6-1-2011

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Recommended Citation

Tubbs, T., Palazotto, A. N., & Willis, M. (2011). Biological investigation of wing motion of the manduca sexta. International Journal of Micro Air Vehicles, 3(2), 101–117. https://doi.org/10.1260/1756-8293.3.2.101

Biological Investigation of Wing Motion of the Manduca Sexta

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Received on 13 April 2011; Accepted on 26 July 2011

ABSTRACT

An investigation was conducted assessing the feasibility of reproducing the biological flapping motion of the wings of the hawkmoth, *Manduca sexta* (*M.sexta*) by artificially stimulating the flight muscles for Micro Air Vehicle research. Electromyographical signals were collected using bipolar intramuscular fine wire electrodes inserted into the primary flight muscles, the dorsal longitudinal and dorsal ventral muscles, of the adult *M.sexta*. These signals were recorded and associated with wing movement using high speed video. The signals were reapplied into the corresponding muscle groups with the intention of reproducing similar flapping motion. A series of impulse signals were also directed into the primary flight muscles as a means of observing muscle response through measured forewing angles. This study pioneered electromyographic research on *M.sexta* at the Air Force Institute of Technology with tests conducted with fine wire electrodes. Through this process, the research showed the deformational structural changes that take place when a wing is removed from an insect and proved that muscular stimulation is a viable method for generating wing movement. This study also assisted in developing an understanding related to the role that a thorax-like fuselage could play in future micro aircraft designs. This study has shown that partial neuromuscular control of the primary flight muscles of *M.sexta* is possible with electrical stimulants which could be used to directly control insect flight.

1. INTRODUCTION

The primary objective of this research was to determine the possibility and benefits of reproducing the biological flapping motion of a hawkmoth, *Manduca sexta* (*M.sexta*), with artificial stimulation for the purpose of Micro Air Vehicle (MAV) development. An examination was conducted of the electromyographical (EMG) signals produced by the dorsal longitudinal muscles (DLMs) and dorsal ventral muscles (DVMs) in the adult *M.sexta* using a bipolar intramuscular fixed wire implant. The EMG signals were recorded, and an attempt was made to associate the signals with wing movement using high speed video. The recorded EMG signals were then reapplied into the corresponding muscle group with the intention of reproducing similar flapping motion. Additional impulse signals were then applied to determine the resulting wing response.

This investigation will shed additional light on the biomechanics of insect flight and provide information that could be utilized directly for bioelectric muscle control of an insect, or could be extrapolated for future MAV designs based on the biological response characteristics. An additional aspect of this research is the possibility of using *M.sexta* as a "living" flapping mechanism, which would allow wing movement to be studied with the insect's natural boundary conditions intact. The ability to induce desired wing movements through electro-muscular stimulation has the potential to greatly advance biologically inspired MAV research specifically with respect to control design.

This study focused on the specific biological system of *M.sexta* (Figure 1). There is no question that *M.sexta* is a living Micro Air Vehicle that has developed through evolution for millions of years with the unique attributes that DARPA desires in MAVs by 2030 (Table 1). The qualities that enable functional flapping motion are of great interest, and were examined at length with the intention of first

understanding the biological process, next quantitatively determining the neuromuscular signals, and then experimentally associating flapping motion with signal inputs.

Figure 1: An adult female Manduca Sexta (hawkmoth) from the colony raised by Dr. Mark Willis at Case Western Reserve University

Table 1. MAV Design Requirements

Specification	Requirements	Details
Size	< 15.24 cm	Maximum Dimension
Weight	~ 100 g	Gross Takeoff Weight
Range	1 to 10 km	Operational Range
Endurance	60 min	Loiter Time on Station
Altitude	< 150 m	Operational Ceiling
Speed	15 m/s	Maximum Flight Speed
Payload	20 g	Mission Dependent
Cost	$1,500	Max Cost, 2009 (US Dollars)

2030 DARPA Specifications [1]

This research is intended to evolve and grow to incorporate some form of control mechanism that could be adapted and incorporated into a free flying M.sexta, with the aim to directly control flight through human supplied signals. This would be a biological MAV. This has been successfully accomplished with beetles [2] and has been tested on M.sexta [3] with promising results, but with no free flight capability yet. The data presented in this paper is intended to assist with or inspire the pursuit of the effort.

2. BACKGROUND

2.1. M.sexta Flight Muscles

There are many different biological areas that need to be considered with regard to MAV flight. This research will focus primarily on the two major power producing muscles in flying insects, the dorsal longitudinal muscles (DLMs) and the dorsal ventral muscles (DVMs). The main power-producing muscles are stimulated by a single motor neuron, which means the total number of neurons controlling power production during flight is small [4].

The DVMs are the elevator muscles and indirectly cause the upward movement of the wings in all insects. This occurs because when the DVMs contract, they pull down the tergum (dorsal surface of the thorax), which moves the point of articulation of *M.sexta*'s wing down also (Figure 2A) [4]. In most

insects (including *M.sexta*), the downward wing (depression) is caused indirectly when the DLMs contract; then, the center of the tergum becomes bowed upward, and this moves the upper wing joint upward and the wing flaps down (Figure 2C) [4].

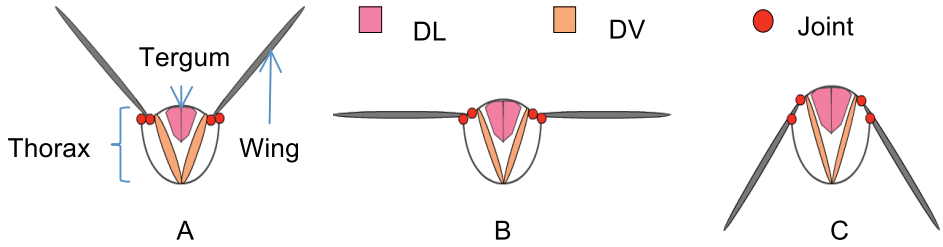

Figure 2: A cross-sectional view of a M.sexta thorax showing the primary flight muscles: A) DVM contracting, causing an up-stroke; B) DVM relaxing, while the DLM begins to contract; C) DLM contracting, causing a down-stroke

2.2. What is Electromyography?

The standard process of recording muscular contraction signals is Electromyography (EMG). EMG is a technique for evaluating and recording the electrical activity produced by muscles [5]. According to the National Library of Medicine, EMG is the measurement of the electrical potential generated by muscle cells while they are electrically or neurologically activated [6]. In insects, as well as vertebrates, biological signals are sent from the central nervous system through specialized cells, called motoneurons, to the muscles [7]. When the muscles receive the electrical impulse, an ionic reaction known as the Action Potential (AP) is triggered. EMG is used to study this electrical impulse during propagation through the muscle. Two electrical leads are used to measure the electrical AP of muscle fibers.

Using EMG on *M.sexta* muscular studies is not a new concept: Johnston and Levine have observed electromyographic activity of the leg muscles synchronized with video-taped recordings in the larval crawling and adult walking stages of *M.sexta* [8]. Wang, Ando, and Kanzaki used EMG to study the flight movement of the wings during free flight in a different species of hawkmoth [9], and Mohseni et al. studied the use of an ultralight biotelemetry backpack for recording EMG signals in *M.sexta* [10].

The research herein is designed to provide an understanding of the signals that are required to induce natural wing movement. A portion of this study is based on research that was carried out by Alper Bozkurt at Cornell University, where surgical implants were directly placed into an *M.sexta*'s muscles during the pupal stage. Bozkurt's work demonstrated that EMG signals could be recorded and that electrical signals could transmitted to the moth, altering movement and behavior [3]. As stated earlier, the primary objective of this paper is to determine what is required to reproduce the biological flapping motion of *M.sexta* with artificial stimulation. Bozkurt has already demonstrated the ability to induce wing movement which indicates that *M.sexta* is capable of being used as biological MAV.

2.3. Biological Flapping Mechanism

An additional benefit for developing a method to quantitatively predict the flapping motion of the *M.sexta* is the ability to study the wing motion while maintaining the natural boundary conditions. The wing hinge is a complicated and difficult mechanical system that is currently impossible to duplicate and not well understood. It has been noticed that many researchers [1], [11] are simply removing *M.sexta* wings from the thorax and testing the wing properties separately. There are two fundamental problems with this approach. First, the wing's properties are time dependent when removed from the body, and second, the boundary conditions are completely altered from what is found naturally.

When removed, the wings begin to immediately desiccate (dry out), as seen in the time-lapse images shown in Figure 3. The most dramatic changes take place within the first three hours of removal, where the curvature of the wing becomes much more pronounced, and the structure becomes more brittle.

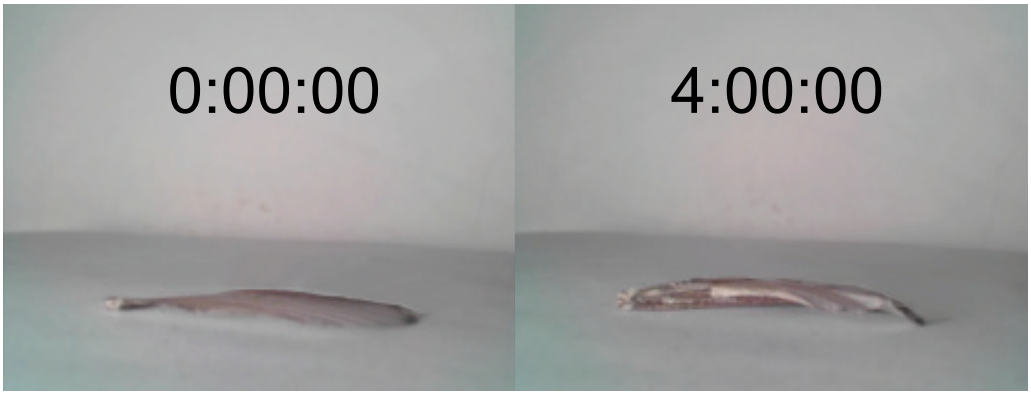

Figure 3: Images taken from time-lapse video showing the structural changes of a severed wing over time. These two images were taken four hours apart, during that time the wing underwent significant structural changes do to desiccation.

Despite the changes of the wing structure, the only way to correctly model the biological movement of *M.sexta* is to account for the boundary condition. The current technique is to remove the wing and clamp it in some sort of flapping device to investigate the flapping properties of the wing. This induces a rigid boundary similar to a beam, while the physiological joint is much more complicated, involving many linkage systems as well as direct and indirect muscles.

The ability to study *M.sexta* wings under different flapping frequencies and movements while eliminating the need to change the boundary conditions or remove the wings would vastly improve the quality of the data. Sending known signals into the appropriate muscles and receiving expected angular displacement would essentially transform the moth into a biological, flapping mechanism with all of the biological processes, mechanisms, and structures still accounted for.

3. EXPERIMENTATION

3.1. Internal Examination

Case Western Reserve University provided multiple specimens to study and experiment with. It was essential for this study to determine the exact location of the muscle groups in an adult *M.sexta* so that the primary flight muscles could be properly tested. In order to gather this information, a Computed Tomography (CT) scan was performed on an anesthetized adult *M.sexta*. The CT images clearly showed the desired muscle structure's shape and location which was vital for proper implant placement. A complete scan of the Manduca was initially performed (Figure 4A) to give an oval understanding of the entire physiological structure. A more detailed and focused scan of the thorax provided valuable information regarding the internal arrangements and structure (Figure 4B & 4C) of the Dorsal Longitudinal Muscle (DLM), which depresses the wings, and the Dorsal Ventral Muscle (DVM), which elevates the wings.

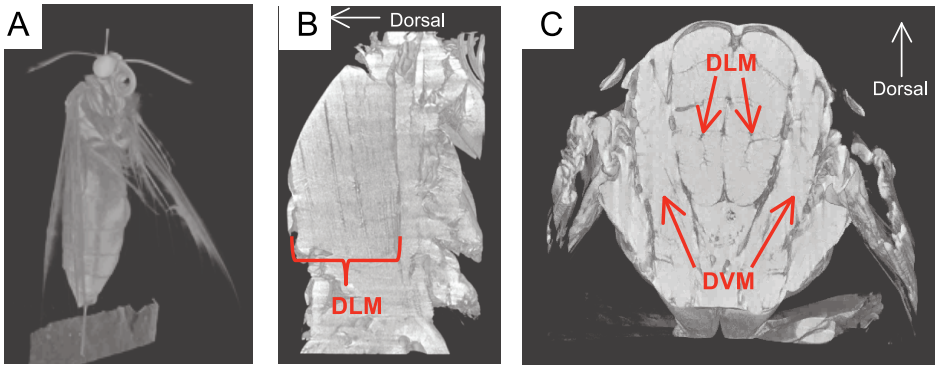

Figure 4: A) Computer Tomography of a *M.sexta*; B) Side view showing the Dorsal Longtudinal Muscle; C) Cross section showing the Dorsal Longitudinal Muscles (DLMs) and Dorsal Ventral Muscles (DVMs)

3.2. Fine Wire Implants

Fine wire implants were placed directly into the flight muscles of *M.sexta* that were observed from the CT scans. Prior to testing, the moths were allowed to fly untethered to ensure the capability of sustained flight. During testing the moths were glued to a fixed stand (also known as a sting). The moths were not allowed to fly freely to ensure the wing movement could be analyzed in detail. It was assumed that the recorded EMG signals and corresponding wing movement when mounted on the fixed stand were similar to those produced during free flight. All EMG signals that were analyzed were of continuous full flapping for longer than five seconds; this helped to ensure similar flight characteristics much as possible. Future research will attempt to use wireless implants which should reduce flight interference.

Fine wire electrodes were placed in the DLMs and DVMs (Figure) as described in the thesis, Biological Investigation of the Stimulated Flapping Motions of the Moth, *Manduca Sexta* [12]. The silver wire electrodes were then connected to a low-pass filtered amplifier. The differential was recorded using the analog to digital converter, NI USB-6229, which was connected to a Dell laptop computer. The EMG signals that were amplified were ~50 mV.

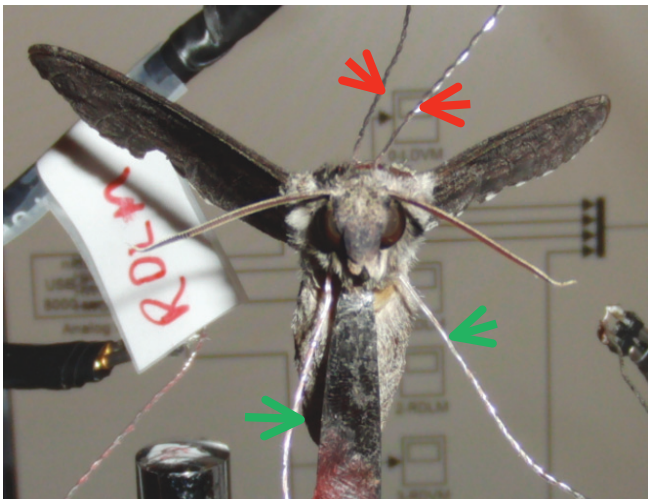

Figure 5: Implanted *M.sexta* in the DLM (red arrows) and DVM (green) using 0.008" silver fine wires coated with Teflon

Matlab's Simulink program was the primary program used for recording the low-pass filtered and amplified EMG signals. After the signals were recorded, analyzed and processed then Simulink was again used to transmit the recorded signals back into the same flight muscles. The implants remained in place throughout the process to ensure no spatial difference so the signals were being supplied in the same location that they were recorded from.

High speed video was taken to record wing movement, whenever signals were transmitted to the moth, as a means to categorize the muscle response. Initially, the recorded EMG signals were sent to the appropriate muscles as a means to determine if EMG recorded signals could illicit similar muscle responses. Next, multiple different signals and frequencies were transmitted in an attempt to assess wing response due to changes in electrical signal conditions. The results are discussed in Chapter IV.

As an alternative means to verify the validity of the signals and collection process the amplified signals were also split off and collected using the commercially available hardware know as Fast Track Pro. Fast Track Pro is designed to record analog audio inputs but it also proved capable of recording amplified electromyography signals. The software supplied with Fast Track Pro, Ableton Live 8.1.4 [13], was used to record the signals. In addition to the supplied software, Audacity 1.3 Beta, a free cross-platform sound editor, was used to record EMG signals from Fast Track Pro [14]. Recording the signals multiple ways demonstrated consistency in the signal collection process, and served to validate the methodology (Figure 6). A depiction of the EMG recording and transmission system can be seen in Figure 7.

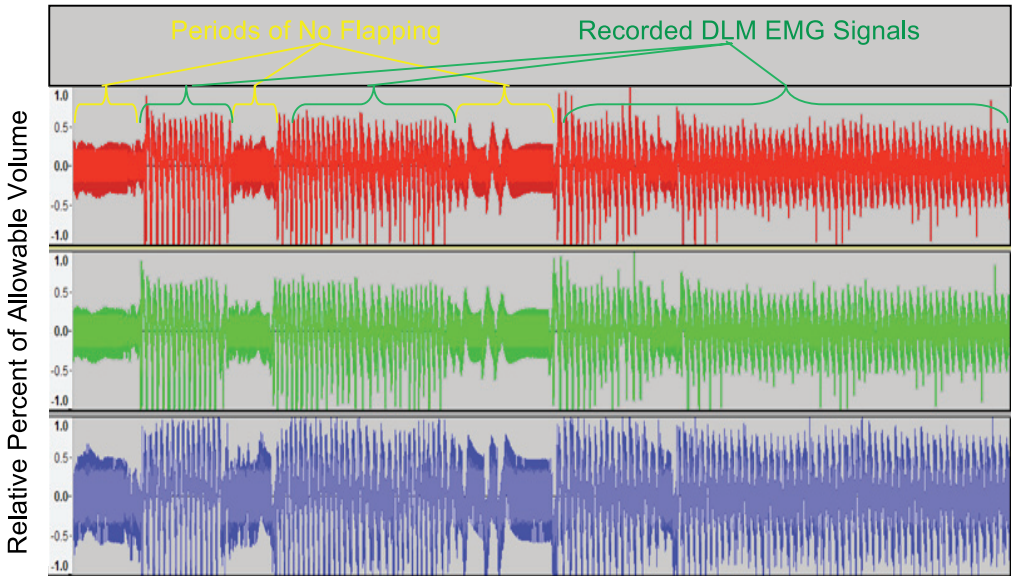

Figure 6: Three different recordings of the same DLM EMG signal. The red signal was recorded using the Fast Track Pro hardware and its associated Ableton software. The green signal was also recorded using the Fast Track Pro hardware but the freeware Audacity was used for the recording. The blue signal was the primary method of recording EMG signals; this process used the analog to digital converter, NI USB-6229 and Matlab's Simulink software.

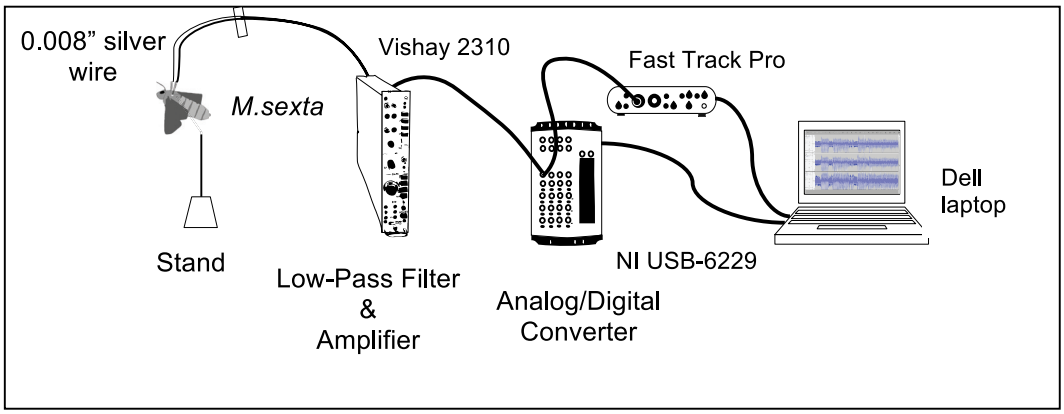

Figure 7: EMG signal recording and transmission process

3.3. High Speed Wing Angles

Collecting the EMG signals provided information on the muscular movement internal to the moth, but this information needed to be associated with structural wing movement. The ability to monitor the actual wing movement is essential to understanding flight characteristics. High speed video taken during flight like flapping motion as well as the corresponding EMG signals, could provide data concerning to the causal relationship of the neuromuscular signals and wing movement.

To calculate the leading edge wing angle to associate with the recorded EMG signals and the transmitted signals, high speed photography at 420 frames per second (fps) was used throughout the research. The first step in this process was to find the resting position of the leading edge of the wing when no signals were being sent.

Adobe After Effects CS3 software [15] provided the ability to place a blue dot on the pivoting joint (as seen in Figure 8-2a), where the wing meets the thorax, of the forewing in a layer superimposed on top of the video frames. A red dot (seen in Figure 8-2c) was placed on top of the leading edge of the wing when it was at rest. The green dot (seen in Figure 8-2b) marks the tip of the wing. The line

formed between the joint (blue dot; a) and the leading edge at rest (red dot; c) marked the base line (zero degrees) by which all other wing measurements were taken.

The Adobe After Effects software provides the ability to track high contrast areas throughout the video, so the leading edge wing tip was marked with a green dot and tracked throughout a video sequence. Often, the pixels were lost during the tracking process, so hours were spent processing the data (frame by frame) to mark the wing tip. This process could have been greatly improved with a higher contrasting background.

When the video sequence was marked appropriately, the background video was removed so that only the three colored dots were visible (Figure 8-3). The three dot video was rendered at 29.97 fps which is the standard for television, and the video was then pulled into the Matlab software. Using Matlab's Red, Green, Blue (RGB) layering system (which breaks colors into their own data set), and the centroid was calculated for each dot. Using the centroid information two vectors were created (Figure 8-4), one for when the wing was at rest (Figure 8-4;) and one that tracked the wing movement (Figure 8-4;). The angle between the baseline, when the wing was at rest, and the wing tip was calculated for each sequenced frame. Despite the time this took, the process proved invaluable for measuring the leading edge wing angle and associating it with the recorded and transmitted signals.

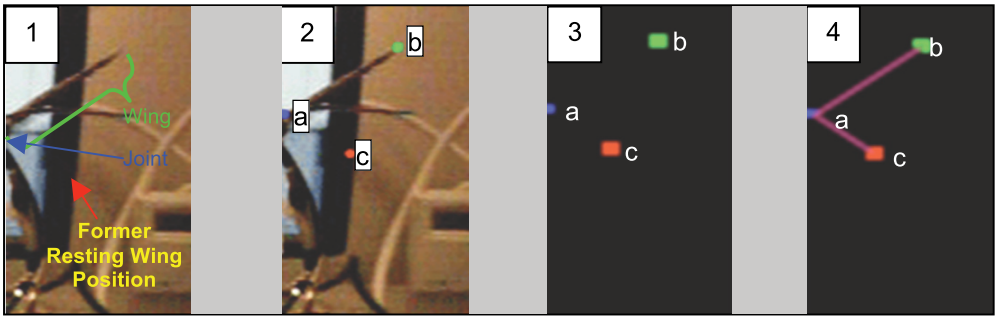

Figure 8: 1) A high speed video image showing highest point on up-stroke being processed through Adobe After Effects and Matlab. 2) The blue dot (a) on the edge of the picture represents the wing joint; the green dot (b) follows the leading edge throughout the flapping motion; the red dot (c) indicatis the wing position at rest. 3) Background removed so only the colored dots remain. 4) The vector formed from blue (a) to red (c) represents the wing at rest by which wing angular displacement vector, blue (a) to green (b) was measured against.

3.4. EMG and Flapping Motion

The EMG signals were recorded and reapplied to the muscles. This enabled a comparison between the observed "natural" wing movement that the moth produced during flight and the artificial induced signals that could be applied to the muscle. The differences between the flight patterns were of primary interest. The amplified signals were transmitted to the moth because of insect muscles do not propagate the Action Potential, increasing the voltage resulted in better and more consistent results.

After sending the recorded EMG signals into the respective muscles, an attempt was made to determine the wing response to a simple impulse signals. To associate the EMG signal and artificial stimulating signal with wing movement the position of leading edge was measured and the difference between the leading edge position and the resting wing position was recorded as the wing flapping angle. Because this is an initial investigation, it was determined that measuring the change in the wing angles was sufficient to verify the process.

The importance of this work is to demonstrate control over wing movement. This ability to induce known wing responses provides the potential capability of controlling free flight behavior of a moth, in essence making it a biological MAV. This control could aid in studying manufactured wings, which could be attached to the moth and analyzed. Understanding how to control wing movement could also provide insight into the design and control of synthetic MAVs. But before wing control can be established, a clear connection between signal input and wing movement must be identified

4. RESULTS AND DISCUSSION

4.1. Unstimulated Flapping

An unimplanted moth was glued to the stand and high speed video recorded the natural flapping motion of the wings. The resting position of the wing served to mark the zero point by which all angles were measured. When the moth flapped its wings the angle difference between the current wing position and the initial resting position were recorded. The flapping angles were plotted over time to determine if this was a viable wing angle measurement process compared to *M.sexta* wing movement published by Willmott and Ellington [16].

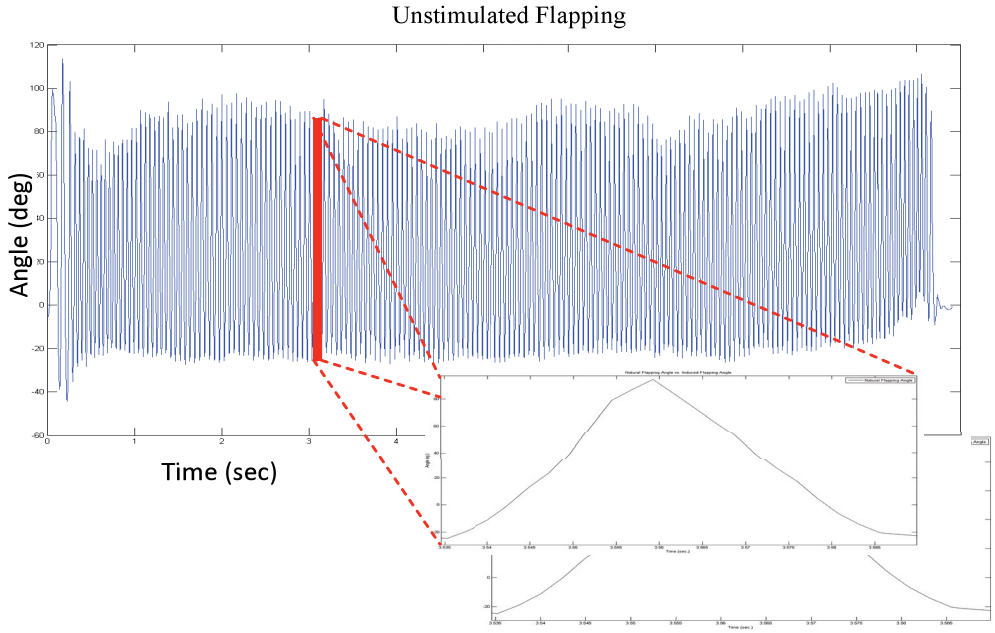

Figure 9: M.sexta wing angles, the fly-out shows a closer view of one complete flapping cycle.

A random cycle of the flapping angles found during this research was overlaid on the experimental data found Willmott and Ellington [16] and appeared to be very similar (Figure), the frequency (25Hz) was nearly identical so the results show very similar angular wing movement, this validates the process used to record angular wing movement using high speed video and colored marker overlay. The data suggests that the tested moth in this study was not depressing its wings as much as experimental data. There are many different explanations for this. Some possible reasons could be: variability between individual specimens, implanted with fine wires versus a moth that was unimplanted, different angle measurement processes, fixed versus free flight flapping, atmospheric or environmental factors, or muscle fatigue.

4.2. Transmitted EMG Signal

The recorded EMG signals were transmitted back into the muscles of the implanted moth after switching the BNC cables onto the output ports of the NI USB-6229. The flapping angle was measured (Figure 11) and compared to the unstimulated flapping angles (Figure 9). Based on these findings, it appeared that the overall input caused a greater net force in the DLMs because all of the angles were measured below what was deemed the resting angle. This indicated that *M.sexta* would respond to input signals, but clearly not in a similar fashion as it did when the EMG signals were collected. The moth would not perform the same flapping motion that generated the EMG signal when that same signal was reapplied to the muscle.

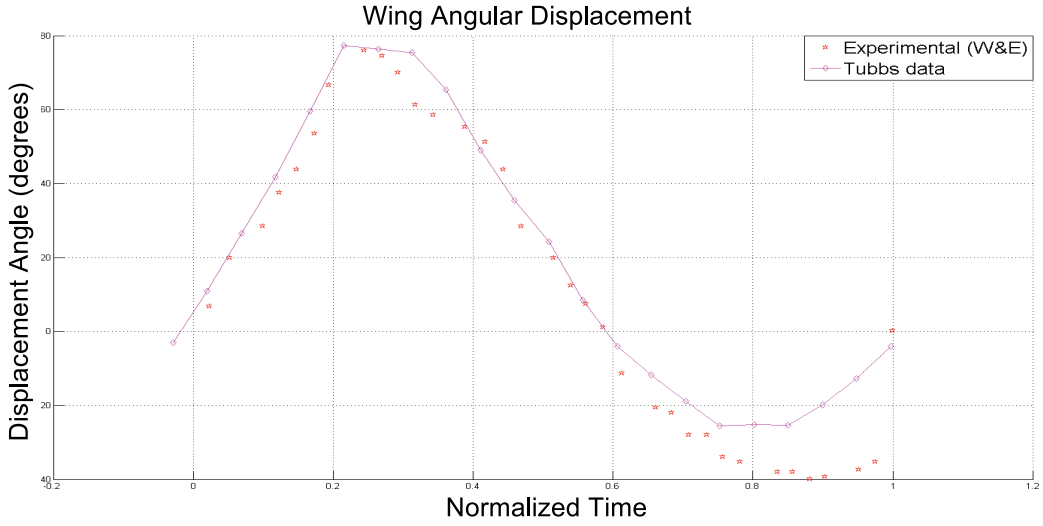

Figure 10: Overlay of flapping angles with those found experimentally from Willmott and Elington [16]

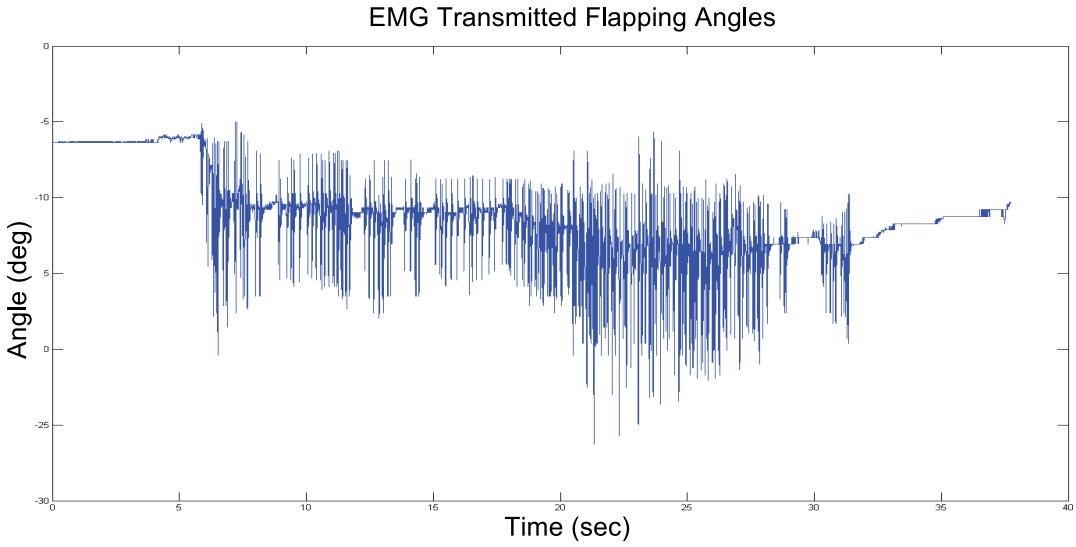

Figure 11: Flapping angles found when stimulated with previously recorded EMG signals

It is not surprising that the EMG signals transmitted back into the muscles did not produce the same flapping angles because insect muscle fibers do not propagate the depolarization Action Potential like motor neurons [17]. The EMG signal supplied by the electrodes was a localized stimulation, versus using the distributive properties of multi-terminal innervation and the transvers tubular system to ensure that the entire muscle was reacting to the signal. Another reason it may have responded differently could have been attenuation through the muscle, as well as the noise that was inherited through the collection process.

4.3. Transmitted Impulse Signals to Individual Muscles

It was understood that for insects, the entire muscle would not react to the EMG signal because of their inability to propagate the depolarization signal. So, a known signal was supplied to the individual muscles in an attempt to assess what the muscle response was to the given signal. A 5V impulse signal with a period of 1.9 seconds and a signal length of 0.19 seconds was transmitted from the computer,

using Simulink through the NI USB 6229 hardware, out through the fine wire electrodes into the left dorsal ventral muscle (LDVM). The responding wing movement was collected from the high speed video images and processed. The 5V impulse signal was generated asynchronously and the timing was adjusted to correspond with estimated transmit time.

Figure 12 shows the impulse signal in red superimposed upon the responding wing angle (in blue). It is clear that there was direct muscular response to the signal which indirectly induced wing movement. By using Matlab to offset the resting angle to zero and then averaging the maximum angle between each pulse (beginning after the initial flapping, between 0 and 3 seconds), the average angular wing displacement was found to be about 5.2 degrees.

The initial flapping (between 0 and 3 seconds, as indicated by the arrow) was not used in the average wing angular response calculation because these angles were the moth's neurologically directed wing movement due to an electrical signal being sent to its muscles. The signals within the green bracket are the wing angles generated due to the muscle twitch response to the electrical signal not the moth's nervous system, so these are the signals of interest.

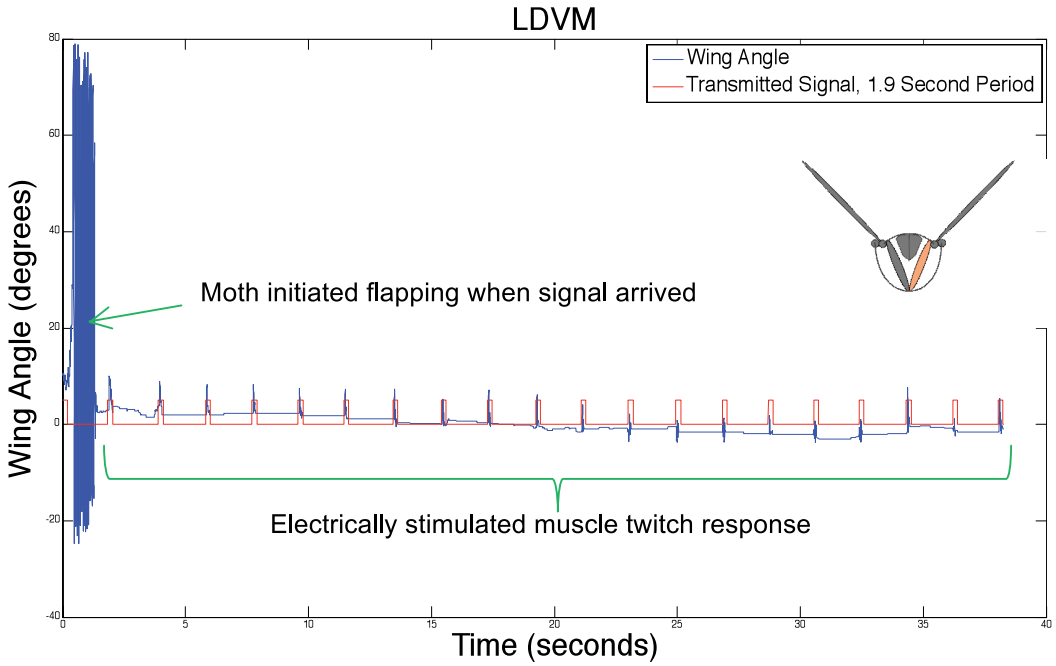

Figure 12: 5V signal directed into the LDVM at a 1.9 second interval; the impulse was 0.19 seconds long

This process was repeated for each of the primary flight muscles. The average wing angular response, from a 5V signal directed into the left dorsal longitudinal muscle (LDLM), was found to be -17.9 degrees (Figure 13). This information was again found using Matlab by offsetting the resting angle to zero, and then averaging the minimum angle between each pulse.

A large response was seen when the right dorsal longitudinal muscle (RDLM) were stimulated (Figure 14). The 5V signal with a 1.9 second period and a 0.19 second signal length was transmitted to the muscle, but the response was unique because it caused *M.sexta* to flap its wings in the down stroke position twice for each impulse signal transmitted. The most likely reason for this was that one flap was caused when the signal began, and the second flap was for when the signal stopped. The double flap with each signal could also have to do with the electrode location, meaning that the fine wire electrode may have been placed slightly closer to a motoneuron resulting in a clearer muscular response to electrical stimuli. The desired implant locations can be seen in Figure 15.

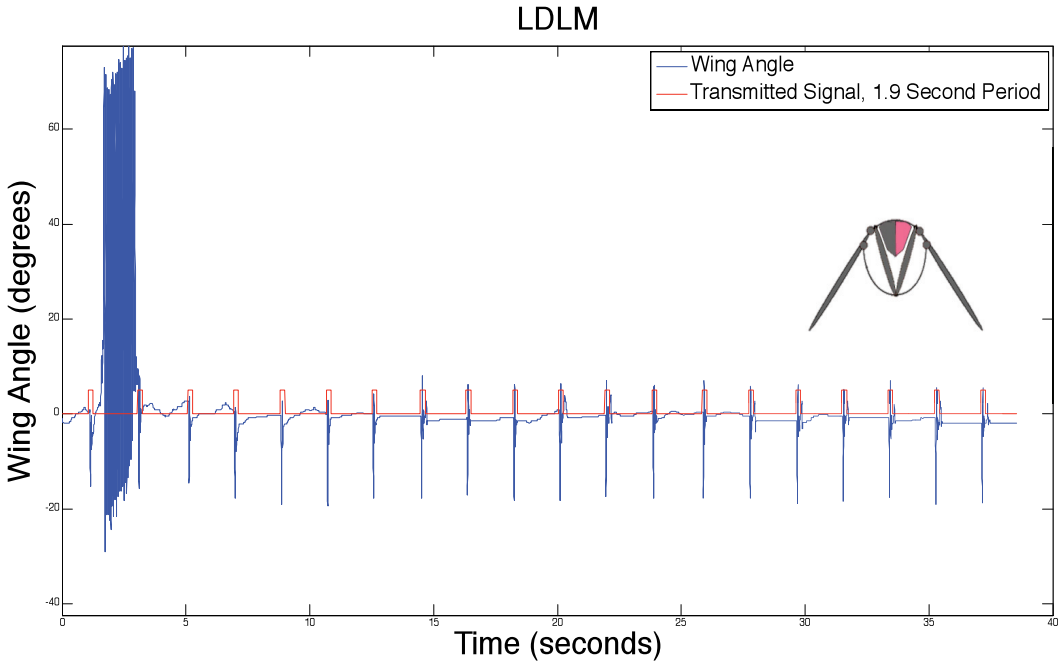

Figure 13: 5V signal directed into the LDLM at a 1.9 second interval; the impulse was 0.19 seconds long

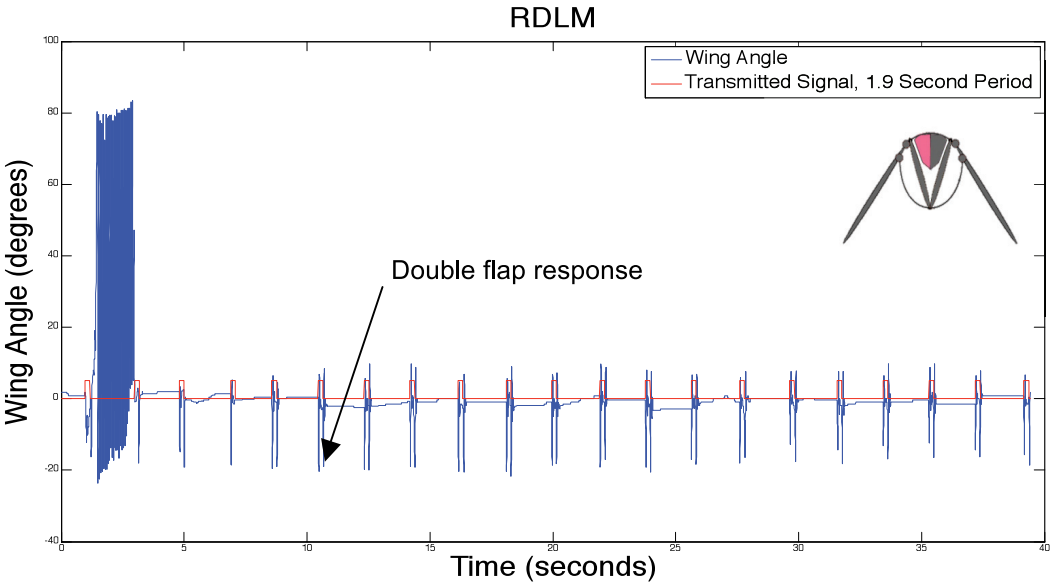

Figure 14: 5V signal directed into the RDLM at a 1.9 second interval; the impulse was 0.19 seconds long

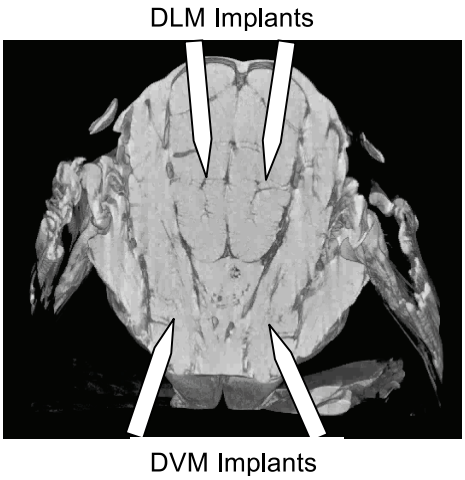

Figure 15: Desired locations for the fine wire implants

Again using Matlab to offset the resting angle to zero, then averaging the minimum angle between each pulse (beginning after the initial flapping between 0 and 4 seconds), the average wing angular response was found to be -18.8 degrees.

The last muscle group to be tested independently with the 5V signal was the right dorsal ventral muscle (RDVM). By averaging the maximum angle between each pulse (beginning after the initial flapping, between 0 and 3 seconds), the wings were elevated to an average of 5.7 degrees with each corresponding impulse (Figure 16). This appeared to be consistent with the LDVM and with the literature, which indicated that the DVMs were not as powerful as the DLMs [18].

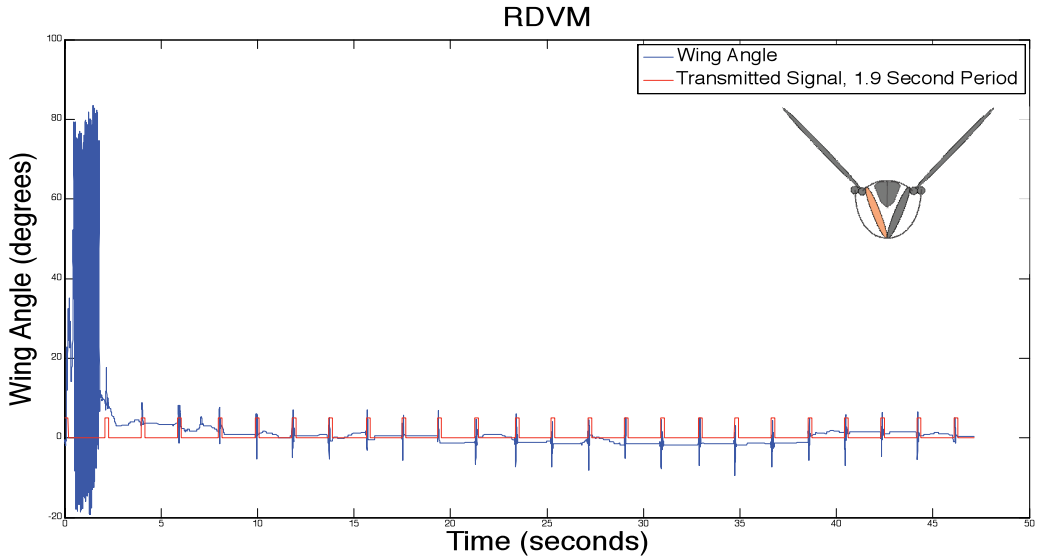

Figure 16: 5V signal directed into the RDVM at a 1.9 second interval; the impulse was 0.19 seconds long

4.4. Transmitted Impulse Signals to DLMs and DVMs

Once the individual muscle responses were isolated and recorded, the same signal was sent to all four of the muscles being studied. The DLM signal was delayed by 0.5 seconds so that the different signals could be detected. Figure 17 shows the angular response of two different impulse signals being supplied to the two main muscle groups. The red line represents the two 5V signals, with a 1.9 second period (pulse width of 0.19 seconds), that were generated asynchronously and the timing adjusted to correspond with the estimated time that the signals were transmitted into the DVMs due to variable computer processing time. For demonstration purposes these signals was superimposed upon the

responding wing angle. The green line represents the same signal, with a phase delay of 0.95 seconds supplied to the DLMs.

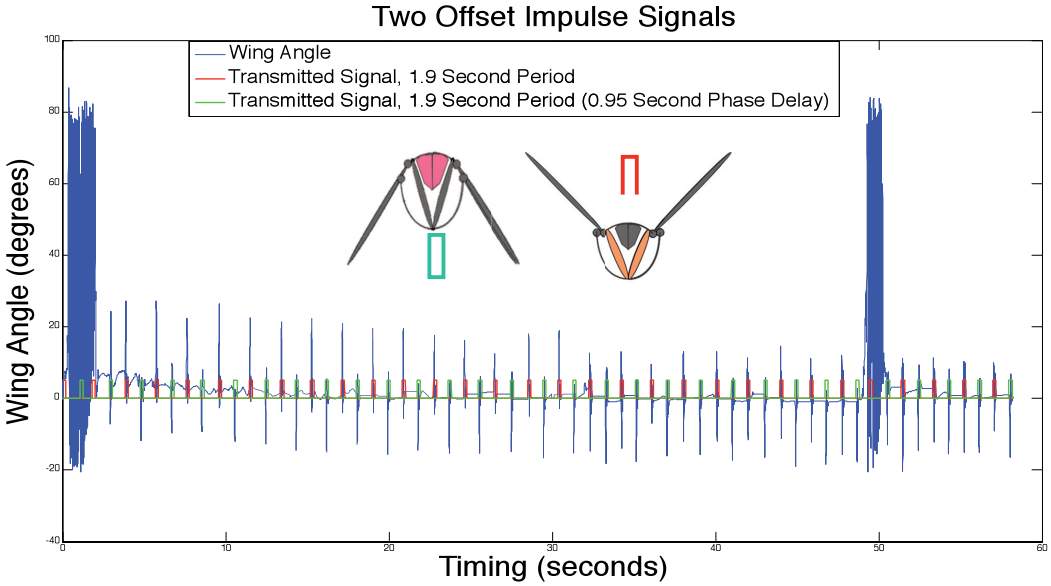

Figure 17: Wing angle response with two different 5V signals, the DLM impulse signal was 180 degrees out of phase

There were two periods of time when the moth flapped under its own volition. The first was at the beginning of Figure 17, when the signals first arrived at the muscle and *M.sexta* immediately responded. This immediate flapping response when the signal first arrived has been seen on the previous four graphs. The second unstimulated flapping occurred around the 49 second mark. After the initial flapping, the beginning of the graph showed a very distinct angular reaction for the two different muscles that were being stimulated. The magnitude of the DLM response gradually became less pronounced over time. This graph clearly indicates that the DLM and DVM muscle groups can be stimulated separately to achieve different wing movements.

4.5. Faster Transmitted Signals

The 5V impulse signal with a 1.9 second period was useful for depicting what each muscle was stimulated to do. However, this was not the rate at which *M.sexta* flapped its wings. To encourage the more realistic flapping movement, the signals were transmitted at a faster rate.

Figure 18 shows the wing angles when supplied with a 5V signal, with a 0.756 second period and a signal length of 0.0756 seconds. The wing movements became much more similar to those observed during independent flapping, when no signal was being supplied, but the range of motion was not as great. The disparity between the two signals still needs to be analyzed to determine what changes could induce the wing to achieve the full range of motion. As with the other signals, the red line was being supplied to the DVMs, and the green line is being directed into the DLMs. This graph justifies the belief that predictable and near natural wing motions can be supplied with artificial signals.

The wing angles appeared to be very similar to natural wing movement when superimposed upon the unstimulated wing movement (Figure 19). The artificially induced wing movement (Figure), seen in green, was superimposed upon the natural wing movement produced without external electrical stimuli, as seen in blue.

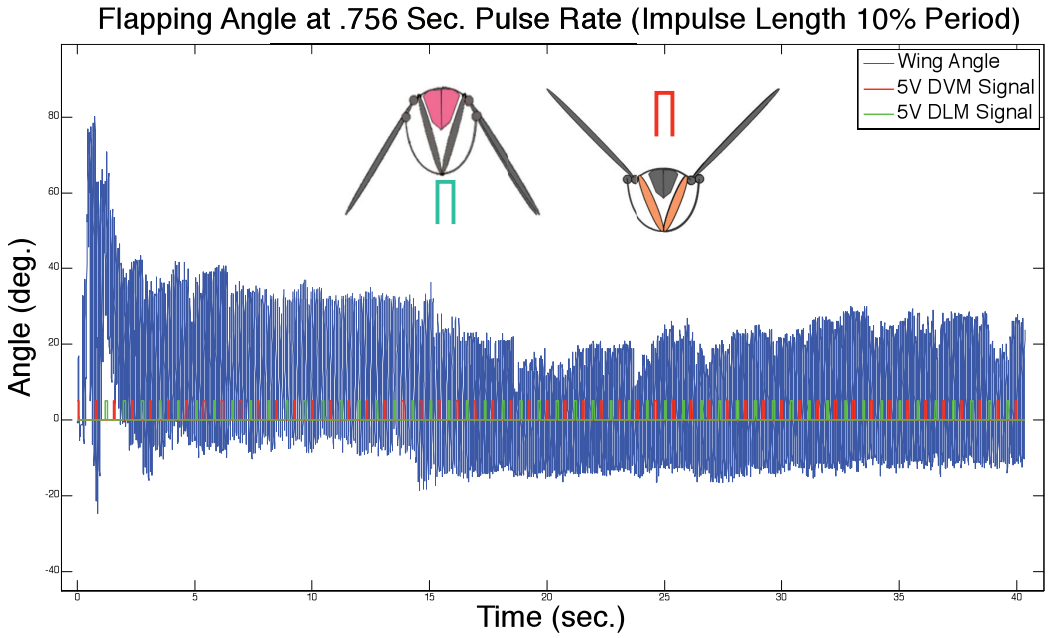

Figure 18: Two different 5V signals supplied with a 0.756 second period and a 0.0756 second signal length; the red is supplied to the DVM and the green is supplied to the DLM

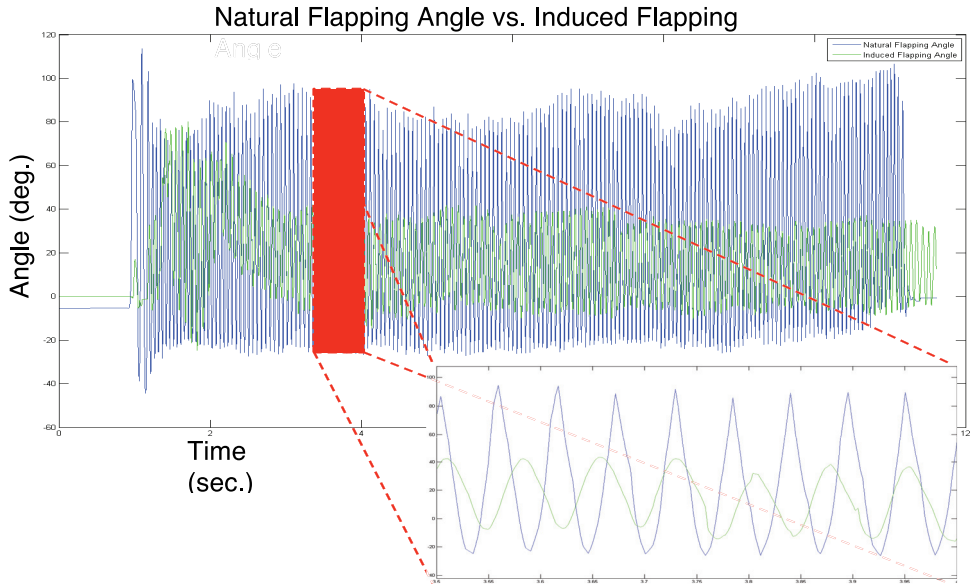

Figure 19: The natural flapping angle from the M.Sexta (blue) and the artificially stimulated signal (green). The fly-out shows that the frequencies and angular range are different.

Figure 19 shows the angular difference that the moth produced naturally (blue) versus what was stimulated (green). It was evident that the stimulated wing did not have the full range of motion when compared to natural wing movement. The signal did appear to be similar in cycle length, the time between the down and up-stroke. The most likely reason for reduced wing motion was caused by incomplete contraction of the DLMs, which indirectly increase the wing angle. It could also be caused by the need to stimulate additional flight muscles which were not examined in this research such as the Basalar or Pleuroaxillary muscles. These muscles control finer wing moves and are not considered the primary power producing muscles but additional research is required to determine their role in stimulated flight control [16].

As a means to demonstrate that the artificially transmitted signal caused the flapping motion, the same signal (as seen in Figure 17), which was a 5V signal with a 0.756 second period and a signal length of 0.0756 seconds) was transmitted to the *M.sexta*. During this test, the signal was randomly stopped (Figure 20). The flat green line (arrow) indicates the times when no signal was being supplied. It is evident that the signal was driving wing movement. When the signals were removed, no wing motion took place. This once again justifies the fact that artificial signals can be used to induce known and expected wing movement.

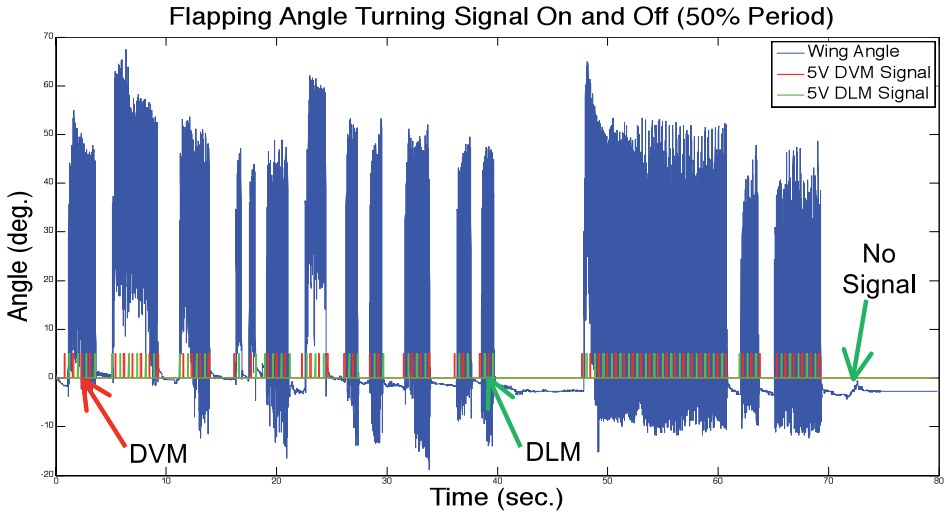

Figure 20: Two different 5V signals supplied with a .756 second period and a .0756 second signal length; the red is supplied to the DVM and the green is supplied to the DLM; during this test, the signals were randomly removed from the test subject (red arrow) to indicate that the supplied signal was in fact causing the motion.

5. CONCLUSIONS

5.1. Reapplying the EMG Signal

Analyzing and experimenting with living organisms with artificial constraints may introduce possible errors because these conditions are not found in the natural environment. It is impossible with current technology to record EMG signals without affecting the natural movement of M.sexta. The fine wire technique used in this research was chosen because of the availability and functional capabilities of the equipment. The results generated from the fine wire process are acceptable and prove that this technique can reproduce expected neuromuscular signal responses, as measured by the wing angles.

When the actual EMG signals were reapplied to M.sexta muscle, the wing angle demonstrated disjointed and erratic behavior. This was due to the multiple motoneuron signals that were recorded during the EMG process. Another problem with reapplying recorded EMG signals is that the muscle fibers do not react the same as motoneurons because the electrodes affect only a localized area, rather than distributing the signal throughout the muscle. One reason that the voltage needs to be increased is to induce more muscle fibers to reach their threshold, which in turn will increase muscular contraction the signals transmitted to the moth during this investigation most likely were activating the motor neurons which then activated the muscles. The EMG signals that are recorded give a basis of understanding about how the muscles are working to generate lift; these signals can then be processed into a simpler impulse signal that can be used to stimulate wing movement.

As Figure 19 showed, the full range of motion was not achieved through stimulation; the frequencies did not match up as well. Bozkurt's experimentations [3] were able achieve much more accurate results as far as mimicking the moths natural flight patterns. More time is needed to fine tune the signals that are being transmitted into the muscles for maximum range of motion and improved frequency control.

The recording of EMG signals from an M.sexta is an intrusive process; as technology improves, it may be possible to gather this information in a different way which will not affect the natural movement

of M.sexta. This could be done with some device that is so sensitive that the muscle movement could be detected from a distance, removing the need for implants or any equipment touching the test subject. Despite the problems in collecting them, the EMG signals proved to be very valuable in determining the electrode placement, phase shift, and flapping frequency. Based on this EMG information specific impulse signals were selected and reapplied to the flight muscles.

5.2. Relevance of the Current Investigation

There are three main benefits with having the ability to categorically induce known wing movement through artificial stimulation. First, using M.sexta as the flapping mechanism maintains the biological integrity under which MAV research at AFIT is conducted. Secondly, this provides quantitative data that can be used in artificial MAV designs. Lastly, this research could evolve into the ability to successfully control the movements of M.sexta as a biological MAV. This paper establishes procedures for fixed wire EMG signal acquisition and signal transmission into M.sexta muscles. It also acquired preliminary data associating wing angle responses with a known signal.

There are many different flapping designs and mechanisms that attempt to simulate wing movement. The best way to ensure that the variables remain constant is to avoid changing them. The best way to see how an M.sexta would move its wings under a given condition is to induce the desired movement in M.sexta. How the wings of an M.sexta function and react in their natural environment (attached to the insect) is more precisely understood by studying wing movement from a known impulse. The data in this paper proves that it is possible to generate wing movement with simple impulse signals sent into the muscle. This process also removes the time variable condition of the wing desiccation that was seen previously in Figure 13.

This research has great application for MAV research. Currently, there is very little understanding about how to power MAVs with the constraints that have been established. It is believed that further insight into this problem will be generated by studying the flight muscles and their impact on wing movement.

5.3. Final Statement

This research successfully accomplished its objective, which was to determine the possibility and benefit of reproducing the biological flapping motion of a Manduca sexta with artificial stimulation for the purpose of MAV development. This investigation has clearly shown that it is possible to generate specific flapping movement of M.sexta through direct muscle stimulation with fine wire electrodes. The ability to accurately reproduce biological movement has three primary benefits, which are to maintain the integrity of wing and joint during testing, provide useful data for MAV design, and to create the possible design of living M.sexta MAVs. This research directly applies to the future of biologically inspired MAV work, and with further study, could greatly improve current understanding about wing movement.

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ACKNOWLEDGEMENTS

The authors wish to express gratitude to Dr Doug Smith (AFOSR) and Dr Richard Snyder (AFRL) whose support made this research possible. Thank you to H. Cahoon, J. Climber (AFIT), and R. Cobb (AFIT) for extraordinary assistance in on this work.

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