ flies will result in fru^{3}/fru^{4} mutant flies.

Table 2.1: Parents and progeny of mutants used in this study.

Crosses:		**Progeny**:
***fru* hypmorphs:**		
fru^{1}/TM6	*fru^{1}/TM6*	*fru^{1}/fru^{1}*
fru^{4}/TM6	*fru^{4}/TM6*	*fru^{4}/fru^{4}*
***fru* deficiencies:**		
fru^{sat15}/TM3	*fru^{440}/TM3*	*fru^{sat15}/fru^{440}*
P14/TM6	*fru^{440}/TM3*	*P14/fru^{440}*
fru^{MIR} mutants:		
fruGAL4/fruGAL4	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (I*)*	*fruGAL4/UAS Fru^{MIR}, UASGAL4; I*
fruGAL4/fruGAL4	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (II*)*	*fruGAL4/UAS Fru^{MIR}, UASGAL4; II*
fruGAL4/fruGAL4	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; 0.1/0.1 (III*)*	*fruGAL4/UAS Fru^{MIR}, UASGAL4; III*
w/+; fru16GAL4 (19A)	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (I)*	*Fru16GAL4/UAS Fru^{MIR}, UASGAL4; I*
w/+; fru16GAL4 (19A)	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (II)*	*Fru16GAL4/UAS Fru^{MIR}, UASGAL4; II*
w/+; fru16GAL4 (19A)	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (III)*	*Fru16GAL4/UAS Fru^{MIR}, UASGAL4; III*
Sp/CyO; fruP1GAL4/MKRS	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; UAS Fru^{MIR} 0.1/ UAS Fru^{MIR} 0.1 (I)*	*FruP1GAL4/UAS Fru^{MIR}, UASGAL4; I*
Sp/CyO; fruP1GAL4/MKRS	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; 0.1/0.1 (II)*	*FruP1GAL4/UAS Fru^{MIR}, UASGAL4; II*
Sp/CyO; fruP1GAL4/MKRS	*UAS Fru^{MIR} 6.2,UASGAL4/CyO; 0.1/0.1 (III)*	*FruP1GAL4/UAS Fru^{MIR}, UASGAL4; III*

Table 2.1 (continued): Parents and progeny of mutants used in this study.

***fru*-masculinized females:**		
fruGAL4/fruGAL4	*UAS fru^{M}; Df(3R)fru^{AJ96u3}/TM3, ftz-Lacz*	*fruGAL4/UAS fru^{M}; Df (3R) fru^{AJ96u3}*
fruGAL4/fruGAL4	*UAS fru^{M}; Df(3R) fru^{sat15}/TM6*	*fruGAL4/UAS fru^{M}; Df (3R)fru^{sat15}*
fruGAL4/fruGAL4	*UAS fru^{M}; In(3R)fru^{440}/TM6*	*fruGAL4/UAS fru^{M}; Df (3R)fru^{440}*
fruGAL4/fruGAL4	*UAS fru^{M}; In(3R)fru^{1}/TM3, ftz-Lacz*	*fruGAL4/UAS fru^{M}; In (3R) fru^{1}*
***dxs*-masculinzed females:**		
CantonS wild-type	*y/y+; dsx^{1}/TM6B*	*y/y+; dsx^{1}/+*
$B^{s}Y$; dsx^{M}/TM6B	*y/y+; dsx^{1}/TM6B*	*y/y+; dsx^{M}/dsx^{1}*

*** I, II and III refer to independent recombination lines of the *UASGAL4* onto chromosomes with the same *UAS fru^{MIR}* insert (0.1 and 6.2) refer to independent insertions of the RNAi construct. (Manoli et al 2005).**

2.2 Determining locomotor activity in adult Drosophila

To see if *fru* function is needed for a male-specific activity pattern in *Drosophila*, crosses were made between different flies lacking *fru* function or misexpressing the male-specific protein to generate specific *fru* mutant flies (Table 2.1). Flies collected within a few hours of emergence were held in individual vials under controlled conditions (12h:12h light cycle, sucrose-cornmeal-yeast medium, 25°C) for three to five days. Then, each fly was transferred to an individual tubes (70mm by 3mm in diameter), sealed with a small plug of food at one end and cotton at the other, and placed into a Drosophila Activity Monitor (Trikinetics, Waltham, MA). When a fly walks or runs up and down the tube, it interrupts a red laser light beam, which shines perpendicular to the axis of the tube. The data were collected as the number of interruptions in 15-minute bins during a twenty-four hour period and the locomotor activity is measured continuously over five days.

2.3 Analysis of Data for Activity Levels

Raw data was collected from the DAM and uploaded into a Microsoft Excel spread sheet. The average activity level for each fly over the five days was calculated for each bin to generate the activity level per bin for an individual fly. For each genotype, the individual activity levels were averaged. These data were displayed graphically for each genotype (mean +/- SEM) and then used for further statistical analysis. For each genotype, subsets of the data were averaged to determine: total activity, total activity during the day versus night, as six four-hour bins (the first bin starting at 7:00am and ending at 11:00am or Zeitgeber Time (ZT) 22-2), two hours before lights on (7:00am-

9:00am or ZT 22-0) versus two hours after lights on (9:00am-11:00am or ZT 0-2), and two hours before lights off (7:00pm-9:00pm or ZT 10-12) versus two hours after lights off (9:00pm-11:00pm or ZT 12-14). These categories of total activity were imported to SigmaStat 3.0 for further analysis. SigmaStat was used to perform one-way ANOVA tests within the total activity categories to determine differences between *CantonS* males and *CantonS* females and among the *fru* mutants. In addition to the ANOVA tests, t-tests were used to analyze differences between *CantonS* male and *CantonS* female flies.

2.4 Determining *fru* mutant Phenotype of Chaining in Males

Five male test flies of the same genotype were placed in small plexiglass chambers. The chamber was then placed into an incubator of appropriate temperature (25°C or 28°C) with a Canon VIXIA HF100 video camera placed over the top. Flies were recorded for five minutes twice a day (once at 7:30am and again at 7:30pm) over a period of five consecutive days. Recordings were done at this time because flies show relatively high levels of overall activity just before lights on and just prior to lights off in a 12h:12hr LD cycle. After recording sessions, videos were uploaded to a MacBook using the software, VLC videoplayer, for analysis.

2.5 Analysis of Data for Chaining Behaviors

Video analysis was done manually. Observations of courtship chains were recorded, including time of initial chain formation, number of chaining events, and time spent in a chain. A chain was defined as three or more males courting each other in any kind of group (e.g., a linear array of flies). A Chaining Index was calculated as the

percent of time the males spent chaining (Villella et al. 1997). These data were imported into Microsoft excel and graphs of the average the Chaining Index over the recorded five days were created.

RESULTS

Because *dsx* and *fru* are direct outputs of the sex-determination cascade, I hypothesized that those genes should regulate the sex-specific differences in the locomoter activity patterns. Under conditions in which locomotor activity is entrained by a light-dark cycle, I was able to confirm that males and females have sex-specific differences in aspects of their activity rhythms. In addition, I found that *fru* mutant males were altered in some aspects of their male activity rhythm, although the most prominent effect was a large decrease in overall activity levels. I was not able to show any behavioral masculinization of the female activity rhythm by expressing the male forms of *fru* or *dsx* proteins in females

3.1 Male and Female Wild-type *CantonS* Flies

Under a standard 12:12 LD condition, wild-type male and female flies have a bimodal activity pattern with an early morning peak centered around lights-on and an evening peak of activity centered around lights-off. Following both peaks, a dramatic decrease in activity occurred followed by periods of little locomotor activity (Helfrich-Förster 2000)(Fig. 3.1-3.2). In my experiments, females were more active overall (P=0.010; t-test) (Table 3.1). This sex-specific difference is due to a higher level of activity during the light phase (9:00am-9:00pm or ZT 0-12) (P=0.004; t-test) (Table 3.2).

Two sex-specific differences have been reported in some temporal aspects of these general activity patterns (Helfrich-Förster 2000). First, the morning peak of activity can be divided into pre- and post-lights on periods with males showing significantly more activity than females in the two hours leading up to lights on (ZT 22-0; P=0.015; t-

test)(Table 3.3). Interestingly, males were equally active in the pre- and post-lights on period whereas females were more active in the post-lights on period ($P<0.001$; t-test)(Table 3.3). However, the two hours after lights on do not differ significantly between males and females. During the peak of activity around lights off, both males and females were more active in the two hours prior to lights off than in the two hours after lights off and were not different from each other (Table 3.3). Second, males are much less active than females during the middle of the day, specifically between the hours of 11:00am and 3:00pm ($P=0.004$; t-test)(Table 3.4).

Table 3.1: Average CS male vs. CS female activity for one 12L:12D cycle.

Genotype	n*	Activity (mean +/- SEM)
CS Male	25	570.94 ± 36.22
CS Female	10	757.69 ± 57.96

*** n=number of flies tested in this and subsequent graphs.**

Table 3.2: Average CS male vs. CS female day (ZT 0 - ZT12) and night (ZT12-ZT24) activity.

Genotype	n	Day (mean +/- SEM)	Night
CS Male	25	284.49 ± 21.94	307.98 ± 26.54
CS Female	10	432.62 ± 37.68	341.82 ± 39.84

Table 3.3: CS male vs. CS female lights on and lights off activity in a 12L:12D cycle.

		Lights on		Lights off	
Genotype	**n**	**ZT 22-0**	**ZT 0-2**	**ZT 10-12**	**ZT 12-14**
CS Male	25	165.85 ± 25.61	186.38 ± 20.88	79.34 ± 8.61	150.63 ± 15.23
CS Female	10	75.11 ± 14.19	170.95 ± 13.22	91.44 ± 7.50	172.99 ± 26.40

Table 3.4: CS male vs. CS female activity distributed in four-hour intervals in a 12L:12D cycle.

Genotype	n	ZT 22-2	ZT 2-6	ZT 6-10	ZT 10-14
CS Male	25	242.4 ± 15.0	35.3 ± 6.72	39.4 ± 8.5	208.44 ± 16.09
CS Female	10	246.1 ± 18.3	117.4 ± 16.4	77.5 ± 17.5	247.7 ± 25.6

Genotype	n	ZT 14-18	ZT 18-22
CS Male	25	22.1 ± 6.49	42.5 ± 6.32
CS Female	10	53.7 ± 15.1	54.6 ± 18.7

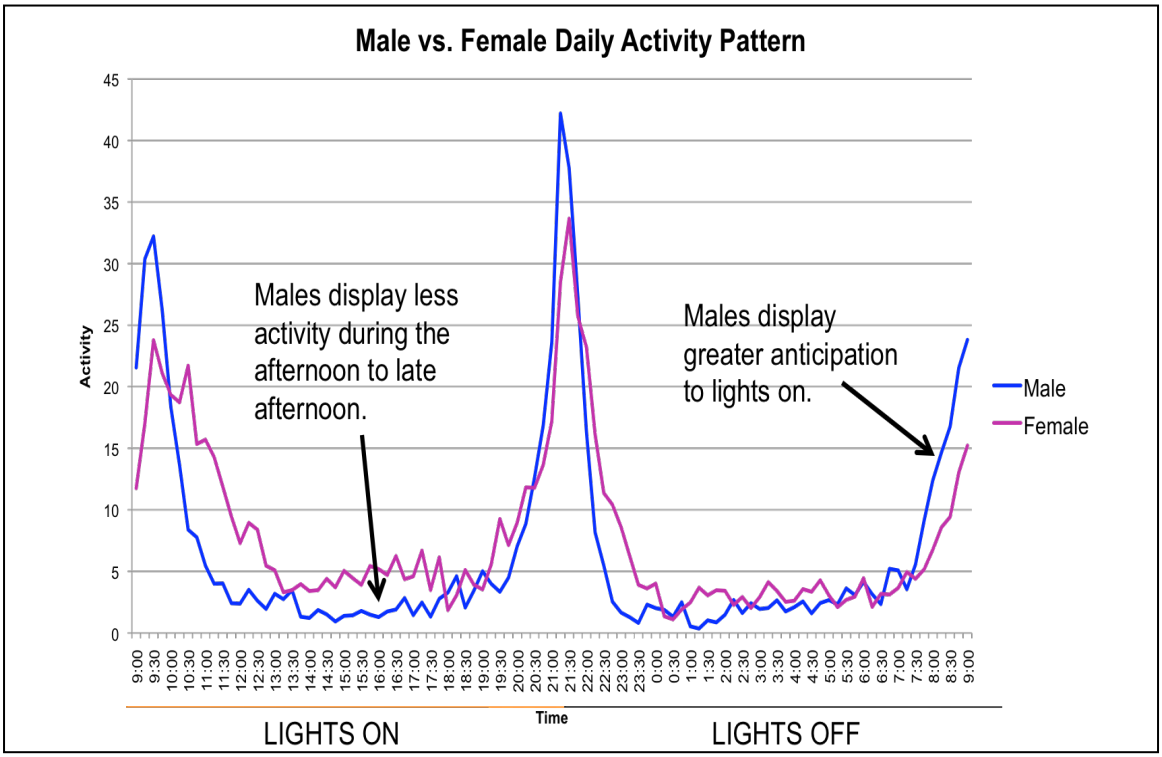

Figure 3.1: CS male vs. CS female Activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

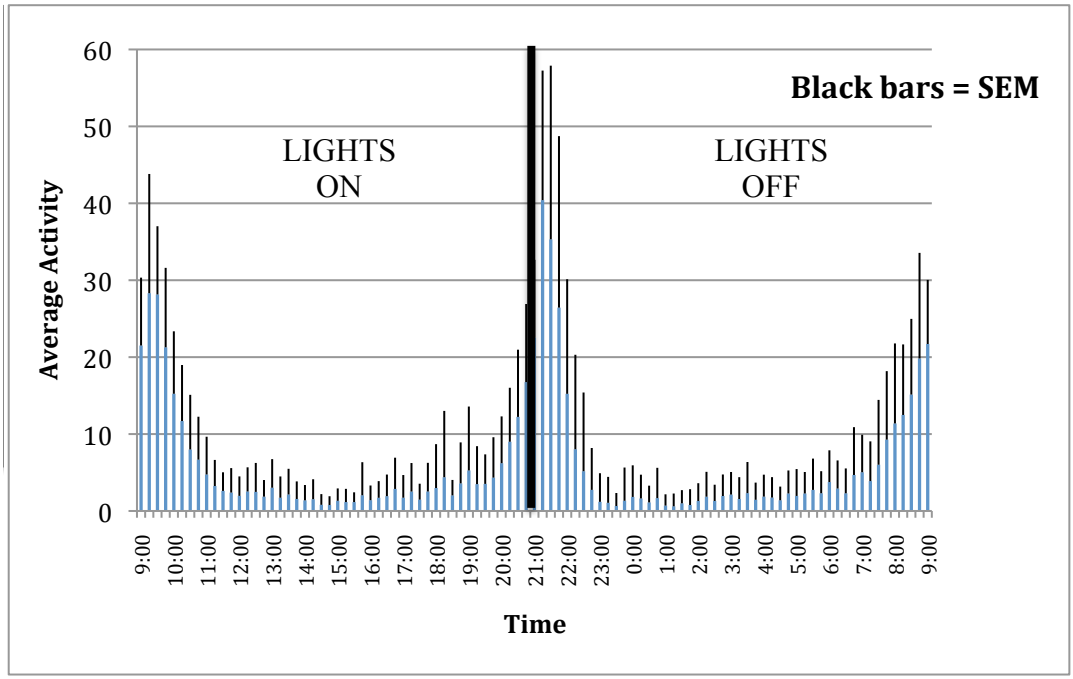

Figure 3.2: Average CS Male Activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

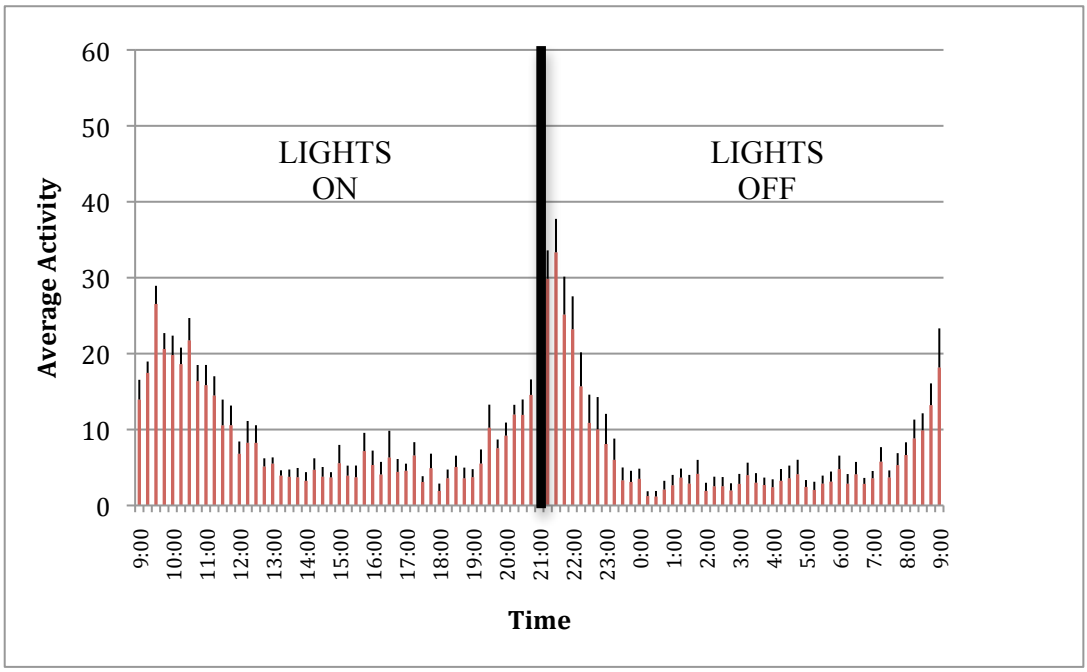

Figure 3.3: Average CS Female Activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

3.2 *fru* Mutants Show Less Overall Activity Than Wild-type Flies

To test the role of the *fru* gene in male-specific aspects of the locomotor pattern, I tested many genotypes of *fru* loss-of-function alleles. The fru^{1} and fru^{4} mutant males showed a bimodal pattern of daily locomotor activity. The one sex-specific difference I found was that males mutant for *fru* hypomorphic alleles showed a reduced anticipation for lights on (Fig. 3.2-3.3). However, the overall activity level of all *fru* mutant males was dramatically decreased. When compared to wild-type flies, fru^{1} males and fru^{4} males had a significant decrease in total activity (P-value<0.001; One-way ANOVA, Dunn post-hoc test). In a similar fashion, fru^{4} females were also less active than wild-type females (P-value<0.001; One-way ANOVA, Dunn post-hoc test)(Table 3.4).

To produce males, that lacked all Fru^{M} protein, I generated *fru* mutant flies caused by deficiencies. These males also showed a decrease in overall activity in both males and females (Fig. 3.5-3.7). Their activity levels were so depressed that many males and females did not have an entrained 24-hour rhythms in a periodogram (data not shown). The fru^{440}/fru^{sat15} and $fru^{440}/P14$ males compared to wild-type males (P-value<0.001) and females compared to wild-type females (P-value<0.001) all show a significant decrease in overall activity (Table 3.5). However, when the activity levels of these *fru* null mutant males were compared with the other *fru* hypomorphs, there was no significant difference among the genotypes. This suggests that there may be some effect of the loss of *fruitless* function because these flies all have different genetic backgrounds. Because both male and female *fru* mutants have less activity than wild-type flies, it is possible that the common functions of *fru* may play a role in how well animals are able to produce locomotor activity rather than the sex-specific functions of *fru*.

Table 3.5: *fru* mutant activity in a 12L:12D cycle

Genotype	**n**	**Total Activity**
Males:		
CS Male	25	570.94 ± 36.22
fru^{1}	15	380.17 ± 40.99
fru^{4} to^{+}	17	208.86 ± 25.41
fru^{440}/fru^{sat15} male	11	245.72 ± 44.51
$fru^{440}/P14$ male	8	207.49 ± 52.40
Females:		
CS Female	10	757.69 ± 57.96
fru^{4} to^{+}	7	411.51 ± 62.67
$fru^{440}/P14$	5	155.25 ± 37.11

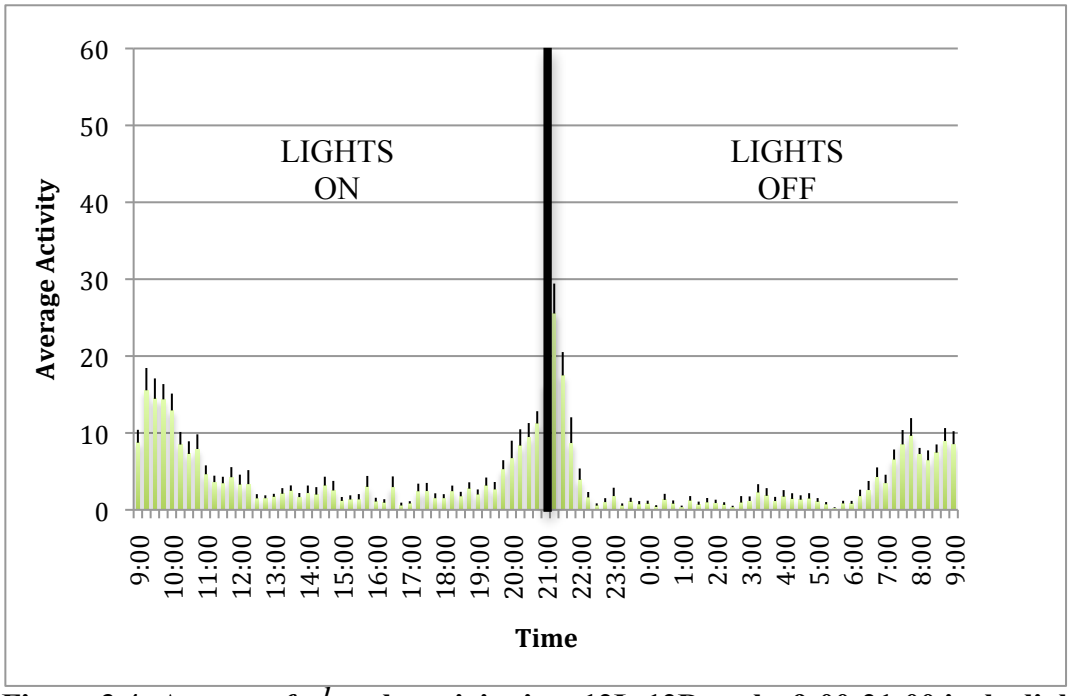

Figure 3.4: Average *fru*[1] male activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

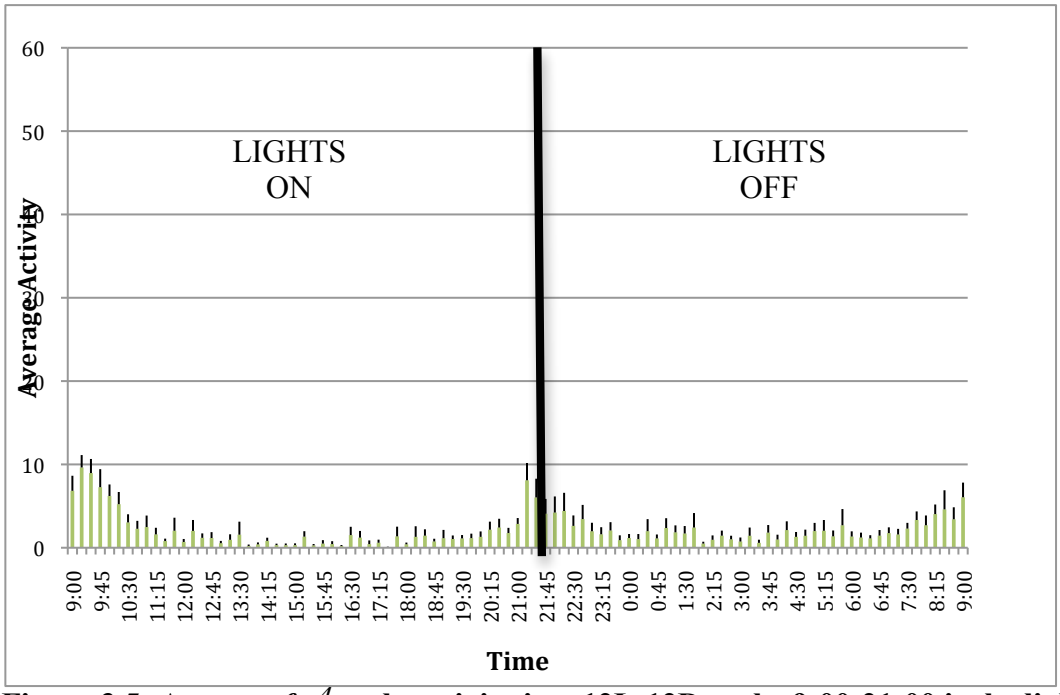

Figure 3.5: Average *fru*[4] male activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

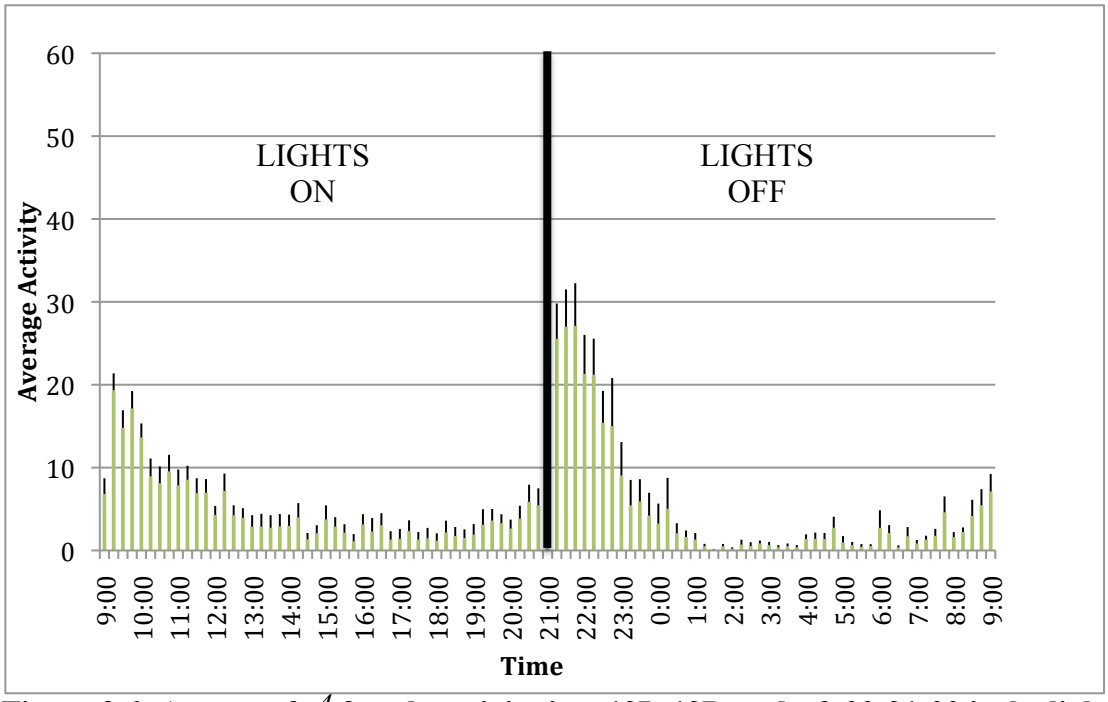

Figure 3.6: Average *fru*^{4} female activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

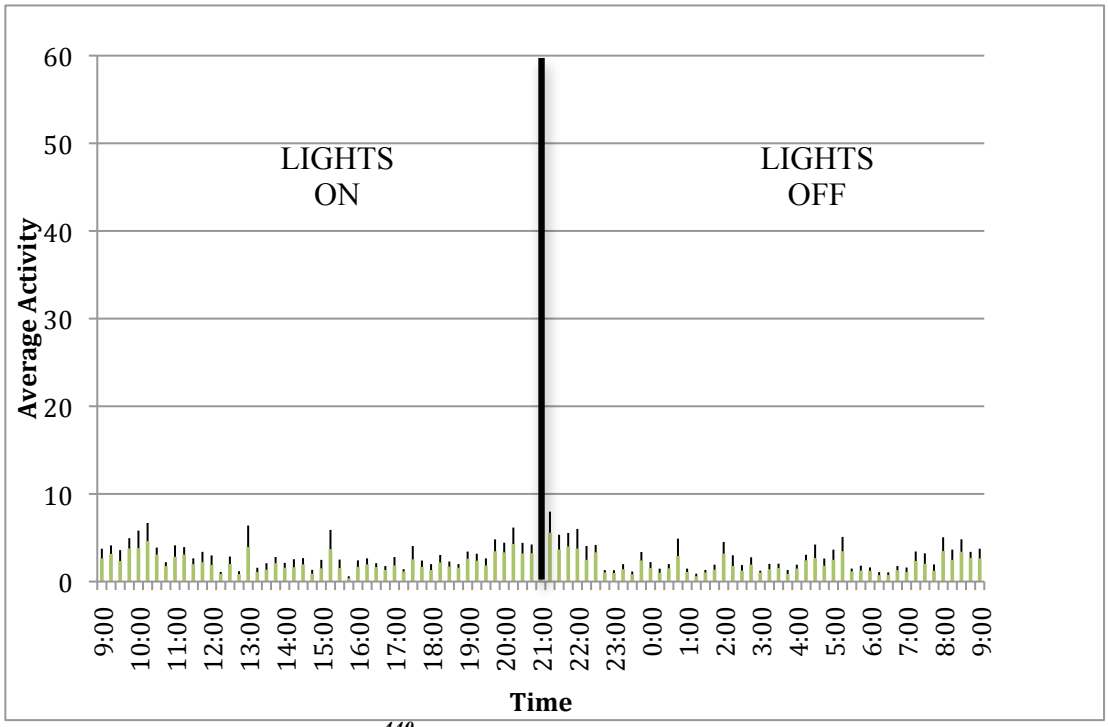

Figure 3.7: Average *P14/fru*^{440} male activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

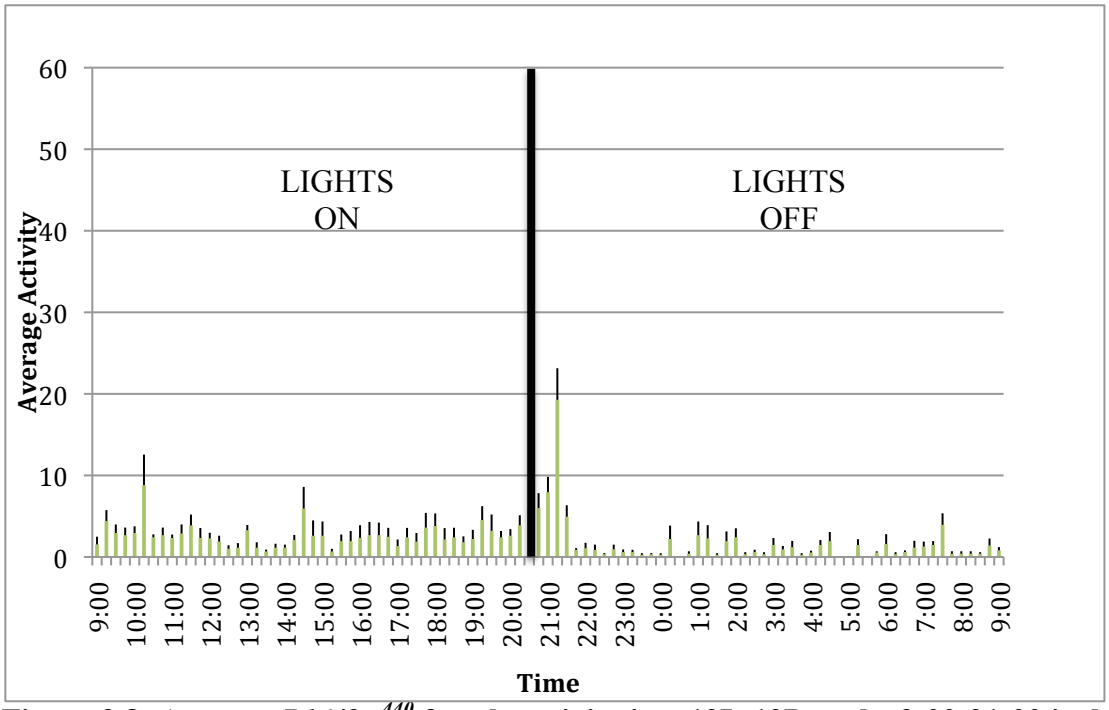

Figure 3.8: Average *P14/fru*^{440} female activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

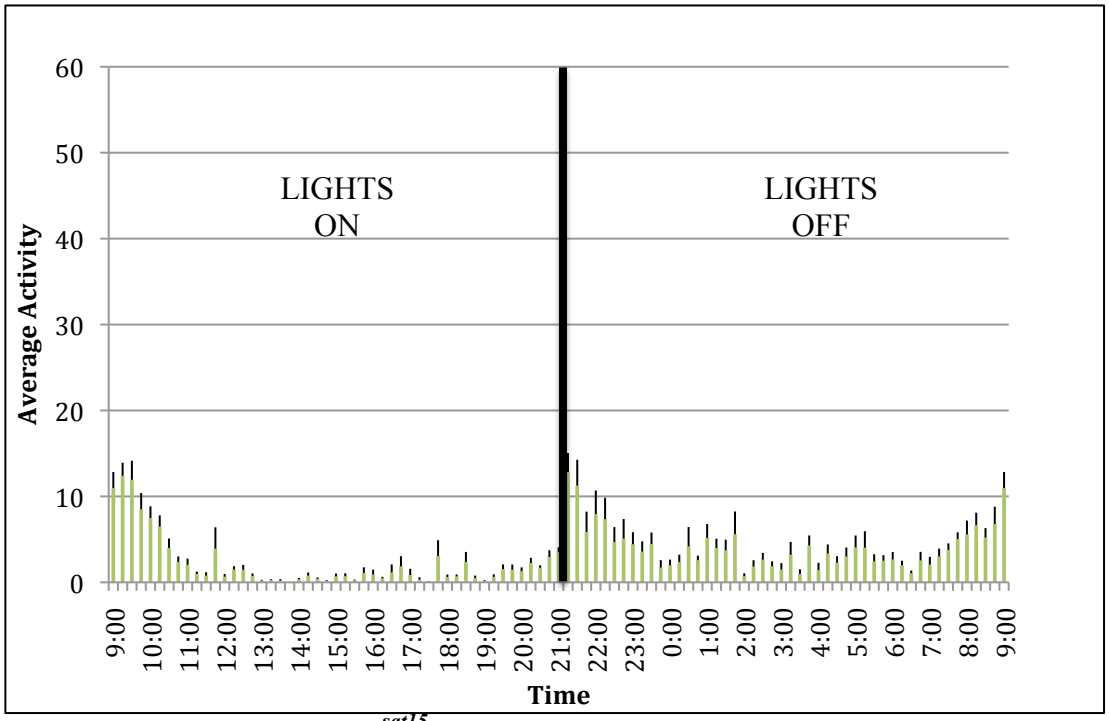

Figure 3.9: Average *P14/fru*^{sat15} male activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

In order to use a different technique to remove *fru* male-specific function, I generated mutant flies in which the yeast protein GAL4, expressed in the normal *fru* pattern, was used to express *fru* RNAi constructs, *UAS-fru*^{MIR} (Manoli et al. 2005). These *fru* mutants are made by a process called RNA interference, or RNAi. This process allows small fragments of double-stranded RNA to silence genes whose transcripts contain homologous sequences. Once these fragments of double-stranded RNA enter the nucleus, an enzyme known as dicer begins to cleave the fragment into 21-22 nucleotide fragments. These fragments are then incorporated into the RNA-induced silencing complex (RISC). From here, RISC cleaves complementary single-stranded RNA transcripts, which are then enzymatically degraded and destroyed (Hartl and Jones 2006). In order to assess whether these flies had *fru* mutant phenotypes, I assayed for the presence of male chaining behavior, in which male flies court other male flies, after these males were grouped together for several days. Unfortunately, these males did not perform chaining *fru* mutant behaviors. Therefore, I have not included these genotypes in my analysis.

3.3 *dsx*-Mutants Show More Overall Activity Than Wild-type Flies

As another approach to determine the role of *dsx* and *fru* in sex-specific aspects of locomotor rhythms, I generated female flies that were masculinized for both *dsx* and *fru* functions. When female flies are only able to produce the Dsx^M protein, as when they are dsx^M/dsx^1, they develop as males physically and biochemically. In order to provide a control genotype, I created dsx^1/+ flies. Although the general pattern of activity remained the same as for wild-type *CantonS* flies, the overall activity level of $dsxM/dsx^1$ (975.43 ± 167.67)(Fig. 3.8) and dsx^1/+ females significantly increased (1172.62 ± 164.21)(Fig. 3.9)

FruGAL4; UAS Fru^M/fru^- flies should be masculinized due to the mis-expression of Fru^M in the central nervous system. These flies also showed as overall increase in locomotor activity (846.87 ± 219.66) but did not cause any sex-specific changes in the pattern of activity.

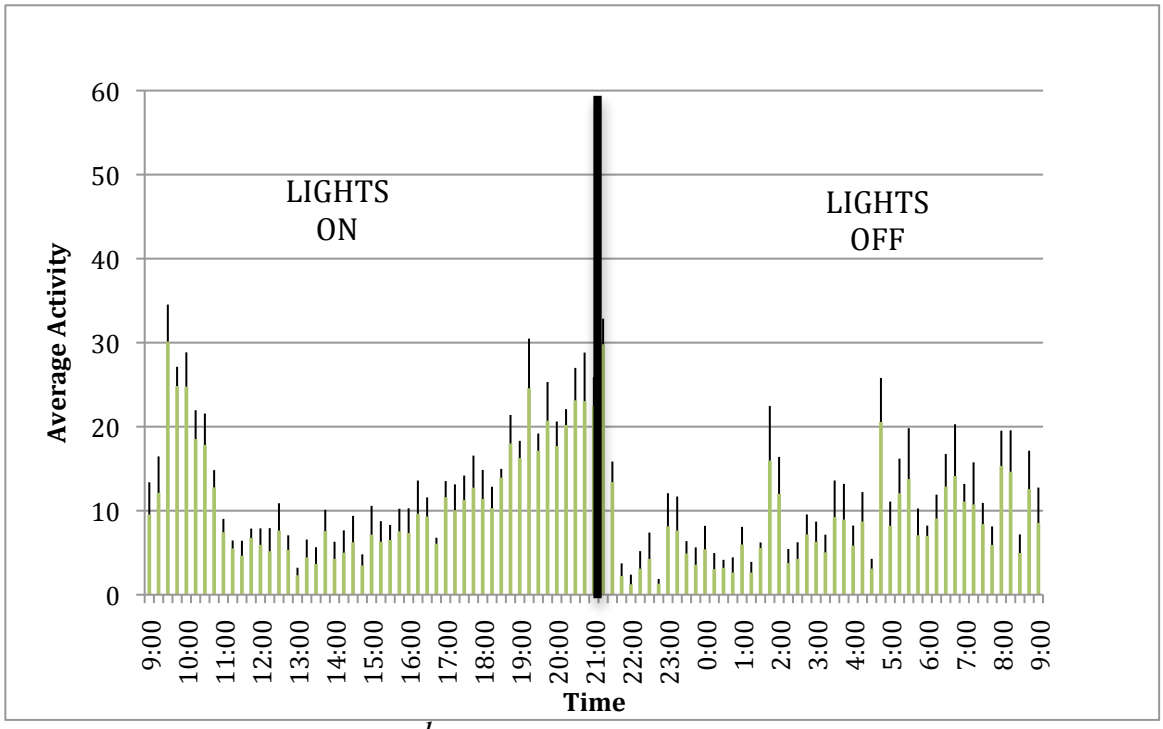

Figure 3.10: Average *dsxM/dsx*^{1} female activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

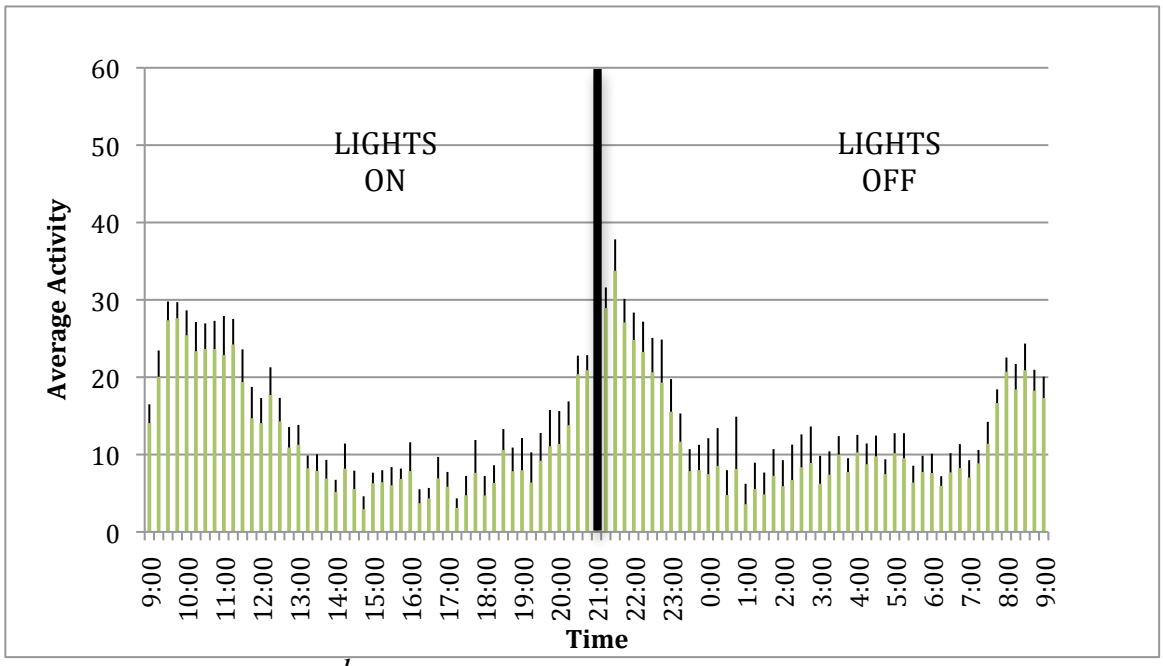

Figure 3.11: Average *dsx*^{1}*/+* female activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0).

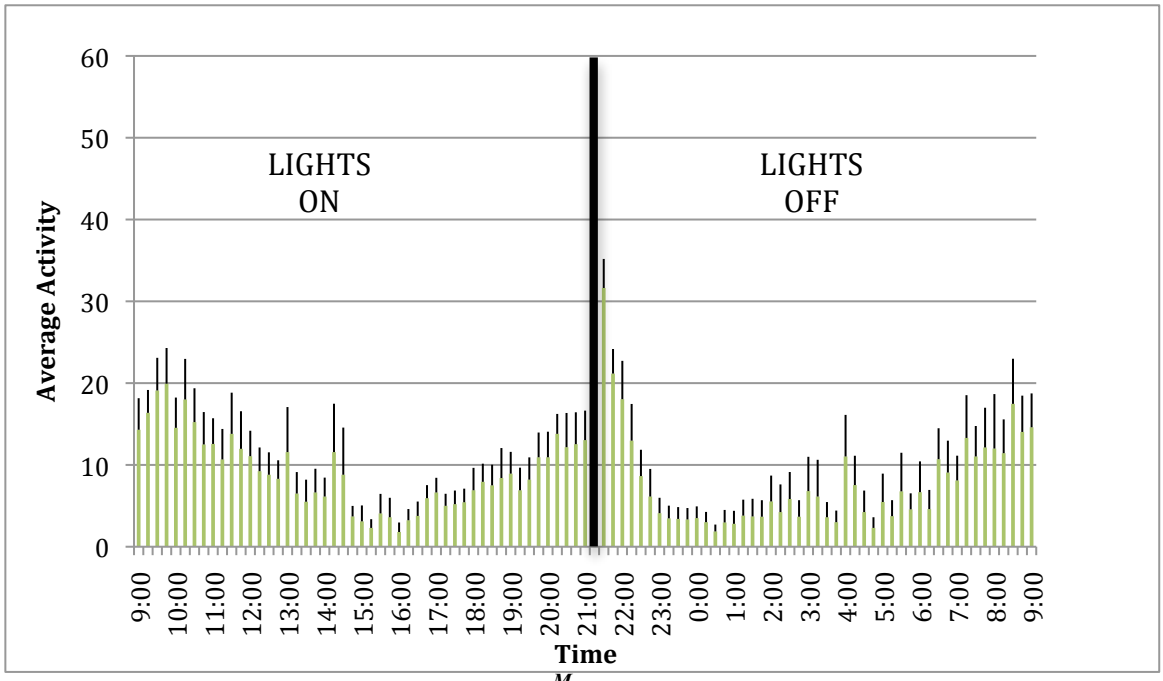

Figure 3.12: Average *fruGAL4;UAS-Fru^{M}/fru^{-}* female activity in a 12L:12D cycle. 9:00-21:00 is the lights on period (or ZT 0-12) and 21:00-9:00 is the lights off period (or ZT 12-0)

DISCUSSION

4.1 *CantonS* Wild-type Activity Patterns Matched Previous Work

CantonS wild-type flies were used to determine sex-specific differences in the daily activity patterns in males and females. Under light-dark conditions, all flies showed biomodal activity with a morning peak around lights-on and an evening peak around lights off. Distinct sex-specific characteristics of the activity pattern were described. Males show a much lower level of activity in the afternoon and greater activity before lights on. Females had a greater overall level of activity compared to males with more activity during the day (lights on) (Fig. 3.1). These results were comparable to those obtained in previous studies (Helfrich-Förster, 2000). In addition to the differences stated above, there was a slight difference in total activity found between males and females. The most significant difference between males and females found in the 12L phase can be found during the mid-day (ZT 2-6). During this phase, males have lower mid-day activity when compared to females (P=0.004; t-test).

4.2 Differences in Activity Patterns in *fru* Mutants

A total of thirteen types of male *fruitless* mutants and seven types of female *fruitless* and *doublesex* mutants were analyzed by a Drosophila Activity Monitor, an instrument used to determine activity levels of flies, to see if *fru* or *dsx* was involved in determining sex-specific activity level patterns. Of the two male-specific locomotor behaviors, some genotypes of *fru* males showed a loss in the anticipation to lights on peak of activity suggesting there may be a role for *fru* in this behavioral response. I was unable to detect a difference between the *fru* males and wild-type males in the mid-day

siesta period. It is clear that the *fru* mutant males did not express the higher activity as would be expected if they had been feminized. However, one confounding variable was the large difference in activity levels among *fru* mutants and wild-type males and females. When comparing total daily activity, wild-type males showed significantly more activity than fru^{4} mutants and both deficiency genotypes, $fru^{440}/P14$ and fru^{440}/fru^{sat15}. These *fru* mutants did not show very much activity during the normal lights on and lights off peak periods but because there were not many animals analyzed it was difficult to reach statistical significance for these time points.

However, a concern is that these males are not as active as the wild-type flies, so it may be that the lack of anticipation is a secondary effect of generally lower activity levels.

4.3 Differences in Activity Patterns in *dsx* Mutant Females

No significant differences in activity level were found when comparing *dsx* mutant females to CS females. However, there were significant differences when comparing the highly active $dsx^{1}/+$ and dsx^{M}/dsx^{1} females to the least active *fru* mutants. This could suggest that both sex-determination genes affect activity levels.

4.4 Comparing Expected Results to Actual Results

It was suggested that if Fru^{M} is responsible for the male activity pattern, then removing *fru* function should result in the loss of male pattern and perhaps the gain of the female pattern of activity in male flies, and mis-expressing Fru^{M} should cause a gain of male activity pattern in females. I have concluded that Fru^{M} may have a role in the full

level of male activity associated with the anticipation of lights on. However, a female pattern of activity is not gained in *fru* mutants. Likewise, mis-expressing Fru^M did not cause a gain of male activity pattern in females. Interestingly, this Fru^M mis-expression was associated with an increase in the general level of activity in females. However, we cannot rule out some explanations of these *fru* mutant differences, such as overall low activity in *fru* mutants compared to CS wild-types. Also, there may be other background genotype effects that affect our ability to interpret the data.

4.5 Future work

Future work will be down to improve the strength of the results found. In these experiments, too few flies for some genotypes were used. In the future, more flies will be tested to strengthen results and to improve data for statistical analysis. More work will need to be done with the *fruGAL4* drivers to see why they did not work for these experiments. These drivers will be very useful in creating new genotypes to test in the future. As mentioned above, there were possible background effects that might have affected the data. In the future, these flies should be Cantonized to reduce any possible background effects.

4.6 Significance of Results

The proposed research has broad implications for the understanding of the interaction between the central nervous system and behavior. It is known that there are substantial physical, personality, and behavioral consequences, of mutating a single gene in both *Drosophila* and humans. It is known that mutating the *fruitless* gene has drastic

effects on courtship in flies. In addition, there are robust mating circadian rhythms as well as locomotor rhythms in *Drosophila*. However, these rhythms are antiphasic and are dependent on females (Sakai 2001). However, if fundamental behaviors, such as feeding, daily activity, and sleep are also sex-specific, then this has profound implications for the neuronal circuits that are needed for these behaviors as well as considerations of how to treat disorders of these behaviors. The genes that were studied in this project have counterparts in the human genome that are not well understood. By gaining a better understand of these genes in *Drosophila*, a better understanding of the human genome could be achieved.

BIBLIOGRAPHY

Anand et al. (2001). Molecular genetic dissection of the sex-specific and vital function of the *Drosophila melanogaster* sex determination gene fruitless. *Genetics* **158**, 1569 – 1595.

Baker, B.S., and K. Ridge. (1980). Sex and the single cell: On the action of major loci affecting sex determination in *Drosophila melanogaster*. *Genetics* **94**: 383-423.

Baker, B. S., Taylor, B. J. and Hall, J. C. (2001). Are complex behaviors specified by detected regulatory genes? Reasoning from *Drosophila*. *Cell* **105**: 13-24. Web. 1 Dec 2009.

Chan, Y. B., and E. A. Kravitz. (2007). Specific subgroups of FruM neurons control sexually dimorphic patterns of aggression in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **104**: 19577–19582.

Chen S., Lee A.Y., Bowens N.M., Huber R., Kravitz E.A. (2002). Fighting fruit flies: A model system for the study of aggression. *Proc Natl Acad Sci USA* ***99****:5664–5668.*

Grima B., Chelot E., Xia R., and Rouyer F. (2004). Morning and evening peaks of activity rely on different clock neurons of the Drosophila brain. *Nature* **431**:869. Web 6 July 2009.

Harbison, ST., and Sehgal,A . (2008). Quantitative Genetic Analysis of Sleep in Drosophila melanogaster. *Genetics* **178**: 2341-2360. Web. 6 July 2009.

Helfrich-Förster, C. (2000). Differential control of morning and evening components in the activity rhythm of *Drosophila melanogaster*—sex-specific differences suggest a different quality of activity. *Journal of Biological Rhythms* **152**: 135-154. Web. 6 July 2009.

Helfrich-Förster, C, Winter C., Hofbauer A., Hall J.C., and Stanewsky R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* **30**:249.

Hendricks, J. et al. (2001). A non-circadian role for cAMP signaling and CREB activity in *Drosophila* rest homeostasis. *Nature Neuroscience*. **4**:1108-1115.

Kume, K., Kume, S., Park, S.K., Hirsh, J., Jackson, F.R. (2005). Dopamine is a regulator of arousal in the fruit fly. *The Journal of Neuroscience*. **25**: 7377-7384.

Lee, G., and J. C. Hall. (2000) A newly uncovered phenotype associated with the *fruitless*

gene of *Drosophila melanogaster*: aggression-like head interactions between mutant males. *Behavioral Genetics* **30:** 263-275.

Manoli, Devanand S., Margit, F., Villella, A., Taylor, B.J., Hall, J.C., Baker, B.S. (2005). Male-specific *fruitless* specifies the neural substrates of *Drosophila* courtship in behavior. *Nature* **436**: 395-400.

Nilsen, S.P., Chan, Y.B., Huber, R., and Kravitz, E.A. (2004). Gender-selective patterns of aggressive behavior in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **101**: 12342–12347.

Sakai, T., and Ishida N. (2001) Circadian rhythms of female mating activity governed by clock genes in *Drosophila*. *PNAS* **98**: 9221-9225.

Sosnowski, B.A., J.M. Belote, M. McKeown. (1989). Sex-specific alternative splicing of RNA from the *transformer* gene results from sequence-dependent splice sit blockage. *Cell* **58**:449-459.

Stoleru, D., Peng, Y., Agosto, J., Rosbash, M. (2004). Coupled oscillators control morning and evening locomotor behaviour of *Drosophila*. *Nature* 431: 862-868.

Villella, A., Hall, J.C. (1996). Courtship anomalies caused by *doublesex* mutations in *Drosophila melanogaster*. *Genetics* **143**:331-334. Web 9 July 2009.

Williams, J.A., S. Sathyanarayana, J.C. Hendricks and A. Sehgal (2007). Interaction between sleep and the immune response in *Drosophila*: a role for the NFkB *Relish*. *Sleep* 30: 389-400.

Yuan, Q., Joiner, W., Sehgal, A. (2006). A sleep-promoting role for the *Drosophila* serotonin receptor 1A. *Current Biology* **16**:1051-1062.