THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE EFFECTS OF BREAKING THE SPECTRIN NETWORK, USING TOBACCO ETCH VIRUS PROTEASE, ON HEART FUNCTION IN *DROSOPHILA*

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ABSTRACT

Spectrin is an important cytoskeletal protein that forms a network of tetramers along the intracellular side of the plasma membrane. This provides a scaffold that is vital for maintaining plasma membrane integrity and structure. In previous studies, defects in the spectrin based membrane skeleton (SBMS) were shown to cause cardiac arrhythmia and irreversible damage to cardiac tissue. The goal of my research is to look for any impairment of heart function in *Drosophila* caused by the cleavage of the spectrin tetramer by Tobacco Etch Virus protease (TEV). Cleavage was confirmed with western blot, and fly hearts were analyzed *via* semi-automatic heartbeat analysis (SOHA).

Once induced, the TEV protease produces steady cleavage of α-spectrin is present 0 min to 8 hours after heat shock. The amount is α-spectrin cleavage is greatest at 4 hours. Multiple heat shock inductions do not show an increase in the production of cleaved α-spectrin. Also, a longer heat shock length is correlated with a higher yield of cleaved α-spectrin. Experimental flies with cleaved α-spectrin experienced tachycardia and paralysis due to heat shock induction.

Since the spectrin network is conserved in both humans and flies, the results from this project will provide a better understanding of the role spectrin plays in human muscle functionality especially during loss of oxygen in ischemic penumbra, caused by stroke, where spectrin is known to breakdown.

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Chapter 1

Introduction

Spectrin Structure and Importance in Cytoskeleton

The spectrin based membrane skeleton (SMBS) is a network of tetramers which functions as a scaffold for proteins that localize at plasma membrane (Bennett and Baines, 2001). α– and β–spectrin subunits associate to form antiparallel heterodimers (Figure 1). Two dimers then, combine to form flexible tetrameric proteins. Spectrin tetramers are 200-280 nm in length and contain an actin-binding domain at either end (Bennett and Healy, 2008). The SMBS is found in brain, muscle and epithelial cells of many metazoan animals, including *Drosophila* melanogaster (Bennett and Baines, 2001; Burridge *et al.*, 1982; Goodman *et al.*, 1981). In vertebrates, spectrin is found in costameres, which are sites for cellular mechanotransduction in heart and skeletal muscle (Ayolan *et al.*, 2011). Costameres have been characterized in *Drosophila* as a key muscle stability complex (LaBeau-DiMenna *et al.*, 2012). In addition, spectrin has been found in vertebrate intercalated disks, which coordinate cardiomyocytes by linking them at their ends (Bennet, 2012).

The SBMS is important for cell shape determination, membrane protein localization, and membrane integrity (Bennett and Healy, 2008; Elgsaeter *et al.*, 1986). When the SMBS was disturbed in erythrocytes using heat and urea, strong membrane distortion has been observed. Furthermore, the spectrin-deficient erythrocytes developed irregular protrusions and appeared to be flatter than the typical biconcave red blood cell.

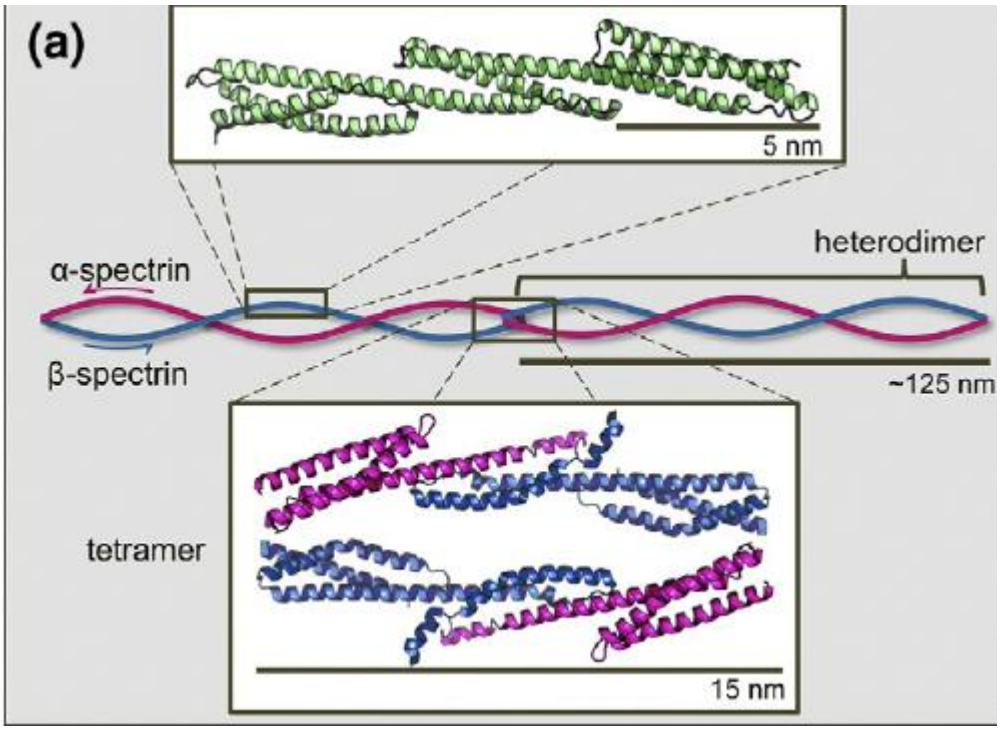

Figure 1: This figure shows 2 heterodimers meeting at a tetramerization domain, where the C-terminal tail of the β-spectrin (blue) binds and folds with the N-terminal tail of α-spectrin (pink) of the opposing heterodimer (Hill *et al.* 2014).

When exposed to a shear force, normal red blood cells normally display elasticity and recover their shape. Spectrin-deficient cells could still be stretched with a shear force, but they took several minutes to revert back to the original shape while normal cell shape recovery was nearly instantaneous (Schmid-Schönbein *et al.*, 1986).

The SMBS is also required at the axon initial segment and nodes of Ranvier to propagate action potentials in neurons. These areas of the neuron contain high densities of voltage-gated sodium channels, and they are vital to maintaining an action potential movement along a neuron. β_{IV}-spectrin is known to colocalize with ankyrin-G, which is a membrane associated adapter protein that bind to β-spectrin, and these ion channels. Loss-of-function mutations in the β_{IV}-spectrin gene cause alterations in ion channel localization in myelinated nerves. This has

been known to causes a reduction in the amplitude of action potentials in the auditory brainstem response of mice (Davis *et al.*, 2001; Parkinson *et al.*, 2001).

Spectrin is also known to be involved in the trafficking of Ca^{2+} homeostasis proteins. Ankyrin adapters are known to bind to inositol 1,4,5-trisphosphate (IP3) and ryanodine receptors, both of which are known to trigger Ca^{2+} transport from the sarcoplasmic reticulum into the cytosol of muscle cells. A loss of ankyrin-B has been shown to result in a mislocalization of these Ca^{2+} homeostatic proteins (Truvia *et al.*, 1999). Furthermore, loss of β_{II}-spectrin has also been shown to directly affect Ca^{2+} transport. This has been shown to alter the calcium dependent contraction of heart muscles leading to arrythmia (Smith *et al.*, 2015).

In addition, β_{II}-spectrin is involved in Smad translocations and signaling. Smad signaling is important to proper cardiomyocyte development; thus, a defective β_{II}-spectrin can lead to dramatic changes in the localization of membrane proteins and defects in heart structure (Lim *et al.*, 2014). The normal binding sites for plasma proteins, and the spectrin tetramer are shown in figure 2.

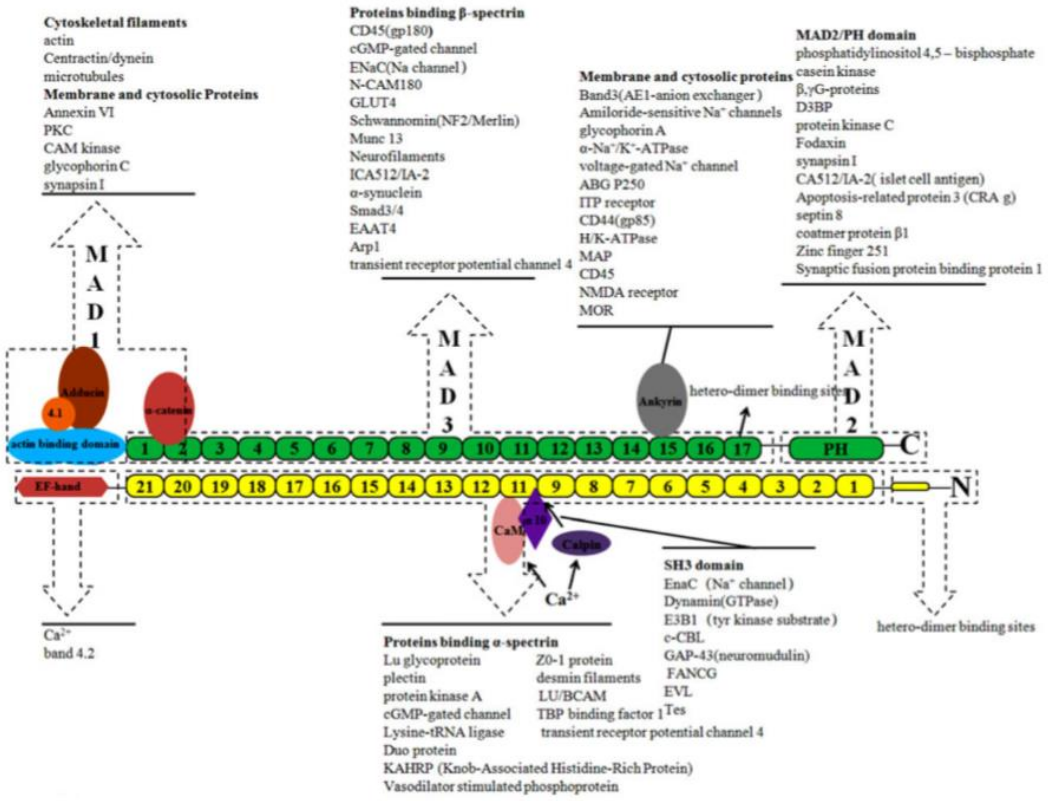

Figure 2. The site of interactions between various plasma membrane proteins and spectrin is shown. This figure shows an anti-parallel heterodimer with α-spectrin shown in yellow and β-spectrin shown in green (Taken from Zhang *et al.*, 2013).

Spectrin Breakdown During Ischemic Stroke

Calpain is a Ca^{2+} dependent protease that is ubiquitous in many plants and animals and exist as tissue-specific isoforms in higher organisms (Czogalla and Sikorski, 2005). The activity of this protease is amplified when bound to calmodulin, a calcium binding messenger protein. α_{II}-spectrin is known to have a calmodulin-binding site, which will allow the calpain-calmodulin complex to bind. Once bound, calpain cleaves a peptide bond between Tyr 1176 and Gly 1177 of the 11^{th} spectrin repeat unit. This creates 2 halves of around 150 kDa. Necrosis occurs when cells are physically or chemically damaged beyond repair during ischemia. This is linked with a massive influx of Ca^{2+} and calpain activation. Thus, the cellular pathology caused by a loss of oxygen leads cleavage of α_{II}-spectrin into highly stable products which have been observed and quantified in neurons just 10 min after ischemic event (Czogalla and Sikorski, 2005). Also, β_{II}-spectrin is cleaved by calpain into 165 and 125 kDa fragments (Lofvenberg and Backman, 1999). This breakdown of spectrin has been shown to cause osmotic fragility and membrane blebbing (Armstrong *et al.*, 2001).

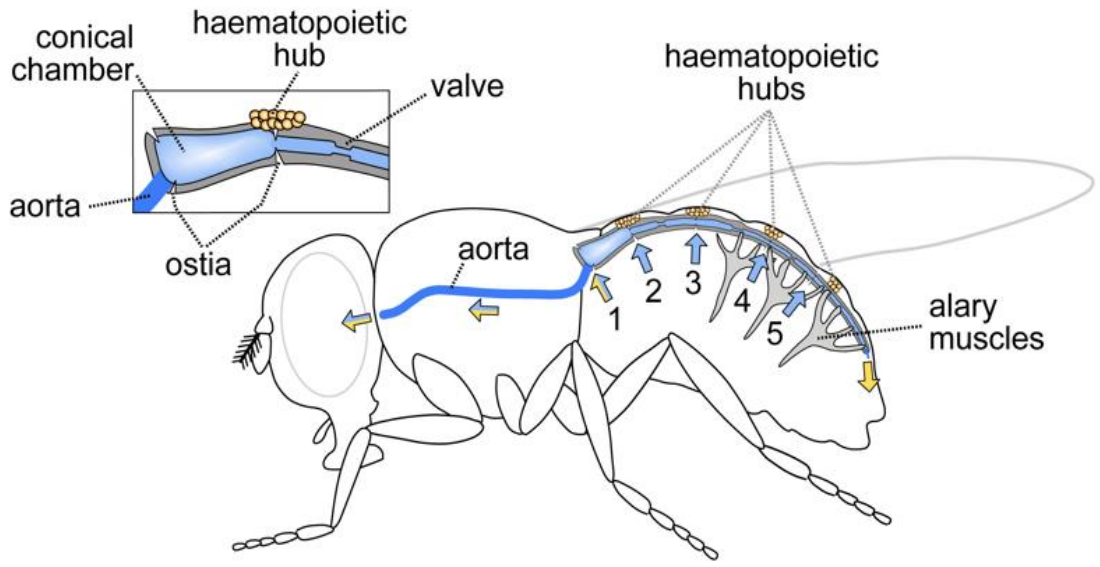

Figure 3: This figure shows the fly heart. The blue arrows indicate deoxygenated blood and its entry into the ostias. The yellow arrows indicate oxygenated blood flow (Prokop, 2015).

Drosophila Heart Model

The adult fly heart is a narrow tube that runs from the head to abdomen (Figure 3). The abdomen is where blood enters the heart *via* valve-like openings called ostia. There is a conical chamber between the thorax and abdomen which function like a ventricle to pump hemolymph both toward the head and abdomen. The thorax contains the majority of the aorta, which carries hemolymph toward the head. This thesis discusses the role of α-spectrin in the fly heart, but this invertebrate model contains other developmental and functional homologies to the vertebrate heart (Fink *et al.*, 2009).

Firstly, dystrophin is a protein found between the sarcolemma and the outermost layer of myofilaments in the myofiber. It is found in fly and vertebrate hearts and is vital to cardiac

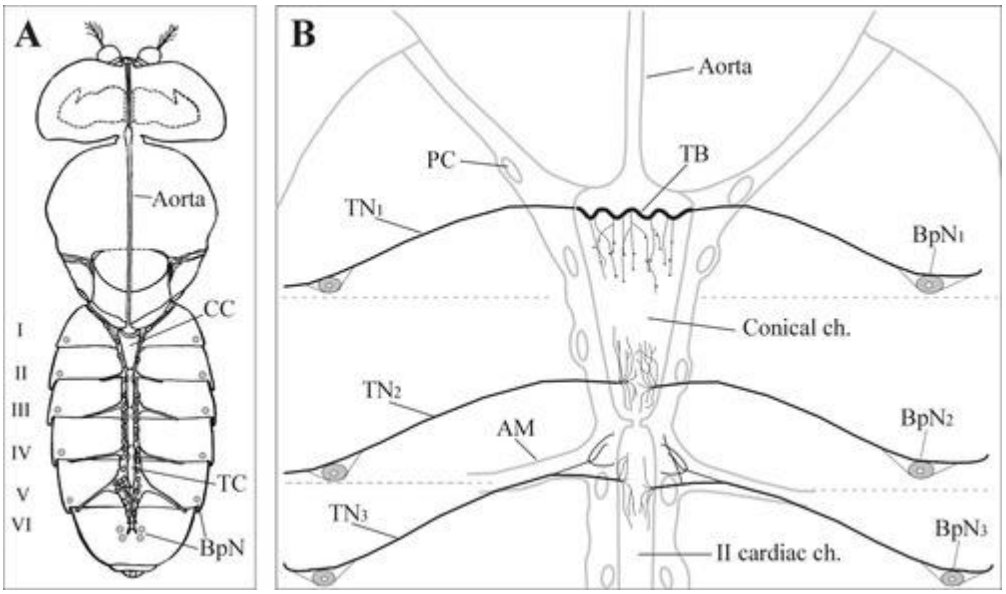

Figure 4. This is figure shows a diagram of the fly heart and its enervation. Transverse nerves (TN), bipolar neurons (BpN), the transverse bridge (TB), aorta, alary muscles (AM), and a representative pericardial cell (PC) are labeled (Dulcis *et al.*, 2003).

development. The transcription of the dystrophin gene is regulated by Smad signaling, which has already been shown to require functional β_{II}-spectrin. When β_{II}-spectrin is not present cardiomyocytes have been shown to down-regulate dystrophin. In affected mouse embryos, a defect in the wall dividing the left and right ventricles was detected (Lim *et al.*, 2014).

The fly heart is innervated by nerve projections known as the bipolar neuron cluster, which functions to release Crustacean Cardioactive Peptide (CCAP) into the posterior of heart. This regulates the forward heartbeat in which the hemolymph flows from abdomen to head. In addition, there are glutaminergic neurons that innervate the heart bilaterally to regulate periodic reversal of hemolymph flow back into abdomen (Piazza and Wessells, 2013). The human heart is also innervated at the pacemaker region. These neurons are sensitive to neuropeptides and trophic factors and are crucial to modulating the autonomic heartbeat. Thus, the fly heart model can also be used to study pathologies involving autonomic nerve function.

The fly heart can also be used to study age-related deterioration of cardiac tissue caused by decreased Insulin/IGF signaling in humans since insulin-like peptides and insulin-like growth factor homologs have been found in flies. Furthermore, the KCNQ potassium channel is conserved in flies and humans and is vital to maintaining heart rhythmicity and stress tolerance (Fink *et al.*, 2009).

Lastly, cardiac pathologies such as congenital arrhythmia, dystrophies, aortopathies, acquired and congenital forms of heart failure, and possibly sudden cardiac death have all been associated with alterations in β_{II}-spectrin. Furthermore, the β_{II}-spectrin/ankyrin-B complex, which is found near T-tubules of cardiac myocytes, interacts with other membrane-associated proteins such as Na^+-K^+-ATPase and Na^+/Ca^{2+}exchanger and can disrupt electrical conduction in the heart if it is defective (Derbala *et al.*, 2018).

Tobacco Etch Virus Cleavage Mechanism

The Tobacco Etch Virus (TEV) protease has high specificity and cleavage efficiency towards its substrates, and it has been previously used for cleavage of fusion proteins *in vitro* for removal of affinity tags during protein purification (Chen, 2013). TEV protease has also been used to cleave RAD21 protein *in vivo* in *Drosophila* (Pauli *et al*, 2008). To allow inducible breakdown of the SBMB in the fly heart, the α-spectrin gene has been modified with by attaching a TEV binding site. The modified α-spectrin contains 3 tandem cleavage sites which have high affinity for TEV protease. Since the TEV protease only recognizes the sequence, ENLYFQ(G/S), it will only cleave at this site in α-spectrin because the sequence is not found anywhere else in the fly proteome. The TEV protease is under the control of the HSP70 promoter, which will allow the expression of the protease to be controlled *via* heat shock induction at 37 ^{o}C (Harder *et al.*, 2008).

Hypothesis

Spectrin is known to form a complex with ankyrin-B, the Na^+/K^+ ATPase, the Na^+/Ca^{2+} exchanger, and the IP3 receptor in a specialized microdomain in cardiomyocyte T-tubules (Bennett and Healy, 2008). Thus, the cleavage of spectrin can cause an imbalance of sodium and calcium. Since sodium ions are essential for the propagation of action potentials and calcium ions, which are essential for muscle contractions, an imbalance of these ions can affect contraction rhythm in cardiomyocytes. In addition, spectrin breakdown can cause membrane blebbing, osmotic fragility, and its cleavage is correlated with the transition from reversible to irreversible ischemic injury in the heart (Armstrong *et al.*, 2001). Lastly, since spectrin is known to bind the ankyrin-G adapter at the axon initial segment. A loss-of-function mutation in β_{IV}-spectrin is known to reduce the action potentials due to mislocalization of sodium ion channels, so cleavage of the homologous spectrin in *Drosophila* may similarly result in faulty pacemaker cell signals, which can cause arrythmia. (Bennett and Healy, 2008)

Since spectrin is needed to maintain cell membrane integrity, and its breakdown has been shown to affect nerve action potentials, my hypothesis is that its cleavage will lead to arrhythmia in the treated flies.

Materials and Methods

Hemolymph Solution

Hemolymph consists of 108mM Na^+, 5mM K^+, 2mM Ca^{2+}, 8mM $MgCl_2$, 1mM NaH_2PO_4, 4mM $NaHCO_3$, 10mM sucrose, 5mM trehalose, 5mM HEPES. This sterile solution was stored at -20°C without the sugars. The sucrose and trehalose was stored separately at -20°C and added immediately prior to use. Before use, the hemolymph solution was equilibrated to room temperature.

Heart Dissection

Flies are first immobilized by placing them in ice. Then, a thin layer of petroleum jelly is placed on a silicone coated petri dish. The fly is placed with the ventral side up onto the petroleum jelly. The wings and legs can be pressed into the jelly to ensure that the fly is stationary during dissection. Once stuck, the fly is submerged in artificial hemolymph solution, and a single incision is made along the mid-sagittal plan on the ventral surface of the fly abdomen (Figure 5). Since the heart is bound to the exoskeleton on the dorsal side of the abdomen it is vital to make the cut as shallow as possible. The guts and all other organs are removed by gentle tugging with tweezers.

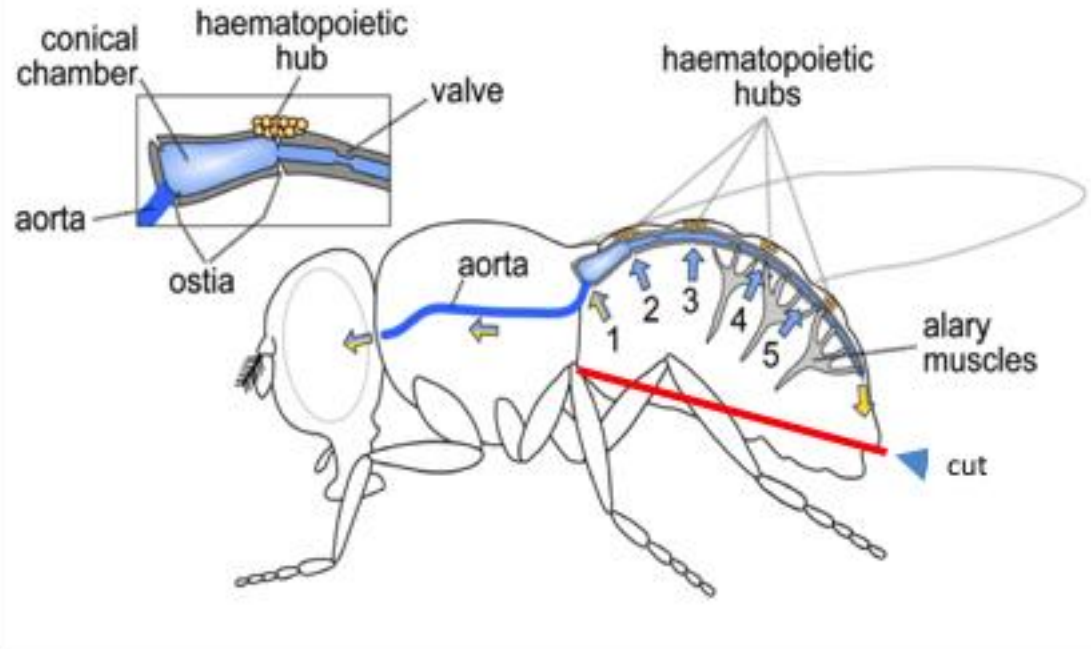

Figure 5: This is a lateral view of the fly with the heart shown in blue. The dissection is made long the line indicated in red. (Prokop, 2015).

SOHA Heart Beat Visualization

The exposed fly heart, submerged in hemolymph solution, was imaged at approximately 30 frames per second using a 40x lens. Then, the pictures will be compiled into an M wave, which shows the movement of the heart via an elongated region of interest (ROI) over a 30 second interval. Using the FIJI multi-kymograph function, a line of 1-pixel width was selected perpendicular to a major moving region of the heart in the ROI and turned into a kymograph. Therefore, the M-wave tracks the movement of a feature along this line selection. The heart rhythm has previously been observed *via* video analysis of partially dissected flies in artificial hemolymph solution (Fink *et al.*, 2009; Ocorr *et al.*, 2008).

Western Blot

Experimental flies were heat shocked at 37°C in water bath for 1 hour to induce the TEV protease transgene and therefore spectrin cleavage. Protein extracts were analyzed after various treatments and times after heat shock to assess the optimal conditions for spectrin cleavage. The following variations were tested: time after heat shock, time of heat shock and multiple heat shock. The proteins were separated along an 8% SDS-PAGE gel. The gel was transferred to nitrocellulose, stained with a mouse anti-α-spectrin primary antibody and non-fluorescent secondary goat anti-mouse antibody. The western blot was submerged in chemiluminescent, high-sensitivity ECL reagent and visualized.

Chapter 3

Results

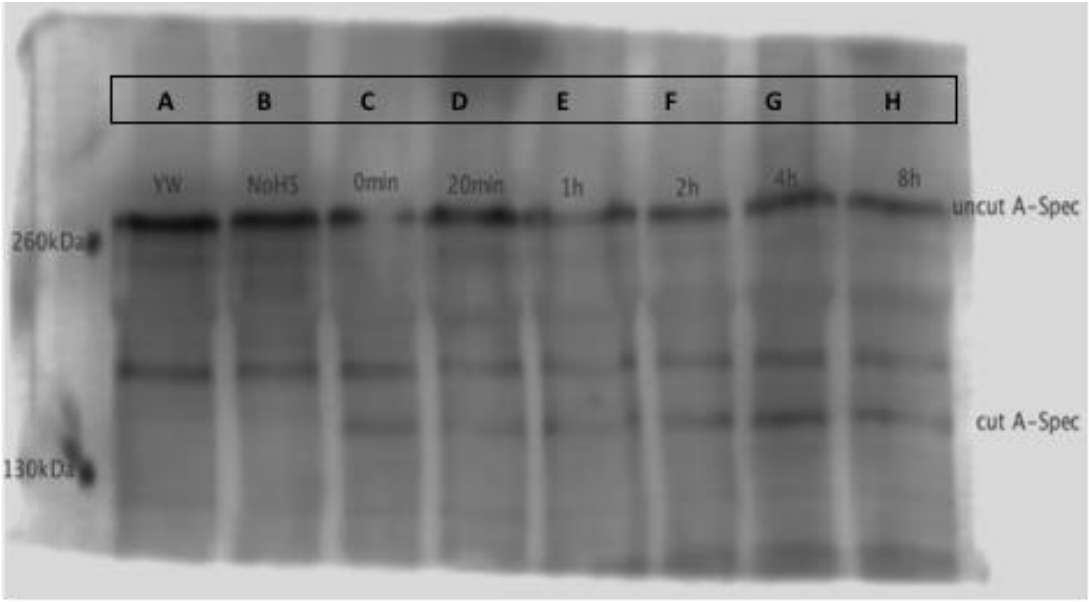

Figure 6: Time course of spectrin cleavage following heat-shock. A- yellow-white (YW) flies were used as a no TEV protease, heat-shock control and were collected 0 min after heat shock. **B**-Experimental flies collected without heat shock. **C**-Experimental flies collected 0 min after heat shock. **D**- Experimental flies collected 20 min after heat shock. **E**-Experimental flies collected 1 hour after heat shock. **F**-Experimental flies collected 2 hours after heat shock. **G**-Experimental flies collected 4 hours after heat shock. **H**-Experimental flies collected 8 hours after heat shock. Notice the production of a cut α-spectrin band of the expected size (cut A-spec).

To determine how fast α-spectrin is cleaved after TEV protease induction, flies were analyzed *via* western blot at varying times after heat shock. Yellow-white (*yw*; wild type) flies were used as the control. The uncut α-spectrin (280 kDa) and cleaved α-spectrin (~140 kDa) are indicated. In figure 6, cleavage of α-spectrin is shown 0 min after TEV protease induction and is maintained until 8 hours. Similar amounts of cleaved α-spectrin are present at all times; however at 4 hours after TEV protease induction, the level cleaved α-spectrin is highest. The YW control and flies with no TEV protease induction did not produce cleaved α-spectrin.

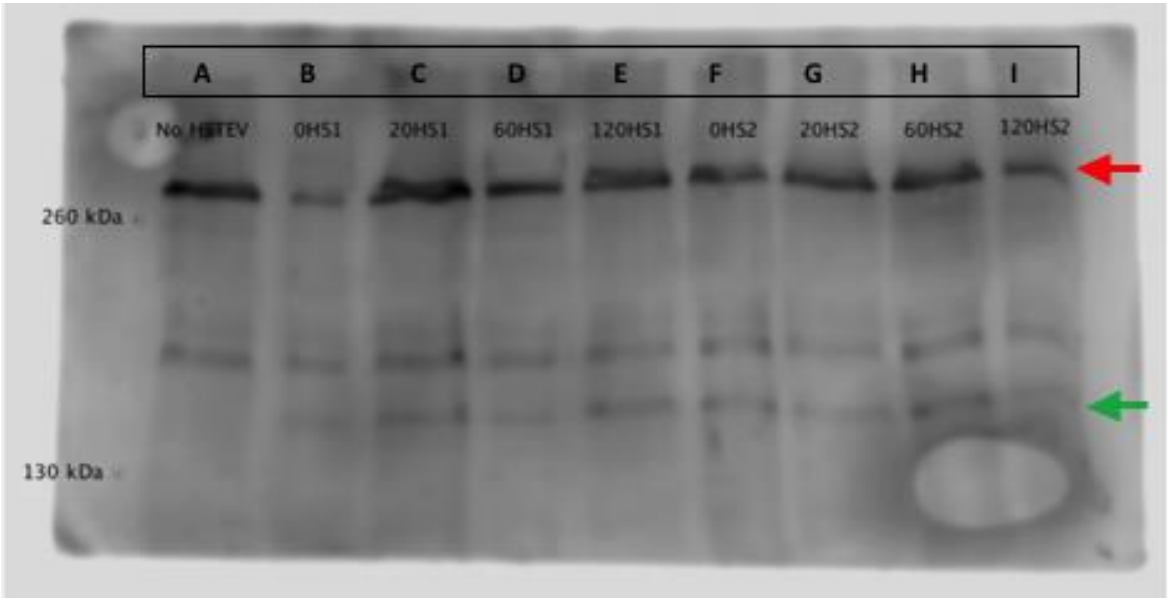

Figure 7: Multiple heat shock inductions of cleaved α-spectrin. A-Experimental flies collected without heat shock. **B**-Experimental flies collected 0 min after first heat shock. **C**-Experimental flies collected 20 min after first heat shock. **D**-Experimental flies collected 60 min after first heat shock. **E**-Experimental flies collected 120 min after first heat shock. **F**-Experimental flies collected 0 min after second heat shock. **G**-Experimental flies collected 20 min after second heat shock. **H**-Experimental flies collected 60 min after second heat shock. **I**-Experimental flies collected 120 min after second heat shock. The red arrow indicates uncut α-spectrin (280 kDa), and the green arrow indicates cleaved α-spectrin

To determine the effect of multiple heat shocks, flies were analyzed *via* western blot for 2 hours after 2 consecutive heat shocks. The No HSTEV control was collected without heat shock. The first set of heat shocking (HS1) contained samples analyzed 0 min, 20 min, 60 min, and 120 min after heat shock. The second set of heat shocking (HS2) was conducted 120 min after the first heat shock, and the same time points were visualized. In figure 7, similar quantities of cleaved α-spectrin are present at all times tested after the first heat shock. The second heat shock shows similar levels of cleaved α-spectrin as the time points tested in the first heat shock. The No HSTEV control showed no cleavage of α-spectrin.

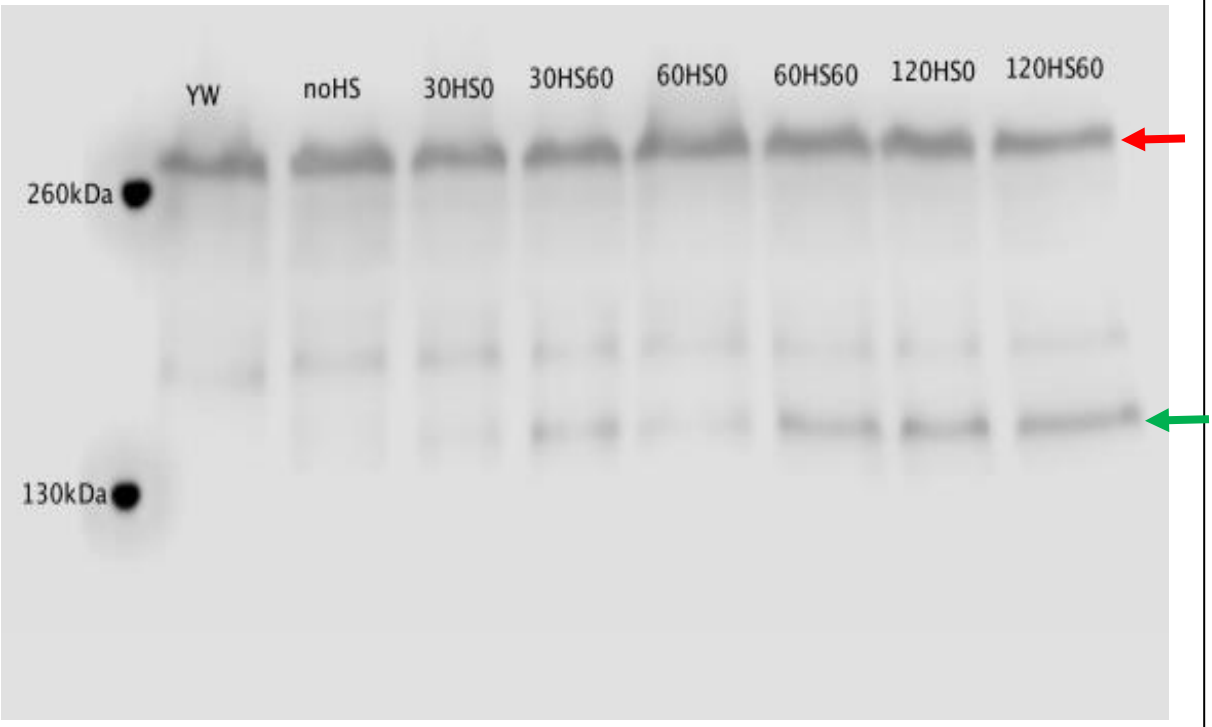

Figure 8: Cleavage of α-spectrin after varying lengths of heat shock induction. A-YW flies collected 0 min after heat shock. **B**-Experimental flies collected without heat shock. **C**-Experimental flies collected at 0 in after a 30 min heat shock. **D**-Experimental flies collected at 60 in after a 30 min heat shock. **E**-Experimental flies collected at 0 in after a 60 min heat shock. **F**-Experimental flies collected at 60 in after a 60 min heat shock. **G**-Experimental flies collected at 0 in after a 120 min heat shock. **H**-Experimental flies collected at 60 in after a 120 min heat shock. In each lane the first number indicates minutes of heat shock, and the second number indicates minutes after heat shock that the sample was collected. The red arrow indicates uncut α-spectrin (280 kDa), and the green arrow indicates cleaved α-spectrin (~140 kDa).

To determine the effect of varying lengths of heat shock on cleavage of similar quantities, flies were analyzed *via* western blot after 30 min, 60 min, and 120 min heat shock lengths. Yellow-White (wild type) flies and experimental flies with no heat shock were used as the controls. In figure 8, cleaved α-spectrin was present at all times and lengths of heat shock tested; however, more α-spectrin cleavage was seen in longer heat shocks. In addition, more cleaved α-

spectrin was seen 1 hour after TEV protease induction than the 0 min after induction in all tested heat shock lengths.

Table 1: Observations of the effects of heat shocking on fly mobility after varying times.

Fly Type	0 min	20 min	1 hour	2 hours	4 hours	8 hours
Yellow-white (control)	Mobility unaffected	Mobility unaffected	Mobility unaffected	Mobility unaffected	Mobility unaffected	Mobility unaffected
Experimental	Cannot fly or walk. Flies are able to stand back on legs after being flipped.	Cannot fly or walk. Flies are able to stand back on legs after being flipped.	Cannot fly or walk. Flies are able to stand back on legs after being flipped.	Flies couldn't fly but could walk when agitated.	Flies couldn't fly but were freely walking.	Flies couldn't fly but were freely walking.

To determine the mobility of flies after TEV protease induction, they were observed a 0 min, 20 min, 1 hour, 2 hours, 4 hours, and 9 hours after heat shock. The ability to stand on legs after being flipped, ability to walk, and ability to fly were observed in YW (control) and experimental flies. As seen in table 1, the YW flies showed no loss of mobility due to heat shock. The experimental flies were only able to stand on legs after being flipped but could not walk or fly until 2 hours after heat shock. At 2 hours, the experimental flies only walked when agitated. At 4 hours and 8 hours, experimental flies were able to walk freely, but flight was not recovered in the 8 hours tested.

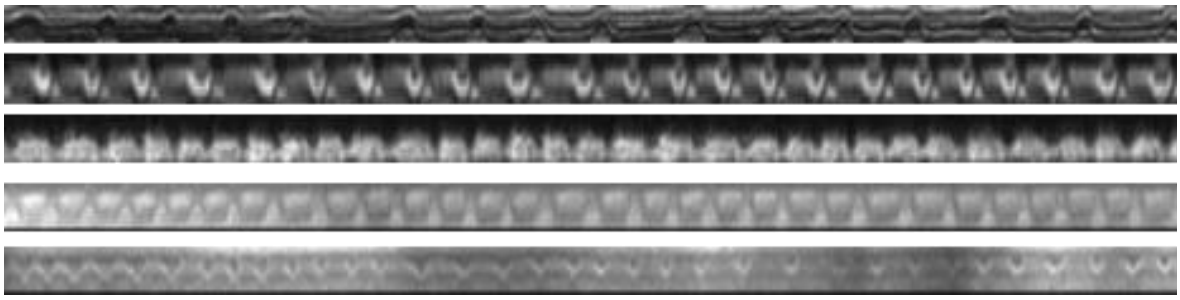

Figure 9: M-waves of YW flies without heat shock. Figure 9 shows, the heart rhythms of 5 yellow-white (*yw*; wild type) flies. Each peak represents a heartbeat.

Yellow-white flies were dissected and immediately analyzed *via* SOHA without heat shock to serve as a control for the heat shock procedure. The first m-wave is slightly arrhythmic, but the rest were regular in figure 9. Overall this experiment showed the most regular and slowest heart rate.

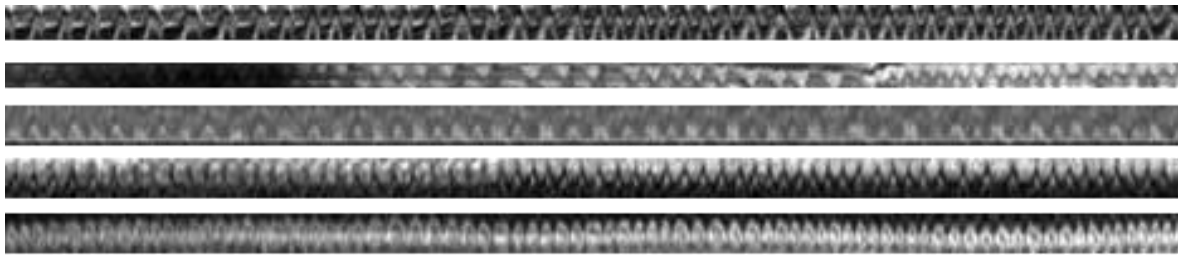

Figure 10: M-waves of experimental flies without heat shock. Figure 10 shows, the heart rhythms of 5 experimental flies. Each peak represents a heartbeat.

Experimental flies were dissected and immediately analyzed *via* SOHA without heat shock to serve as a control for the heat shock procedure. The first and second m-waves are arrhythmic, while the rest show a normal rhythm in figure 10. The overall heart rate is more than what was observed in yellow-white flies after heat shock.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 1 hours after heat shock. In figure 11, kymographs 2, 6, 7, 8, 9, and 10 show a more regular heart rhythm, but kymographs 1, 3, 4, and

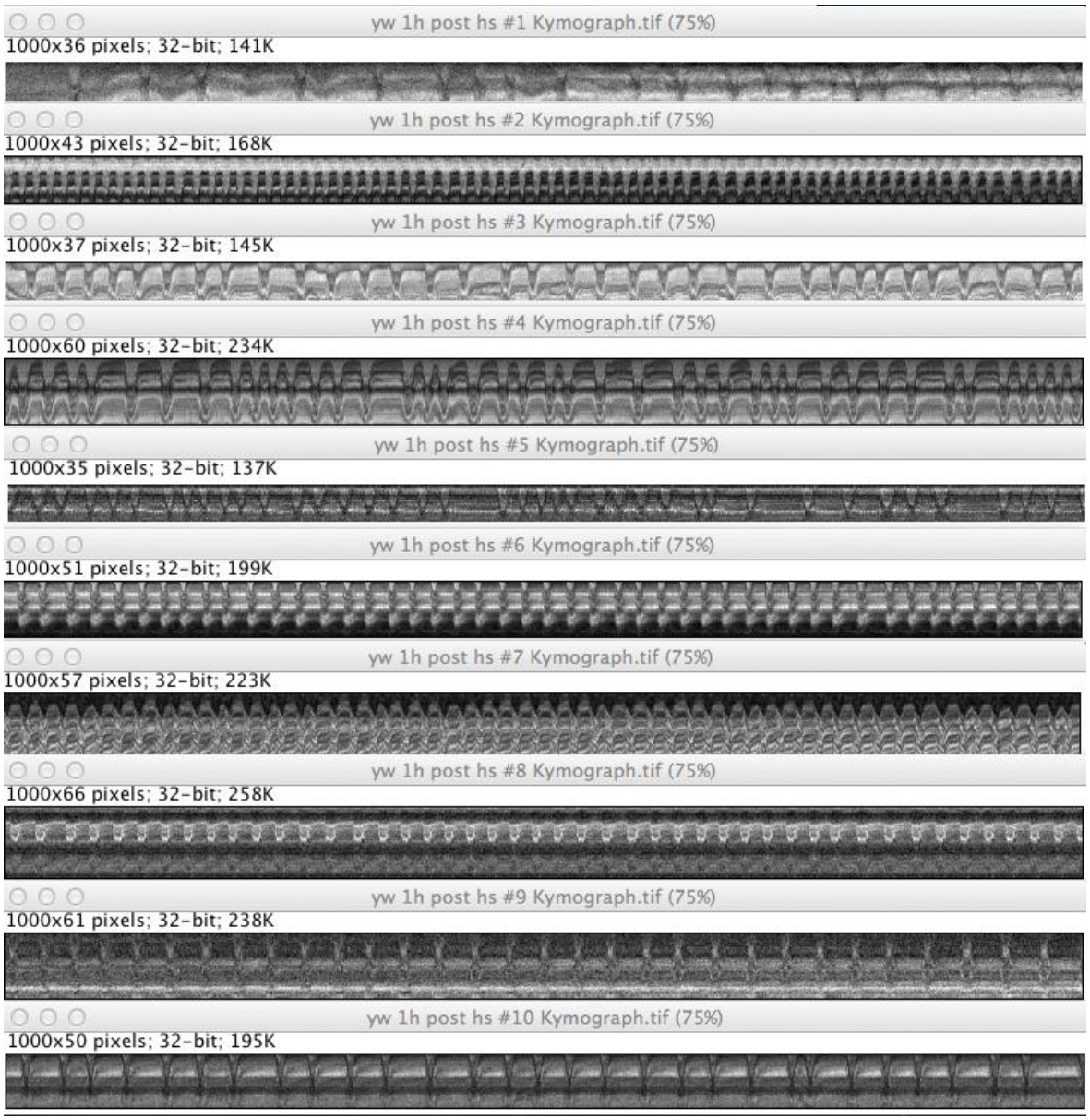

Figure 11: M-waves of yellow-white (control) flies 1 hours after heat shock. Figure 11 shows, the heart rhythms of 10 experimental flies. Each peak represents a heartbeat.

5 are arrhythmic. Overall, the average heart rate was higher than the yellow-white tests with no heat shock, but the heart rate is lower than all heat shocked experimental tests.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 1 hours after heat shock. All kymographs show

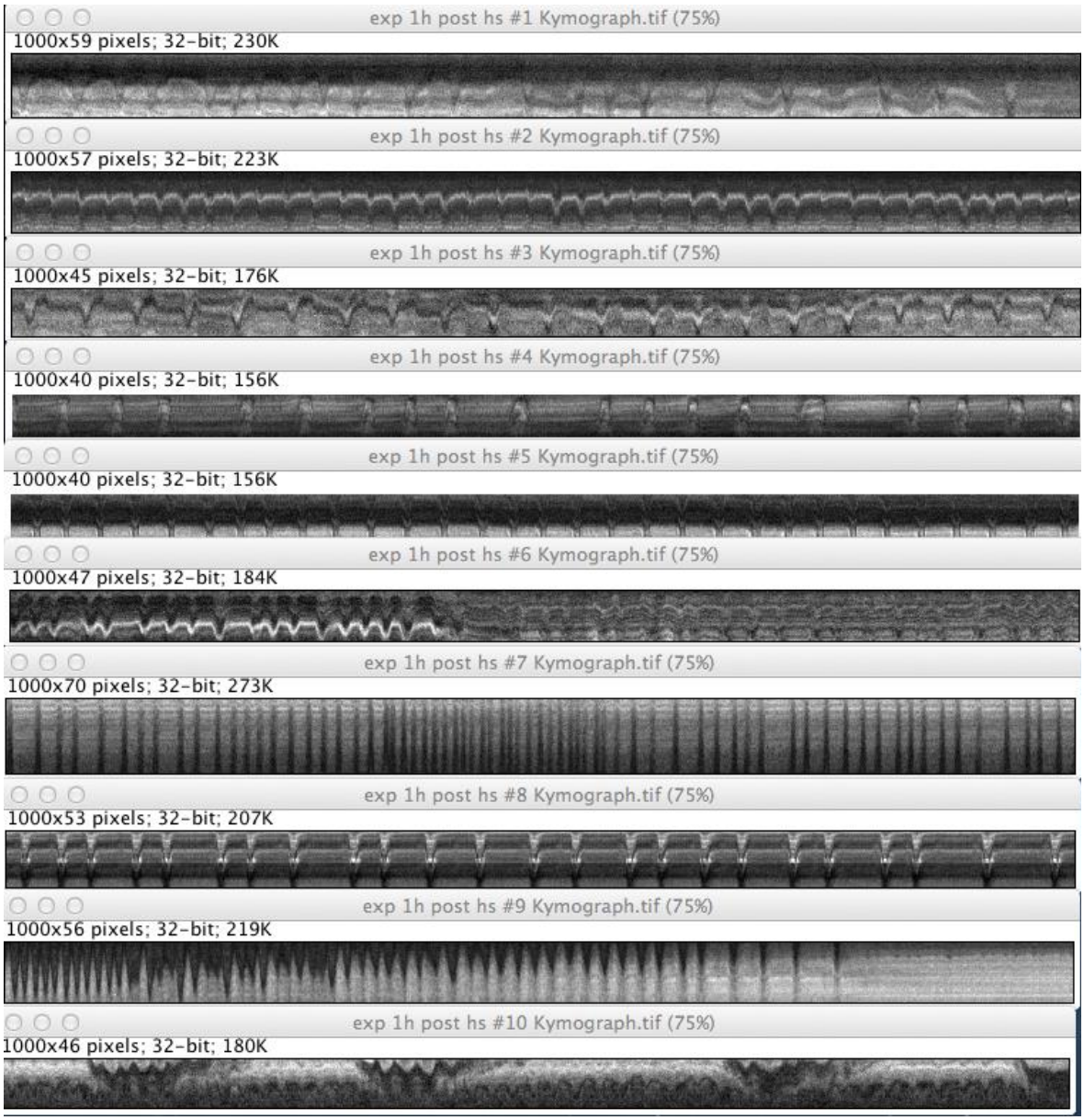

Figure 12: M-waves of experimental flies 1 hours after heat shock. Figure 12 shows, the heart rhythms of 5 experimental flies. Each peak represents a heartbeat.

arrhythmia. Overall, the experimental flies in figure 12 experienced a faster and more arrhythmic heart beat than the yellow-white flies at 1 hour after heat shock.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 2 hours after heat shock. In figure 13, the third

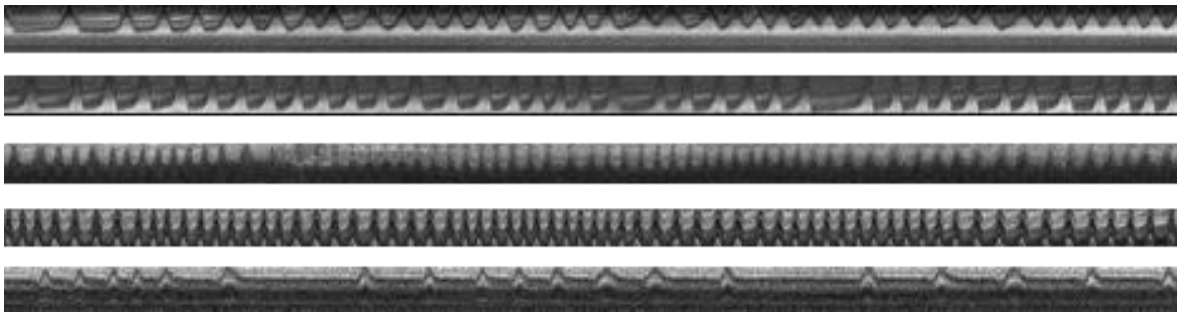

Figure 13: M-waves of yellow-white (control) flies 2 hours after heat shock. Figure 13 shows, the heart rhythms of 5 experimental flies. Each peak represents a heartbeat.

and fourth m-wave is regular while the rest are arrhythmic. The overall heart rate is slower than the experimental fly heart rates 2 hours after heat shock, but its faster than the yellow-white flies without heat shock.

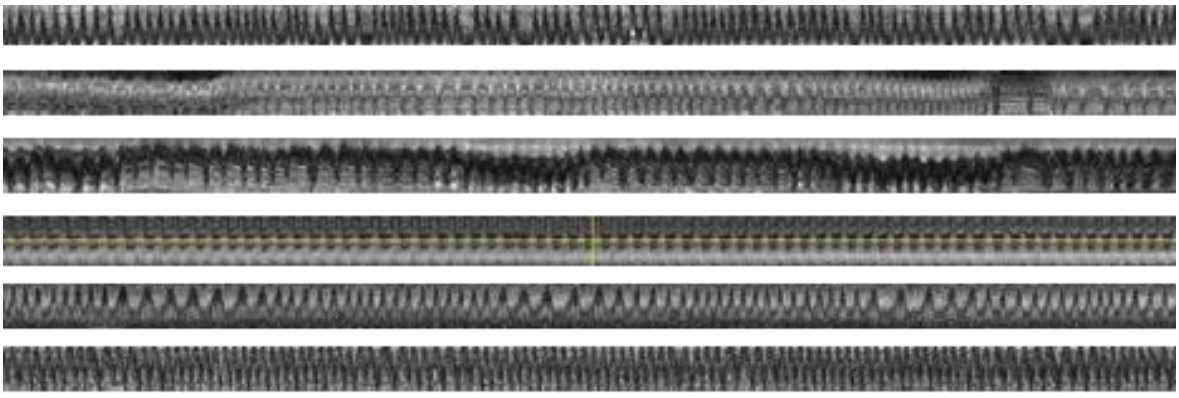

Figure 14: M-waves of experimental flies 2 hours after heat shock. Figure 14 shows, the heart rhythms of 6 experimental flies. Each peak represents a heartbeat.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 2 hours after heat shock. In figure 14, the fourth m-wave is regular while the rest are arrhythmic. Overall the experimental flies had a much faster and more irregular heart beat than the yellow-white flies at 2 hours after heat shock.

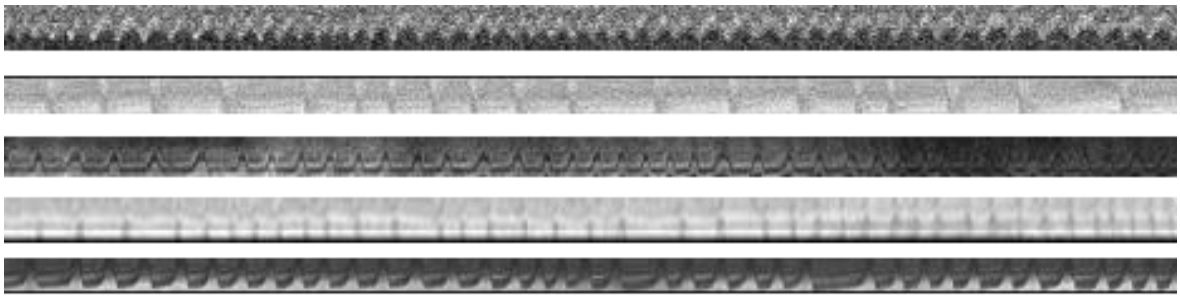

Figure 15: M-wave of yellow-white (control) flies 5 hours after heat shock. Figure 15 shows, the heart rhythms of 5 experimental flies. Each peak represents a heartbeat.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 5 hours after heat shock. In figure 15, the first and third m-waves are regular while the rest are arrhythmic. Overall the heart rate is lower and more arrhythmic than the experimental flies at 5 hours after heat shock. The heart rate is similar to the yellow-white flies tested 2 hours after heat shock.

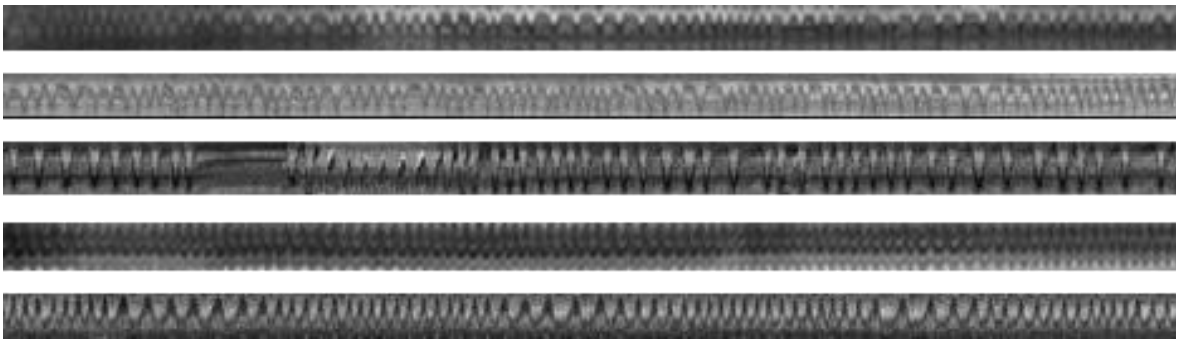

Figure 16: M-wave of experimental flies 5 hours after heat shock. Figure 16 shows, the heart rhythms of 5 experimental flies. Each peak represents a heartbeat.

To visualize the effects of TEV protease induction on fly heart rhythm, flies were dissected and immediately analyzed *via* SOHA 5 hours after heat shock. In figure 16, all m-waves are arrhythmic. Overall the experimental flies had a much faster and more irregular heart beat than the yellow-white flies at 5 hours after heat shock.

Table 2: Average heart rate and rhythmicity of YW and experimental Flies

Condition	Heart Rate (beats/min)
YW flies with no heat shock	54 (regular)
Experimental flies with no heat shock	120 (mixed regular/arrhythmic)
YW flies 1 hour after heat shock	81.6 (mixed regular/arrhythmic)
Experimental flies 1 hour after heat shock	100.7 (arrhythmic)
YW flies 2 hours after heat shock	84 (mixed regular/arrhythmic)
YW flies 5 hours after heat shock	76 (mixed regular/arrhythmic)
Experimental flies 2 hours after heat shock	180 (mixed regular/arrhythmic)
Experimental flies 5 hours after heat shock	170 (arrhythmic)

Table 2 shows the average heart rate after various treatments of experimental and YW flies. The peaks from the M-waves were counted for each fly and double to determine heart rate. The overall rhythmicity of each test condition is shown in parentheses next to heart rate.

Chapter 4

Discussion

To establish the optimal conditions for heat shock western blots were used to analyze the yield of cleaved α-spectrin. In figure 6, protein samples were collected at varying times after heat shock. The yellow-white flies show no cleavage in α-spectrin after heat shock as expected. The experimental flies which contain both TEV protease and α-spectrin with TEV protease binding site also showed no sign of cleavage without heat shock. This shows that the TEV protease expression was effectively controlled. When heat shocked, the experimental flies show consistent cleavage from 0-8hours.

Figure 7 shows the effects of multiple heat shocks on α-spectrin cleavage. Again, experimental flies showed no cleavage of α-spectrin without heat shock. There is no considerable difference in the amount of α-spectrin that is cleaved due to the second heat shock.

Figure 8 shows the effects of changing the length of heat shock. The 30 min and 60 min heat shock showed minimal cleavage at 0 min, but there was an increase in cleaved α-spectrin at 60 min. The 120 min heat shock resulted in the largest yield in cleaved α-spectrin.

To analyze the heart rate and rhythm of fly hearts SOHA was used, and heart rhythms were visualized in M-waves. Both yellow-white (control) and experimental flies were heat shocked and dissected at 2-hour and 5-hour intervals. From Figures 9-16, it is evident that both *yw* and experimental flies had arrythmia. Furthermore, the experimental flies showed tachycardia 2 hours and 5 hours after heat shock while the heart rate for *yw* flies was lower at both time points as shown in Table 2. The heart rate for *yw* flies slightly increased as a result of the heat shock procedure, but the experimental flies had a more significant increase in heart rate due to

heat shock. This shows evidence that TEV protease induction correlated with an increase in heart rate.

Paralysis was also observed and recovery after heat shock was shown in Table 1. *yw* flies had no change in mobility due to heat shock, but experimental flies showed severe paralysis until 2 hours after heat shock. At 2 hours, flies would walk when agitated which indicated partial recovery. At 4-8 hours, the flies had fully recovered in their ability to walk, but flight was never recovered in the 8 hours of observation.

From these results, paralysis and tachycardia were key effects that were only found in the experimental flies. Previous studies show that a loss-of-function mutation in βH-spectrin is known to reduce the action potentials due to mislocalization of sodium ion channels in mice; thus, cleavage of α-spectrin may lead to similar pathology in the neuromuscular junction (Bennett and Healy, 2008). This could also explain the observed paralysis phenotype. Furthermore, Spectrin is known to form a complex with ankyrin-B and Na^+/K^+ ATPase, Na^+/Ca^{2+} exchanger and the IP3 receptor in a specialized microdomain in cardiomyocyte T-tubules (Bennett and Healy, 2008). Since IP3-mediated Ca^{2+} regulation is vital to muscle contractions, it is possible that cleavage of α-spectrin alters this pathway resulting in tachycardia.

To further assess the cause of paralysis, future experimentation is necessary. The neuromuscular junction of experimental flies can be stained with an α-spectrin antibody to observe how cleavage affects its localization. In addition, visualization of IP3 and the Na^+/Ca^{2+} exchanger is also necessary to better understand the precise defects causing paralysis. In addition, due to large variability in the M-wave data, more data is necessary to better observe the tachycardia phenotype.

Appendix A

Fly Crosses

♀ *HSTEV / HSTEV ; kr / cyo ; d / TM6, tb, hu* X ♂ *w / 7 ; + / + ; α-specRG41, Ubα-specTEV, e / TM3, p^{w+}, sb, e*

↓ *d+ hu+ sb+*

♂ *HSTEV / 7 ; + / cyo ; α-specRG41, Ubα-specTEV, e / TM6, tb, hu* X ♀ *w / w ; + / + ; Df(3L)R^{R2} / TM6, tb, hu*

↓ *hu+ p^{w+}*

(experimental) ♀ *HSTEV / W ; + / cyo ; α-specRG41, Ubα-specTEV, e / Df(3L)R^{R2}*

(stock) ♂ *HSTEV / 7 ; + / + ; α-specRG41, Ubα-specTEV, e / TM6, tb, hu* X ♀ *w / w ; + / + ; Df(3L)R^{R2} / TM3, sb, e*

↓ *hu+ sb+*

(experimental) ♀ *HSTEV / W ; + / cyo ; α-specRG41, Ubα-specTEV, e / Df(3L)R^{R2}*

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ACADEMIC VITA

AMOGH KIRAN

PROFESSIONAL PROFILE

- Gained 4 years of research experience culminating in an honors thesis on Cardiac pathologies with Drosophila model
- Assisted students learning in lecture and led recitation of introductory chemistry at Penn State
- Developed strong leadership skills in the Schreyer Honors College Leadership Jumpstart program

EDUCATION

THE PENNSYLVANIA STATE UNIVERSITY *Graduation – May, 2019*

- B.S. in Biochemistry and Molecular Biology with Honors
- Schreyer Honors Scholar

ACADEMIC HONORS AND AWARDS

SCHREYER HONOR COLLEGE ACADEMIC EXCELLENCE AWARD. *August 2015 – May 2019*
DEAN'S LIST. *December 2015, 2016, 2017. May 2016, 2017, 2018*
PRESIDENT'S FRESHMAN AWARD. *February, 2016*

LEADERSHIP ACTIVITY

LEADERSHIP JUMPSTART. *July, 2015 – December, 2015*

- Trained in leadership skills
- Led public service project to connect State College residents to local business
- Organized educational event at local farmers market and on campus HUB

VOLUNTEER AND COMMUNITY SERVICE ACTIVITIES

Holy Spirit Hospital, Camp Hill, PA: Outpatient surgery and nurse department. *June, 2013 - present (240 hours)*
Pinnacle Hospital, Harrisburg, PA: Outpatient surgery department. *June, 2014 to August, 2014 (50 hours)*

RESEARCH

Research in Drosophila spectrin network. *March 2016 – present*

- Gained familiarity with *Drosophila* model
- Mastered lab techniques: qPCR, PCR, Western Blotting, SOHA, fly dissection, and fly husbandry
- Presented research in honors thesis
- Received Erickson Discovery Grant

INTERESTS AND ACTIVITIES

THON Donor and Alumni Relations committee. *September 2015 – present*
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Learning Assistant for chemistry. *January, 2016 to December, 2016*
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